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PREFACE

The United States Air Force Summer Faculty Resarch Program (USAF-SFRP) is designed to introduce university, college, and technical institute faculty members to Air Force research. This is accomplished by the faculty members being selected on a nationally advertised competitive basis for a ten-week assignment during the summer intersession period to perform research at Air Force laboratories/centers. Each assignment is in a subject area and at an Air Force facility mutually agreed upon by the faculty members and the Air Force. In addition to compensation, travel and cost of living allowances are also paid. The USAF-SFRP is sponsored by the Air Force Office of Scientific Research, Air Force Systems Command, United States Air Force, and is conducted by Universal Energy Systems, Inc.

The specific objectives of the 1989 USAF-SFRP are:

(1) To provide a productive means for U.S. faculty members to participate in research at Air Force Laboratories/Centers;

(2) To stimulate continuing professional association among the faculty and their professional peers in the Air Force;

(3) To further the research objectives of the United States Air Force;

(4) To enhance the research productivity and capabilities of the faculty especially as these relate to Air Force technical interests.

During the summer of 1989, 168-faculty members participated. These researchers were assigned to 23 USAF laboratories/centers across the country. This four volume document is a compilation of the final reports written by the assigned faculty members about their summer research efforts.

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Chi-Ming Tang Associate Prof. New York, State Univ. of Dept. of Math Geneseo, NY 14454 716\245-5386	Degree: PhD Specialty: Mathematics Assigned: Aerospace Medical Research Lab.
Richard Tankin Professor Northwestern Univ. Mechanical Eng. Dept. Evanston, IL 60201 312\491-3532	Degree: PhD Specialty: Mechanical Eng. Assigned: Aero Propulsion Laboratory

NAME/ADDRESS	DEGREE, SPECIALTY, LABORATORY ASSIGNED
Teresa Taylor Assistant Prof. Missouri-Columbia, University of 600 West Mechanic Independence, MO 64050 816\276-1285	Degree: PhD Specialty: Civil Eng. Assigned: Engineering & Services Center
Ebo Tei Professor Arkansas-Pine Bluff, Univ. of Social & Behavioral Sci. Pine Bluff, AR 71601 501\541-6787	Degree: PhD Specialty: Psychology Assigned: Aerospace Medical Research Lab.
Roger Thompson Assistant Prof. Pennsylvania St. University 233 Hammond Bldg. University Park, PA 16802 814\863-0968	Degree: PhD Specialty: Eng. Mechanics Assigned: Astronautics Laboratory
Richard Tipping Professor Alabama, University Dept. of Physics Tuscaloosa, AL 35487 205\348-3799	Degree: PhD Specialty: Physics Assigned: Arnold Engineering Development Ctr.
Phillip Tomporowski Assistant Prof. Alabama, University of Box 870348 Tuscaloosa, AL 35487 205\348-1936	Degree: PhD Specialty: Psychology Assigned: Human Resources Laboratory: Operations Training Division
Ram Tripathi Professor Texas-San Antonio, Univ. of Dept. of Mathematics San Antonio, TX 78285 512\691-5549	Degree: PhD Specialty: Statistics Assigned: School of Aerospace Medicine

NAME/ADDRESS	DEGREE, SPECIALTY, LABORATORY ASSIGNED
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Timothy Troutt Associate Prof. Washington State University of Mech. & Mat. Eng. Dept. Pullman, WA 99164 509\335-4375	Degree: PhD Specialty: Mechanical Eng. Assigned: Frank J. Seiler Research Lab.
Donald Ucci Associate Prof. Illinois Inst.Tech. 3300 S. Federal St. Chicago, IL 60616 312\567-3405	Degree: PhD Specialty: Electrical Eng. Assigned: Rome Air Development Center
George Veyera Assistant Prof. Rhode Island, University of Dept. of Civil Eng. Kingston, RI 02881 401\792-2692	Degree: PhD Specialty: Civil Eng. Assigned: Engineering & Services Center
Hung Vu Assistant Prof. California State University Mech. Eng. Dept. Long Beach, CA 90840 213\985-1524	Degree: PhD Specialty: Applied Mechanics Assigned: Frank J. Seiler Research Lab.
Bonnie Walker Assistant Prof. Central State Univ. Psychology Dept. Wilberforce, OH 45384 513\376-6516	Degree: PhD Specialty: Experimental Psychology Assigned: Aerospace Medical Research Lab.

NAME/ADDRESS	DEGREE, SPECIALTY, LABORATORY ASSIGNED
William Wallace Professor Rensselaer Poly. Inst CII Room 5117 Troy, NY 12180 518\276-6452	Degree: PhD Specialty: Management Science Assigned: Rome Air Development Center
Ji Wang Professor San Jose State Univ. S. 7 St. San Jose, CA 95192 408\924-4299	Degree: PhD Specialty: Mechanical Eng. Assigned: Astronautics Laboratory
Phillip Wapner Associate Prof. Southern Illinois University Dept. of Mech. Eng. Carbondale, IL 62901 618\453-7021	Degree: PhD Specialty: Chemical Eng. Assigned: Astronautics Laboratory
Robert Wheasler Professor Wyoming, Univ. of Box 3295 University Station Laramie, WY 82071 307\766-5126	Degree: PhD Specialty: Engineering Assigned: Aero Propulsion Laboratory
D. Wilkes Assistant Prof. Vanderbilt Univ. Box 1649 Station B Nashville, TN 37235 615\343-6016	Degree: PhD Specialty: Electrical Eng. Assigned: Arnold Engineering Development Center
Robert Willis Associate Prof. Mercer University 1400 Coleman Ave. Macon, GA 31207 912\744-2704	Degree: PhD Specialty: Physics Assigned: Geophysics Laboratory

NAME/ADDRESS	DEGREE, SPECIALTY, LABORATORY ASSIGNED
John Wills Professor Indiana Univ. Physics Dept. Bloomington, IN 47405 812\855-1479	Degree: PhD Specialty: Physics Assigned: Geophysics Laboratory
David Woehr Assistant Prof. Texas A&M Univ. Dept. of Psychology College Station, TX 77843 409\845-2097	Degree: PhD Specialty: Industrial Psychology Assigned: Human Resources Laboratory: Manpower and Personnel Division
Michael Wolfe Assistant Prof. West Virginia Univ. PO Box 6025 Morgantown, WV 26506 304\293-4495	Degree: PhD Specialty: Management Science Assigned: Human Resources Laboratory: Logistics & Human Factors
William Wolfe Associate Prof. Ohio State Univ. 470 Hitchcock Hall Columbus, OH 43210 614\292-0790	Degree: PhD Specialty: Engineering Assigned: Flight Dynamics Laboratory
James Wolper Assistant Prof. Hamilton College Dept. of Math. & Comp. Sci. Clinton, NY 13323 315\859-4417	Degree: PhD Specialty: Mathematics Assigned: Rome Air Development Center
Asad Yousuf Assistant Prof. Savannah State College PO Box 20089 Savannah, GA 31404 912\356-2154	Degree: MS Specialty: Electrical Eng. Assigned: Armament Laboratory

NAME/ADDRESS	DEGREE, SPECIALTY, LABORATORY ASSIGNED
Juin Yu Professor West Virginia Tech. Mechanical Eng. Dept. Montgomery, WV 25136 304\442-3248	Degree: PhD Specialty: Mechanical Engineering Assigned: Flight Dynamics Laboratory
Gregory Zagursky Assistant Prof. Morris College Div. General Studies Sumter, SC 29150 803\775-9371	Degree: MS Specialty: Biology Assigned: Occupational and Environmental Health Laboratory
Lawrence Zavodney Assistant Prof. Ohio State Univ. 209 Boyd Lab. Columbus, OH 43210 614\292-2209	Degree: PhD Specialty: Mechanical Eng. Assigned: Flight Dynamics Laboratory
Yehoshua Zeevi Professor Harvard Univ. Applied Sciences Cambridge, MA 02138 617\495-2850	Degree: PhD Specialty: Electrical Eng. Assigned: Human Resources Laboratory: Operations Training Division
Robert Zerwekh Assistant Prof. Northern Illinois University Dept. of Comp. Sci. DeKalb, IL 60115 815\753-6949	Degree: PhD Specialty: Philosophy Assigned: Human Resources Laboratory: Training Systems
Henry Zmuda Assistant Prof. Stevens Inst Tech Dept. of Electrical Eng. Hoboken, NJ 07030 201\420-5507	Degree: PhD Specialty: Electrical Eng. Assigned: Rome Air Development Center

PARTICIPANT LABORATORY ASSIGNMENT

C. PARTICIPANT LABORATORY ASSIGNMENT (Page 1)

1989 USAF/UES SUMMER FACULTY RESEARCH PROGRAM

AERO PROPULSION LABORATORY (WRDC/APL)
(Wright-Patterson Air Force Base)

1. Mingking Chyu
2. Jerry Clark
3. Wayne Eckerle
4. Frank Gerner
5. Thomas Lalk
6. Baruch Lieber
7. Larry Roe
8. William Schulz
9. Richard Tankin
10. Robert Wheasler

ARMAMENT LABORATORY (ATL)
(Eglin Air Force Base)

1. Peter Armandarez
2. Joseph Brown
3. Roger Bunting
4. Satish Chandra
5. David Cicci
6. George Coleman
7. Boghos Sivazlian
8. Steven Trogdon
9. Asad Yousuf

HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY (AAMRL)
(Wright-Patterson AFB)

1. Ernest Hallford
2. Carl Ingling
3. Charles Kincaid
4. Thomas Lockwood
5. Lewis Lutton
6. Ethel Matin
7. Randy Pollack
8. Michael Stanisic
9. Joseph Szucs
10. Chi-Ming Tang
11. Ebo Tei
12. Bonnie Walker

ARNOLD ENGINEERING DEVELOPMENT CENTER (AEDC)
(Arnold Air Force Base)

1. Brian Beecken
2. Lee Britt
3. Brian Circelli
4. Stephen Cobb
5. John Francis
6. Orlando Hankins
7. Lang-Wah Lee
8. Chun Fu Su
9. Richard Tipping
10. D. Mitchell Wilkes

ASTRONAUTICS LABORATORY (AL)
Edwards Air Force Base)

1. John Kenney
2. M. Inga Kenney
3. Lynn Kirms
4. Mark Kirms
5. Faysal Kolkailah
6. Bipin Pai
7. Vittal Rao
8. Richard Robertson
9. Larry Swanson
10. Roger Thompson
11. Ji Wang
12. Phillip Wapner

AVIONICS LABORATORY (WRDC/AL)
(Wright-Patterson Air Force Base)

1. David Choate
2. R. H. Cofer
3. Larry Crum
4. Mohammad Karim
5. Dar-Biau Liu
6. James Noyes
7. Robert Shock

C. PARTICIPANT LABORATORY ASSIGNMENT (Page 2)

ELECTRONIC SYSTEMS DIVISION (ESD)
(Hanscom Air Force Base)

1. Beryl Barber
2. Ajit Choudhury
3. S. Natarajan

ENGINEERING AND SERVICES CENTER (ESC)
(Tyndall Air Force Base)

1. Jon Anderson
2. William Bannister
3. Emerson Besch
4. Anthony Carlisle
5. Derald Chriss
6. Avery Demond
7. Kirk Hatfield
8. Kim Hayes
9. Deborah Ross
10. Teresa Taylor
11. George Veyera

FLIGHT DYNAMICS LABORATORY (WRDC/FDL)
(Wright-Patterson Air Force Base)

1. Larry Byrd
2. Kenneth Cornelius
3. Dennis Farrell
4. Augustus Morris
5. Arnold Polak
6. Nisar Shaikh
7. William Wolfe
8. Juin Yu
9. Lawrence Zavodney

FRANK J. SEILER RESEARCH LABORATORY (FJSRL)
(USAF Academy)

1. R. Granger
2. L. Hillman
3. John Lanning
4. John Sanders
5. Clay Sharts
6. Timothy Troutt
7. Hung Vu

GEOPHYSICS LABORATORY (AFGL)
(Hanscom Air Force Base)

1. Pradhip Bakshi
2. Chi Chen
3. P. Das
4. Alan Kafka
5. Charles Lishawa
6. Amy Miller
7. Thomas Miller
8. Henry Nebel
9. Hugh Nutley
10. Craig Rasmussen
11. Robert Willis
12. John Wills

HUMAN RESOURCES LABORATORY (HRL)
(Brooks, Williams, and Wright-Patterson Air Force Bases)

1. Christopher Bell
2. Kevin Bennett
3. Kathryn Cochran
4. Deborah Mitta
5. Miles Simpson
6. William Smith
7. Stanley Stephensen
8. P. Tomporowski
9. David Woehr
10. Michael Wolfe
11. Yehoshua Zeevi
12. Robert Zerwekh

C. PARTICIPANT LABORATORY ASSIGNMENT (Page 3)

MATERIALS LABORATORY (ML)
(Wright-Patterson Air Force Base)

1. Karren Brito
2. Donald Chung
3. Kenneth Currie
4. Peter Henrisken
5. Joseph Newkirk
6. Harvey Paige
7. Michael Resch
8. James Sherwood
9. Michael Sydor

OCCUPATIONAL AND ENVIRONMENTAL HEALTH LABORATORY (OEHL)
(Brooks Air Force Base)

1. Barbara Alvin
2. Stewart Maurer
3. Edmund Shearer
4. Gregory Zagursky

ROME AIR DEVELOPMENT CENTER (RADC)
(Griffiss Air Force Base)

1. Charles Alajajian
2. Ian Grosse
3. John Hadjilogiou
4. Henry Helmken
5. Stuart Hirshfield
6. Michael Klein
7. William Kuriger
8. Jay Lee
9. Robert O'Connell
10. Sally Sedelow
11. Khaja Subhani
12. Donald Ucci
13. William Wallace
14. James Wolper
15. Henry Zmuda

SCHOOL OF AEROSPACE MEDICINE (SAM)
(Brooks Air Force Base)

1. Thomas Abraham
2. Robert Blystone
3. Carolyn Caudle-Alexander
4. James Chambers
5. Mark Cornwall
6. Vito DelVecchio
7. Patrick Hannon
8. Cynthia Hardy
9. Jeffrey Himm
10. Gwendolyn Howze
11. Tze San Lee
12. Harold Longbotham
13. Rex Moyer
14. Raymond Quock
15. Paul Scheie
16. Ronald Seaman
17. Ram Tripathi

WEAPONS LABORATORY (WL)
(Kirtland Air Force Base)

1. Thomas Dwyer
2. William Filippone
3. William Grissom
4. Harry Hogan
5. Duc Nguyen
6. Duane Sanders
7. Hugh Siekfen
8. John Silvestro

WILFORD HALL MEDICAL CENTER (WHMC)
(Lackland Air Force Base)

1. Walter Drost-Hansen

RESEARCH REPORTS

RESEARCH REPORTS

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Technical Report Number	Title	Professor
Volume I		
Armament Laboratory		
1	Reactive Compositions Using Light Metals and Metal Alloys	Dr. Peter Armendarez
2	Maneuvering Hard Target Penetrators	Dr. Joseph Brown
3	A Study of Ionic Polymer Membranes for Application as Capacitor Electrolytes and Preliminary Investigations on Photo-Activated Stripline Switches	Dr. Roger Bunting
4	Multisensor Seeker for Medium Range Air-to-Air Missiles	Dr. Satish Chandra
5	Extended Kalman Filter Tuning and Alternative Techniques	Dr. David Cicci
6	Statistical Analysis of Blast Loading in Concrete	Dr. George Coleman
7	A Methodology for Evaluating the Effectiveness of Smart Submunition Systems	Dr. Boghos Sivazlian
8	Shock Wave Initiated Detonation of an Explosive	Dr. Steven Trogdon
9	Distributed Filter Architecture Implementation with VLSI and Expert Systems	Dr. Asad Yousuf
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10	Response of Infrared Detectors to Pulsed Radiation	Dr. Brian Beecken
11	An Analysis of Focal Plane Irradiance Effects on IR Detectors	Dr. Lee Britt
12	Code Development for Design of a High Temperature Hypersonic Facility Mixer	Dr. Brian Circelli

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14	Thermal Analysis of Bodies Subjected to Aerodynamic Heating	Dr. John Francis
15	Diagnostics for Determination of Arc Plasma Parameters of the AEDC HEAT H1 Arc Heater	Dr. Orlando Hankins
16	The Design of Jet Mixers for an Arc Heater: An Experimental Approach	Dr. Lang-Wah Lee
17	Laser Induced Fluorescence (LIF) of Nitric Oxide (NO)	Dr. Chun Fu Su
18	Spectroscopic Monitoring of Exhaust Gases	Dr. Richard Tipping
19	Distributed and Parallel Image and Signal Processing	Dr. D. Wilkes

Astronautics Laboratory

20	Magnetic Perturbations of the Structural Characteristics, Photophysical Properties and Photochemical Behavior of Cryogenic Noble Gas-Alkali Metal Matrices	Dr. John W. Kenney
21	I_{sp} Enhancement Via Adsorption/ Absorption of Small Energetic Molecules on Solid Propellants	Dr. M. Inga Kenney
22	Studies Toward the Synthesis of Pentanitrobishomocubane	Dr. Lynn M. Kirms
23	The Preparation of Poly(imide Siloxane) Polymers: Oxygen Resistant Space Polymers	Prof. Mark Kirms
24	Numerical Presentation of Stress Analysis, Design and Fracture Mechanics for Composite Materials and Structures	Dr. Faysal Kolkailah
25	Fracture Behavior of a Composite Solid Rocket Propellant	Dr. Bipin Pai
26	Robust Control of a L Experimental Grid Using Reduced Order Models	Dr. Vittal Rao

Astronautics Laboratory (continued)

27	A Neural Network Approach to the Adaptive Control of Large Space Structures	Dr. Richard Robertson
28	Cryogenic Heat Pipes	Dr. Larry Swanson
29	Design and Development of a Flexible Multi-Body Dynamics Experiment	Dr. Roger Thompson
30	Synthesis of Active Space Structure Vibration Control Systems for an Astrex Test Article	Dr. Ji Wang
31	Dynamic Mechanical Response of Carbon/ Carbon Composites by Vibrating Reed Measurements	Dr. Phillip Wapner

Electronics Systems Division

32	Carrier Free Radar	Dr. Beryl Barber
33	Detection Performance for Over Resolved Targets with Varying Energy Level in Cells	Dr. Ajit Choudhury
34	Analysis of Testability Concepts and its Application to RSIP	Dr. S. Natarajan

Engineering and Services Center

35	Proposed Innovative Semi-Hard Aircraft Shelter	Dr. Jon Anderson
36	JP-8 Ignitability	Dr. William Bannister
37	Effect of Jet Aircraft Noise on Domestic Goats	Dr. Emerson Besch
38	An Algorithmic System for Subjective Comparisons	Dr. Anthony Carlisle
39	The Study of Alkali-Enhanced Cements and Concretes	Dr. Derald Chriss
40	Prediction of the Capillary Pressure-Saturation Relationships for Aquifiers Contaminated with Jet Fuels	Dr. Avery Demond
41	Contaminant Flux Reduction Through In Situ Solubility Modification	Dr. Kirk Hatfield

Engineering and Services Center (continued)

42	An FT-IR Spectroscopic Investigation of Surfactant Adsorption at the Mineral-Water Interface	Dr. Kim Hayes
43	Biodegradation of Jet Fuel JP-8	Dr. Deborah Ross
44	Further Development of the AFESC Centrifuge Facility	Dr. Teresa Taylor
45	Static and Lynamic Behavior of Compacted Unsaturated Sands	Dr. George Veyera

Volume II
Frank J. Seiler Research Laboratory

46	The Vibration of Thin Leading Edges	Dr. Robert Granger
47	Second Harmonic Generation in Optical Fibers	Dr. Lloyd Hillman
48	Evaluation of Cold Fusion in Molten Salt Systems	Dr. John Lanning
49	High Charge Density Batteries Employing Ionic Liquid Electrolytes	Dr. John Sanders
50	A Convenient Preparation of Nitronium Triflate and its Use for Nitration	Dr. Clay Sharts
51	An Investigation of Dynamic Stall Vortex Characteristics	Dr. Timothy Troutt
52	Modeling of a Structure-Actuator System with Structure-Borne Reaction-Mass Actuators and Optimal Design of Passive Vibration Absorbers	Dr. Hung Vu

Geophysics Laboratory

53	Impulse Approximation Formalism for Atom Molecule Collisions: Exact Theory and Limitations	Dr. Pradip Bakshi
54	A Statistical Analysis of the Geomagnetic Indices, 1932-1989	Dr. Chi Chen
55	Cumulus Parameterization in Numerical Prediction Models: Proposal for a New Parcel-Dynamical Approach	Dr. Phanindramohan Das

Geophysics Laboratory (continued)

56	Estimating Characteristics of Chemical Explosions in New England and Eastern Kazakhstan Using Local and Regional Seismic Data	Dr. Alan Kafka
57	A Study of the Water Vapor Cation-Neutral Reactions	Dr. Randal Lishawa
58	Acidities of Iron Hydride and Various Transition-Metal Compounds; Reactions of Iron and Iron Carbonyl Anions	Dr. Amy Miller
59	Acidities of Iron Hydride and Various Transition-Metal Compounds; Reactions of Iron and Iron Carbonyl Anions (Same Report as Dr. Amy Miller)	Dr. Thomas Miller
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61	Estimating Solar Flare Proton Fluences From 1850 with Tritium Data	Dr. Hugh Nutley
62	Electric Fields in the Middle-and Low-Latitude Ionosphere and Plasmasphere	Dr. Craig Rasmussen
63	Review and Assessment of Carbon Dioxide Pressure Broadening Data	Dr. Robert Willis
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Rome Air Development Center

65	Design Considerations in the Implementation of ACT Programmable Transversal Filters	Dr. Charles Alajajian
66	Automating Finite Element Reliability Assessment of Microelectronic Components	Dr. Ian Grosse
67	Design for Testability: From Components to Systems	Dr. John Hadjilogiou
68	Development of a High Resolution Research Facility	Dr. Henry Helmken
69	Iterative V&V: A Model for Verification and Validation in the Rapid Prototyping Life Cycle	Dr. Stuart Hirshfield

Rome Air Development Center (continued)

70	Capable Neural Networks for Applications in Data Analysis (1988 Participant)	Dr. Oleg Jakubowicz
71	A Study of Interacting Tunneling Units with Possible Application to High Temperature Superconductors	Dr. Michael Klein
72	Design of a Practical Binary Phase-Only Optical Correlator	Dr. William Kuriger
73	A Computer for Temporal Frequency Spectrum of Vegetation Clutter Return	Dr. Jay Lee
74	Material Effects in Photoconductive Frozen Wave Generators	Dr. Robert O'Connell
75	Parallel Processing for Associative Semantic Space Analysis	Dr. Sally Sedelow
76	Characterization of an Optical Switch	Dr. Khaja Subhani
77	Study of a Communication Receiver for Spread Spectrum Signals	Dr. Donald Ucci
78	Tactical Command and Control: A Group Problem Solving and Decision Making Process	Dr. William Wallace
79	Neural Networks for Invariant Pattern Recognition: A Survey of the State of the Art	Dr. James Wolper
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Weapons Laboratory

81	An Experimental Protocol for Line-of-Sight Slewing, Optical Alignment and AFT Body Station Keeping Control Emulation	Dr. Thomas Dwyer
82	Linking the Twodant S_N Code and the MCNP Monte Carlo Code	Dr. William Filippone
83	Simulation of a Spray Reactor for Generating Excited Oxygen	Dr. William Grissom
84	Modeling the Response of Pressurized Composite Cylinders to Laser Damage	Dr. Harry Hogan

Weapons Laboratory (continued)

85	Parallel and Vector Processing for Nonlinear Finite Element Analysis ** To be published at Weapons Lab as Technical Memorandum **	Dr. Duc Nguyen
86	Scattering of Elastic Waves in a Random Inhomogeneous Soil Media	Dr. Duane Sanders
87	A Possible New Source of Negative Hydrogen Ions	Dr. Hugh Siefken
88	The Effect of a Maverick Missile on a Test Antenna at Spacings Less than $2D^2/\lambda$	Dr. John Silvestro

Volume III
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Aero Propulsion Laboratory

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90	Experimental Study of Electronic Excitation of Xenon by Electron Impact	Dr. Jerry Clark
91	No Report Submitted	Dr. Wayne Eckerle
92	Flow Limitations in Micro Heat Pipes	Dr. Frank Gerner
93	Conceptual Design of an In-House Facility for Endothermic Fuels Research (Report is not publishable at this time)	Dr. Thomas Lalk
94	Large-Scale Motion and Coherent Structures in Axisymmetric Swirling Flow of a Dump Combustor	Dr. Baruch Lieber
95	Stability Modification and Flowfield Evaluation of a Ramjet Combustor Model	Dr. Larry Roe
96	Oxidative Thermal Degradation Studies of a Surrogate JP-8 with a Modified Thermal Precipitation Apparatus	Dr. William Schulz
97	Measurements of Droplet Velocity and Size Distributions in Sprays	Dr. Richard Tankin
98	Aircraft Engine Compressor and Fan Rework Practices	Dr. Robert Wheasler

Avionics Laboratory

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100	Ladar Target Detection and Recognition	Dr. R. H. Cofer
101	Toolbox for Image Processing using Distributed Computing	Dr. Larry Crum
102	Analytical Model of a Unique E-O Beam Scanner	Dr. Mohammad Karim
103	Dynamic Task Scheduling for the "ADA Distributed System Evaluation Testbed (ADSET)"	Dr. Dar-Biau Liu
104	Ada Compiler Efficiency Evaluation	Dr. James Noyes
105	Towards a Course-Grained Test Suite for VHDL Validation	Dr. Robert Shock

Flight Dynamics Laboratory

106	Parametric Study of Combined Boiling and Partial Dryout in Liquid Metal Heat Pipe Wicks	Dr. Larry Byrd
107	3-D Analysis of Laser Measurements of Vortex Bursting on Chined Forebody Fighter Configuration	Dr. Kenneth Cornelius
108	Robust Design Using Internal Model Control	Dr. Dennis Farrell
109	Neural Networks and their Role in Visual Object Recognition	Dr. Augustus Morris
110	A Study of Surface Roughness Effects in Hypersonic Flow	Dr. Arnold Polak
111	Life Prediction of Aircraft Transparencies by Accelerated Crazing Tests	Dr. Nisar Shaikh
112	Strain Distribution in Composite Coupons in Tension	Dr. William Wolfe
113	Characteristics of an Osmotically Driven Thermal Transfer Cycle	Dr. Juin Yu
114	The Influence of Viscoelastically Damped Members on the Dynamic Response of Flexible Elastic Structures	Dr. Lawrence Zavodney

Materials Laboratory

115	No Report Submitted	Dr. Karren Brito
116	The in-situ Laser Deposition of Superconducting Thin Film	Dr. Donald Chung
117	An Intelligent Neural Model for Recognition of Input/Output Patterns for a Molecular Beam Epitaxy Process	Dr. Kenneth Currie
118	Scanning Tunneling Microscopy and Ballistic-Electron-Emission Spectroscopy	Dr. Peter Henriksen
119	Evaluation of CR-SI Alloys for Aerospace Structural Applications	Dr. Joseph Newkirk
120	Molecular Modeling and Computational Chemistry: Studies of Additives for Fluids and Lubricants	Dr. Harvey Paige
121	Improvement in the Detection of Microcrack Initiation and Growth During Fatigue Cycling by Surface Acoustic Wave Scattering	Dr. Michael Resch
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Harry G. Armstrong Aerospace Medical Research Laboratory

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126	Comparison of Microsaint and Colored Petri Nets as Modeling Techniques	Dr. Charles Kincaid
127	Degradation of the Renal Peritubular Basement Membrane in Relation to Toxic Nephropathy of Fuels of Military Interest	Dr. Thomas Lockwood

Harry G. Armstrong Medical Research Laboratory (continued)

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130	An Investigation Into Techniques for Landmark Identification on 3-D Images of Human Subjects (Not publishable at this time)	Dr. Randy Pollack
131	Kinematic Mappings Between the EXOS Handmaster Exoskeleton[1] and the Utah/MIT Dexterous Robot Hand	Dr. Michael Stanisic
132	Harness Belt Task	Dr. Joseph Szucs
133	Articulated Total Body (ATB) View Program with Harness-Belt Implementation	Dr. Chi-Ming Tang
134	Insights into Human Factors in Aviation with Emphasis on Non-Canonical Flow Fields	Dr. Ebo Tei
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Human Resources Laboratory

136	Software Development to Support Data Collection and Analysis of Cognitive Task Analysis Studies	Dr. Christopher Bell
137	Computer-based Training for Complex, Dynamic Tasks	Dr. Kevin Bennett
138	Working Memory and Cognitive Structure	Dr. Kathryn Cochran
139	Investigation of Color Appearance within Low Light Levels (1988 Participant)	Dr. Douglas Mandra
140	Fisheye Representation of Information: IMIS User Interface	Dr. Deborah Mitta
141	The Validation of the Occupational Learning Difficulty (OLD) Index as a Predictor of Retrainee Performance	Dr. Miles Simpson

Human Resources Laboratory

142	Assessment of Intelligent Tutoring Systems	Dr. William Smith
143	The Role of the Instructor in Computer Based Training (CBT)	Dr. Stanley Stephenson
144	Evaluation of Air-Intercept Performance: Observer Reliability Issues	Dr. Phillip Tomporowski
145	Career Progression in Air Force Enlisted Personnel: An Examination of Two Alternate Criterion Measures	Dr. David Woehr
146	Development of the "City of Quality (Coq)" Group Decision Support System	Dr. Michael Wolfe
147	Variable Resolution Imagery for Flight Simulators	Dr. Yehoshua Zeevi
148	Neurocomputing the Student Model in an Intelligent Tutoring System	Dr. Robert Zerwekh

Occupational and Environmental Health Laboratory

149	Statistical Analyses of Data Pertaining to Ground Water Contamination and Laboratory Quality Control	Dr. Barbara Alvin
150	Design of an Automated Radiofrequency Radiation Measurement System (ARRMS)	Dr. Stewart Maurer
151	Construction and Use of an Identification Manual for Identifying Fibrous Material by Scanning Electron Microscopy with Attached X-Ray Analyzer	Dr. Edmund Shearer
152	Biological Analysis of Three Ponds at Peterson AFB, Colorado Springs, CO	Dr. Gregory Zagursky

School of Aerospace Medicine

153	Convergence Properties of the Occurrence/Exposure Rate	Mr. Thomas Abraham
154	Transmission Electron Microscopy of Mouse Macrophase RAW 264.7 Cells treated with Lipopolysaccharide, 3-Amino Tryosine, and RFR	Dr. Robert Blystone
155	Effects of Microwave Radiation on Cultured Cells	Dr. Carolyn Caudle-Alexander

School of Aerospace Medicine (continued)

156	The Effects of Three Reputed Carboxyesterase Inhibitors Upon Rat Serum Esterase Activity Using Paranitrophenyl and Naphtbyl Esters as Substrates	Dr. James Chambers
157	Anti-G Suit Inflation Influence on Lower Extremity Muscle Performance during Sustained +G_z Acceleration	Dr. Mark Cornwall
158	PCR Analysis and in situ Detection of Ureaplasma urealyticum and Mycoplasma hominis	Dr. Vito DelVecchio
159	The Influence of Broad Spectrum Illumination on Circadian Neuroendocrine Responses and Performance	Dr. Patrick Hannon
160	The relationship between locus of control, performance on cognitive tasks, and affective states after the consumption of antihistamines in hi- and low-workload conditions among aircrew personnel	Dr. Cynthia Ford Hardy
161	Aspects of the Diffusion of Inert Gases in Biological Systems	Dr. Jeffrey Himm
162	Studies of Interactions Between Microwaves, Melanin and Melanocytes	Dr. Gwendolyn Howze
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FINAL REPORT

PERCEIVED TIME TO CONTACT AS A FUNCTION OF EVENT STRUCTURE DURING SELF MOTION

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Date:	25 Aug 89
Contract No:	F49620-88-C-0053

Perceived time to contact as a function of event structure during self motion

by

Ernest W. Hallford

Abstract

Recent self-motion studies have shown that viewers are sensitive to visual information available in edge and flow rates specifying forward speed and altitude, respectively, although the two specifications may be partially confounded for some viewers. These studies have improved our understanding of visual factors affecting judgment and control of self movements during events involving constant speeds and level flights, as well as accelerations and decelerations, and ascents and descents. The current project extends these studies to questions regarding moving viewers' judgment and control of their time to contact with designated targets, as well as the prediction and control of contacts involving moving objects at a distance from themselves.

Acknowledgements

Thanks are due to The Air Force Systems Command and the Air Force Office of Scientific Research for their support of this project. It should also be noted that Universal Energy Systems was very helpful and accessible in the administration and direction of the program under which the project was developed.

The development of the project was greatly enhanced by the support, encouragement, and scholarship of Dr. Rik Warren, who provided both lab facilities for research and theoretical commentary on the issues of interest. The extensive advice and assistance, discussion of empirical and theoretical questions, and camaraderie provided by Dr. Lawrence Wolpert was invaluable in making my visit both useful and enjoyable. Kim Reardon was exceptionally helpful and supportive in preparing the displays for the experiments, managing the lab, testing participants, analyzing data, and providing technical advice and good humor. Special thanks are also due to Robert Todd, who prepared the program for displaying the scenes and collecting data, and who was notably insightful in asking questions and making suggestions.

I. INTRODUCTION.

The Perception and Control in Low Altitude Flight (PACLAF) laboratory of the Human Engineering Division of the Armstrong Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base has contributed extensively both to the study of the roles of optical flow and edge rates in the perception of egospeed and altitude, and to the study of simulated egomotion. These issues are also relevant to studies of the perception and control of contact, which is, of course, integral to aircraft landings and take-offs, guidance of low-altitude aircraft through cluttered environments, and decision-making activities pursuant to either accomplishing or else avoiding contact.

My own research interests have been directed toward (1) the study of a variety of event components which may affect judgment and control of time to contact, and (2) issues regarding an observer's ability to attend selectively to task-relevant information in the face of related but irrelevant information. My recent work on extrapolative judgments of time to contact for targets at a distance from the observer, using computer-controlled simulations, led to my assignment to the PACLAF laboratory.

II. OBJECTIVES OF THE RESEARCH EFFORT.

My first objective was to develop and review a bibliography of literature relating both general temporal-judgment issues and specific time-to-contact issues to the current studies and interests of the PACLAF laboratory. A second objective was to develop a preliminary proposal for egomotion studies of time to contact, in terms of current PACLAF interests, to be compared to similar studies of time to contact for objects at a distance from the observer. A third objective, added during the course of my summer's research effort, was to design and implement the first of a series of experiments on time-to-contact judgments during egomotion. It was decided that the initiation of this series, paralleled by studies at my own laboratory involving objects at a distance from the observer, should be undertaken during the latter portion of my SFRP, and continued through funding from the Mini Grant Program.

III. OBJECTIVE I: REVIEW OF RELATED RESEARCH.

Time to contact is a major issue in motion studies, since it is relevant to control and guidance of one's own as well as other objects' movements. Its study takes into account (1) whether a contact will be made (Lee, 1976), (2) when the

contact will occur (Hallford & Meagher, 1989; Lee, 1976; Lee & Reddish, 1981; McLeod & Ross, 1982; Schiff, 1965; Schiff & Detwiler, 1979); the nature (hard or gentle) of the contact (Gilden & Proffitt, 1989), and contact-control activities prior to and during the contact (Lee, 1976; Lee & Reddish, 1981; Lee, Young, Reddish, Lough, & Clayton, 1983). Furthermore, the study of judgment and control activities regarding the contact must take into account both situations in which the movement and relevant environmental factors are visually specified throughout the event, and those in which they are continuing but no longer visible. When a plane passes into a cloud bank, for example, it is no longer accessible to someone tracking it visually; personnel on the aircraft, conversely, no longer have visual access to ground-texture information. Although extrapolations of movement are important components of planning and control during both types of events, their accuracy is exceedingly crucial during occlusions, since control adjustments utilizing visual information are no longer possible.

An examination of relevant literature and extensive discussions with PACLAF personnel revealed that the optical specification of an imminent contact is deceptively simple: that is, during egomotion, or else during the approach of an object toward the observer, the optical size of the contact-

target increases while its optical position is fixed. This apparent simplicity is overshadowed, however, by the contact event's potential consequences, which are affected by the velocities of the objects involved; their distances from one another; their relative masses; control actions which may be utilized to avoid, or else to affect the nature of the contact; and time to contact, which greatly influences decision and control activities. Furthermore, contextual factors (such as the sizes and proximities of other objects, the patterning of the terrain, the size of the contact-target, and occlusions of the target), even when irrelevant to true contact time, may affect observer's judgments of contact time, and hence affect both decision and control activities.

Previous and current work on egomotion by FACLAF personnel has shown that both flow and edge rates are related to judgments and control of constant speed, acceleration and deceleration, and altitude (Owen & Warren, 1987; Owen, Wolpert, & Warren, 1983; Warren, 1988; Warren & Owen, 1982; Warren, Owen, & Hettinger, 1982; Wolpert, 1983; Wolpert, 1988; Wolpert, Reardon, & Warren, 1989). Most studies have shown that flow rate is more strongly related to detection of changes in altitude, while edge rate is more strongly related to detection of acceleration (cf., Denton, 1980):

furthermore, variables which are irrelevant to the task sometimes interfere with performance (Wolpert, et al., 1989).

IV. OBJECTIVE II: RESEARCH PROPOSAL.

The preliminary research proposal maps a three-pronged attack on the time-to-contact questions implied by the foregoing review. First, a study composed of several experiments involving constant-speed events will examine the relative contributions of speed, altitude, texture patterning, and occlusions on judgments of time to contact during egomotion. These will be compared to a study composed of similar experiments involving objects at a distance from the observer. Second, parallel sets of experiments will examine the same issues during events involving accelerations and decelerations. Finally, a series of experiments will examine these issues in situations where the observer responds through active control of the event, as opposed to the passive judgments of the foregoing studies. This project is expected to contribute greatly to our understanding of the factors involved in timing, control of contact, and decision making during ongoing events. Insofar as they reveal effects from salient but irrelevant factors, they will also assist in the development of training programs designed to reduce accidents related to control and guidance of moving objects.

V. OBJECTIVE III: EXPERIMENTAL DESIGN AND IMPLEMENTATION.

Experiment 1 from the proposed egomotion series was initiated in the PACLAF laboratory, and involved the generation of a set of constant-speed displays on a Silicon Graphics IRIS 4-D computer. These displays were perspectival views of a tunnel with dark gray rectangles (texture elements) appearing in more or less random patterns on the lighter-gray walls, floor and ceiling. On the floor of the tunnel, 200 ft (simulated) from the observer, was a white stripe, which was the contact-target for the observer. The display screen was located in a darkened viewing room, thus greatly enhancing the observer's sense of self-motion. The design was 3 X 3 X 3 X 2 completely within, for the following variables: Speed, Tunnel Width, Texture Element Size, and Texture Layout. Five volunteers from contracting agencies at AAMRL participated in this pilot run of Experiment 1.

On each trial, the display disappeared from the screen before the observer's simulated contact with the target occurred. The observer's task was to press the space-bar of a keyboard in front of the screen when contact with the stripe would have occurred, and then to indicate confidence (on a scale of 1 to 7, where 7 = greatest confidence) in the accuracy of the

judgment, by pressing one of the numeric keys at the top of the keyboard.

The pilot test with five participants provided information whereby technical problems with displays were dectected and corrected, and served as the basis for developing analysis programs. The first full run of the experiment was underway at the time of the composition of this report.

VI. RECOMMENDATIONS.

The systematic isolation and variation of factors thought to affect judgment and control of time to contact during egomotion, such as edge and flow rates, acceleration and deceleration, texture patterning and density, proximity to other objects, and occlusions, is essential to a full understanding of time-to-contact issues. The preliminary experiment conducted in the FACLAF laboratory was designed to examine several of the foregoing factors in a constant-speed situation. The systematic examination of other factors will require subsequent experiments; in addition, the current results will need to be compared to similar experiments involving objects at a distance from the observer. This will allow the specification of differences in decision and control activities when the observer is moving versus when

the observer is viewing, and possibly controlling, the movements of distant objects.

The first series of experiments involve constant speed events, but many of the movement issues regarding time to contact involve accelerations and decelerations, and, for aircraft, ascent and descent, of the moving self or distant object. The landing of aircraft or braking of land vehicles exemplify these issues, and an understanding of the visual information whereby decision and control activities are initiated and directed during such events is crucial both to training programs and to the design of simulation devices. Thus the second phase of this project is pointed toward time-to-contact judgments and control during accelerations and decelerations, and during ascent and descent movements.

Finally, the foregoing projects should involve both passive judgment and active control environments, since both are relevant to decision and control problems in the guidance of moving objects. Passive judgment tasks provide the opportunity to examine observers' responsiveness to lag times wherein planning and preparatory activities can be carried out prior to contact. Active control tasks, on the other hand, provide the opportunity to study observers' initiations and adjustments of control activities as a function of

particular temporal structures in the time-to-contact sequence of events.

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FINAL REPORT

The Effect of Luminance on the Perceived Saturation of Lights

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Contract No:	F49620-88-C-0053

The Effect of Luminance on the Perceived Saturation of Lights

by

Carl R. Ingling, Jr.

ABSTRACT

The effect of adaptation on the perceived saturation of lights has been studied extensively by Hunt (e.g., Hunt 1952; 1953). Hunt found that as adapting luminance decreases, the perceived saturation of lights decreases. At Hunt's lowest luminances, the chromaticities of dichoptically matched fields shrunk to the middle of the chromaticity chart; at nigh luminances, the chromaticities of the matches plotted near the spectral locus. These results run counter to similar work by Onley (Onley and Ingling, 1962), who found it necessary to add white light to a dimmer field for it to match the saturation of a dichoptically viewed standard. Although there are methodological differences between the studies, there appears to be no obvious explanation for the opposite results. The pilot work reported here verifies Onley's result.

ACKNOWLEDGMENTS

I thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. I also thank Universal Energy Systems for their handling of the administrative aspects of my summer fellowship. Individuals to whom I am grateful for collaborative efforts are Dr. Brian Tsou and Dr. David Post. Finally, special thanks to Mr. Gary Becklar for rapidly writing and cheerfully revising whenever requested the computer programs for controlling the CRT, and Mr. Christopher Calhoun for making the calibrations and colorimetric measurements of the stimuli.

I. INTRODUCTION

My research for the past 20-some years has been in the area of color vision, devoted in particular to quantitative models of color vision. Because of the need for accurate stimulus specification in my research, I also have interests in radiometry, photometry, and colorimetry, particularly in the photometry of optical systems and in the calibration of photometric standards.

Part of the research effort in the AAMRL/HEA is directed toward human factors/vision research. In particular, Dr. Brian Tsou's research on the visual, optical and photometric aspects of the Helmet-Mounted Display, and Dr. David Post's research on chromatic aspects of visual displays both coincided with my own research interests. At the suggestion of Dr. Tsou, I applied for a UES Summer Fellowship to work with him and with Dr. Post.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Recent quantitative models of color vision (e.g.; Guth et al, 1980; Vos and Walraven, 1974; Ingling and Tsou, 1977) transform the signals from R, G, B cones to form two chromatic differencing channels, or opponent-color channels, and one luminance channel. For various stimulus conditions, the relative contributions of the chromatic and achromatic channels are controlled by parameters in the models that are related to particular aspects of the stimulus situation. For example, one can calculate the spectral sensitivity curves predicted from the models for different adaptation conditions. For conditions of strong neutral adaptation (white-adapted) the models produce spectral sensitivities which depart markedly from the standard CIE luminosity function, V_λ . For this condition, the spectral sensitivities show numerous bumps and submaxima, for the reason that the white adaptation suppresses the

luminance channel sensitivity, thus relatively enhancing the contributions of the more narrowly-tuned opponent channels to the sensitivity curve. Similarly, because the opponent channels are postulated to have slower time constants, their contribution is eliminated in these models for any photometric criterion that depends upon temporal resolution; e.g., a flicker criterion (either a heterochromatic-flicker-photometric criterion, or critical flicker fusion).

At present, the relative weights assigned by the opponent-color models to the sensitivities of the luminance and chromatic channels is largely a matter of post-hoc curve fitting. That is, data are selected, and then it is demonstrated that the model can explain these data by weighting the luminance and opponent channels by whatever factors are needed to account for the empirical curves. The models are tested by showing they can account for a wide range of experimental data if proper values are given to the free parameters in the model; in this instance, they can be made to account for spectral sensitivity curves measured under a variety of experimental conditions.

In this connection it is of interest to know what the effect of intensity is on the relative contributions of opponent and luminance mechanisms. Presumably, each channel can be characterized by an input-output function. To give a related example, Judd (1951) explained the Bezold-Brucke hue shift by postulating different gain functions for the y-b and r-g opponent channels. For this particular instance, the idea is that as intensity increases, the y-b channel output increases more rapidly than does the r-g channel output. As a result, at low intensities the spectrum appears dominated by reds and greens, with relatively little blue and yellow. Conversely at high intensities, the spectrum is

dominated by blue and yellow. Violet practically disappears, being replaced by nearly pure blue, while wavelengths which at low intensities appeared nearly pure red now have a marked yellowish component.

Although the relative gains -- that is, the shapes of the input-output functions -- are an important feature of channel models if they are to successfully account for data over any appreciable intensity range, by and large this is a neglected aspect of these models. In general, the models are used to predict results for a particular intensity level and then reparameterized for different levels.

With an eye toward estimating the relative outputs of the chromatic and achromatic channels as a function of intensity, several studies that measured some form of both chromatic and achromatic responses as a function of intensity were reviewed -- for example, Gordon and Abramov (1988) and in particular Hunt (1952, 1953).

Although the studies by Hunt are self-consistent and carefully done, they stand in near-total contradiction to the results of Onley and Ingling (1962). This study was never published (except in abstract form) because of Dr. Onley's untimely death, and the data from the study, as well as the description of the procedures and conditions, are lost. The present author was the junior author of Onley's study, and also one of the observers in that study. Although there were numerous methodological differences between the two studies, the three major differences (recalling in 1989, with the aid of the abstract, a study done in 1961) were: (i) Hunt used a white surround in his study for both the test field and the matching field, whereas Only and Ingling's test and matching fields had a dark surround. (ii) Hunt (for the conditions of interest here) matched the appearance of the test field, presented at various luminances to the left eye, with a matching field provided by a

colorimeter to the right eye, whereas Onley and Ingling presented a chromatic standard at various luminances to the left eye and a (chromatic + white) test to the right eye. The observer matched the saturation of the left-eye standard by varying the amount of white light added to the right-eye test. (iii) Onley and Ingling presented their stimuli in brief flashes. Hunt's adapting fields were continuously viewed, the matching fields being substituted into them at brief intervals.

Because it is not clear what might be the cause of the discrepancy between Hunt and Onley and Ingling, the first objective was to partially replicate the conditions of the studies. Both studies employed a dichoptic matching technique, and both quantified the appearance of a test light presented at a range of intensities by matching it to a standard. The difference in the results is that for Onley and Ingling, as the intensity of the test light increased, it's whiteness content also increased, whereas Hunt found the opposite; in his study, the dimmest lights were the most desaturated.

III. METHODS

Stimuli were presented on a computer-controlled video monitor. This apparatus has been described elsewhere (Post and Calhoun, 1989). The monitor screen was divided by a septum and viewed from a distance of 5 feet. At this distance the split fields comprising the test and matching fields subtended two degrees of visual angle. The stimuli were viewed through a pair of base-out prisms placed before the eyes (see Fig. 1) so that the observer could fuse the fields despite their separation on the monitor screen. Any stimulus within the gamut shown in the UCS diagram of Fig. 2 could be displayed on the monitor. A program was written which allowed the purity of the stimulus displayed on the monitor to be

adjusted at constant dominant wavelength by rolling a track ball. That is, the chromaticities of the stimuli chosen could be moved along a line defined by a particular dominant wavelength and a particuclar white (D 65). The dominant wavelengths of the 4 stimuli selected for the study were based upon previous work (Post and Calhoun, 1988). The dominant wavelengths of the stimuli were: red, 641 nm; yellow, 580 nm; green, 546 nm; and blue, 465 nm. The CRT has greater maximum luminance for mid-spectral lights than for lights at the spectral extremes. Were the maximum luminance available for the blue light used for all the stimuli, the intensity range would have been greatly restricted. For this reason, maximum luminances which were rounded multiples of the highest blue luminance were used. These luminances were : red, 60; yellow and green, 120; and blue, 30 (in cd/m^2).

After testing various viewing and matching strategies, the following procedure was adopted. A series of neutral density filters (Wratten 96) in steps of 0.6 density (factor of 4X in luminance) were placed before the eye at the position of the prisms. For the experiments reported here, the field to one eye was kept constant at the highest intensity and the 0.6 density steps were successively placed before the other eye. After inspecting the stimulus fields, the observer was required to choose one side or the other to which to add white light in order for the apparent saturation of the fields to match. The question to be answered which dictated the choice of which button to push was "to which side, the bright side or the dim side, must I add white light to make it appear more like the other side?". After making this choice and pressing the appropriate button, the observer altered the colorimetric purity of the chosen side by rolling the track ball. As noted, the luminance was automatically held constant during this adjustment. After

making three settings, the luminance of the variable side was decreased again by a factor of 4X and the procedure repeated until the variable field became so dim that the observer was unable to continue. Upon completion of a run, the filters before the eyes were reversed and a second run made in order to counterbalance any effects due either to asymmetry between an observer's eyes, or possible asymmetries in the apparatus. Data from the two runs were averaged.

IV. RESULTS

Figure 2 shows the results for observer CI for 4 colors for an intensity range of 64:1. For all colors except blue, decreasing the luminance of the field produced the judgment that, in order to make the fields match for whiteness content, that white light must be added to the dimmer field. Operationally, this means that the dimmer field must appear more, rather than less, saturated. This is the opposite of Hunt's result.

V. DISCUSSION AND CONCLUSIONS

The results obtained must be interpreted with caution. These results are interim results; the study was not completed because of time limitations. The present findings must be replicated using at least a second observer (although we note that Hunt's results are based on a single observer; Onley and Ingling's results on two observers). Most important, however, a white surround must be added to the stimulus fields in order to replicate Hunt's conditions. Until this is done, speculation as to the reasons for the opposite results obtained in the two studies would not appear profitable.

VI. RECOMMENDATIONS

a) The study should be completed. It is anticipated that when completed, the results will be of significancce both practically and

theoretically. In the practical domain of color appearance, all color specification schemes -- e.g., the Munsell, the OSA Uniform Color Scales, etc. -- exhibit a relationship between the reflectance and the purity of the saturation of the samples that define the space. See, for example, Hunt (1955) who notes the relationshnsip betweeen saturation and lightness in color space. The contradiction being studied here points to a revision of the concept of saturation, at least to its identification with whiteness content. The problem seems to be that the dimmer stimulus appears to have greater "blackness content"; this leads to the paradoxical situation in which it is simultaneously less saturated, because of the dilution of the hue with black, but also requiring that white be added to cancel the blackness and thus make it appear more nearly similar to a brighter light, implying that it is more saturated, rather than less.

At a theoretical level, the results of the research promise to contribute to a theory of the relative gain factors for the achromatic and chromatic, or whiteness and hue, channels of opponent-color models. In fact, Hunt was led, almost presciently, to propose a whiteness or achromatic channel to account for his results well before the widespread vogue of opponent models in color vision.

b) Follow-on research: As recommended above, the study should be completed. This requires (i) finishing the replication of the conditions which simulate Onley and Ingling's lost study; (ii) adding the white surrounds, and (iii) if necessary, controlling the temporal sequence of the stimulus presentations, and finally, (iv) accounting for the results theoretically. This latter point promises to be somewhat of a challenge, inasmuch as it is certainly a strong possibility that the major, and contradictory, results of both studies will be confirmed.

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FIGURE CAPTIONS

Figure 1. Schematic of apparatus. Each of the observer's eyes viewed half the field, which fused to a bipartite matching field. The neutral density dimmed one side of the field. The observer added white (at constant luminance) to whichever side appeared the more saturated in order to match the fields for apparent saturation.

Figure 2. The CIE 1976 UCS chromaticity diagram showing the gamut of chromaticities available on the CRT. The points closest to the spectral locus shows the chromaticities of the red, yellow, green and blue lights used in the experiment. Also shown are the chromaticities of stimuli which appeared to match the saturation of the standard lights when the standard lights were dimmed by the densities indicated next to the points. The saturation of the lights was varied by moving them along the line connecting their dominant wavelengths with the D 65 white.

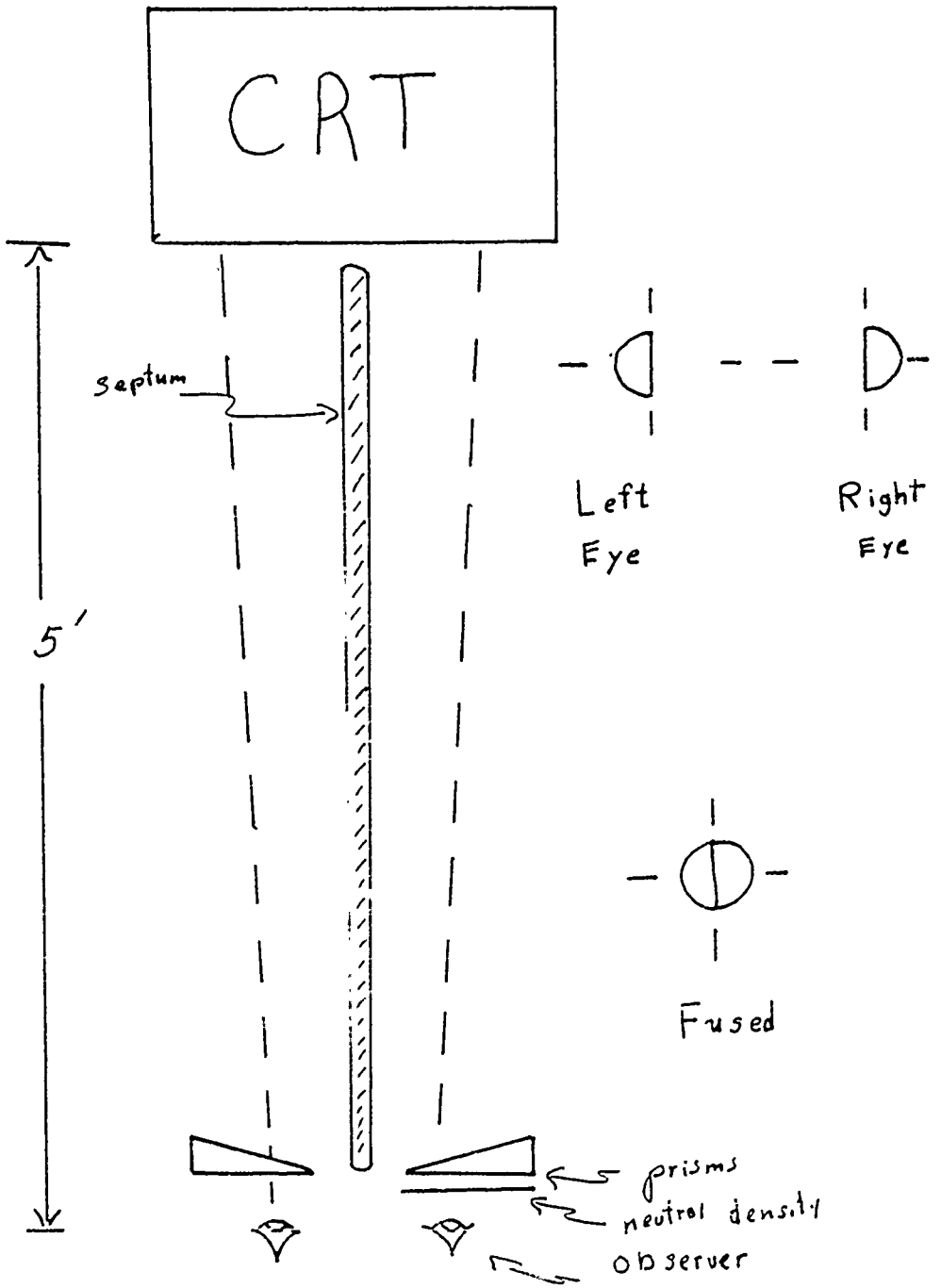

CRT
septum
Left
Eye
Right
Eye
5'
Fused
prisms
neutral density
observer

Fig 1

CARL.DAT 10-AUG-89

BLACK BACKGROUND

NO FILTER ON ONE SIDE

PLOT SHOWS COORDINATES OF ADJUSTED SIDE

CARL ALWAYS ADJUSTED THE FILTERED SIDE

EXCEPTING BLUE (1.2, 1.8),

REDUCING LUMINANCE

CAUSED SATURATION

TO INCREASE

CIE 1976 UCS CHROMATICITY DIAGRAM

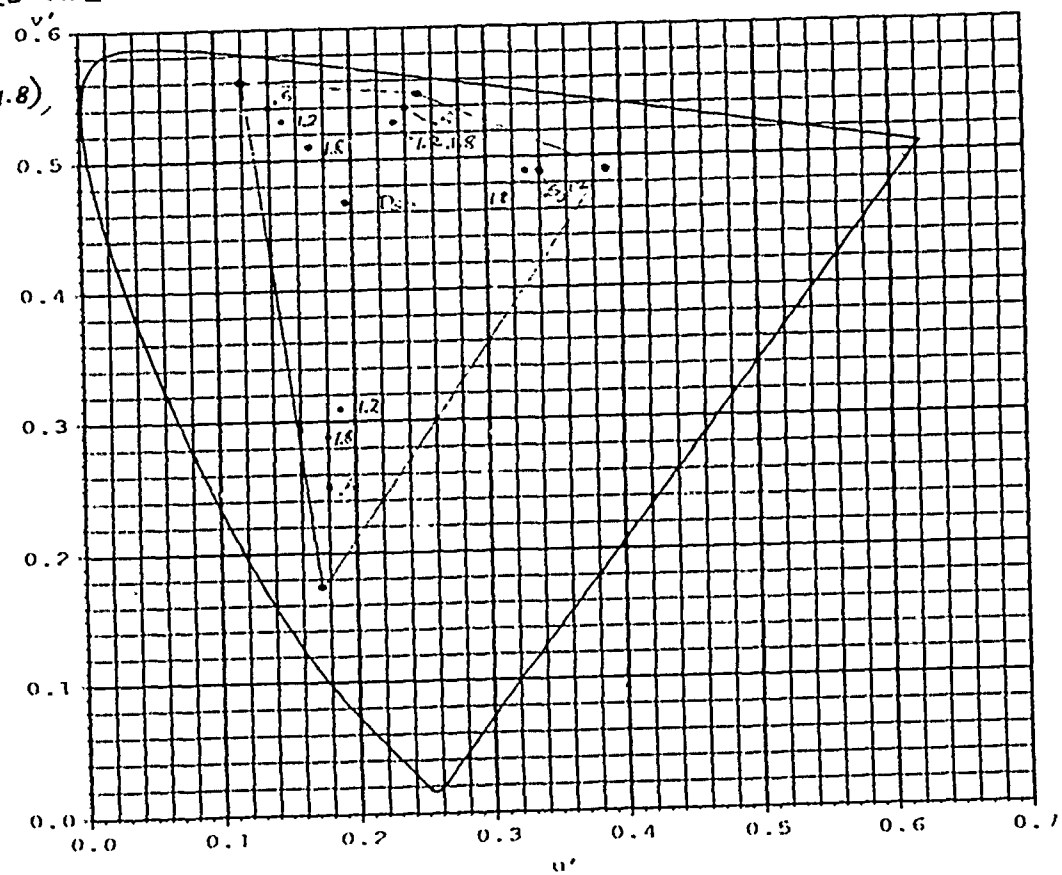


Figure 2

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FINAL REPORT

COMPARISON OF MICROSAINT AND COLORED PETRI NETS AS MODELING TECHNIQUES

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ANALYSIS OF MICROSAINT AND COLORED PETRI NETS AS MODELING TECHNIQUES

by

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ABSTRACT

This paper describes and evaluates two modeling techniques: Task Network theory using MicroSaint, and Colored Petri Nets using Design/CPN.

Both the concepts and the applications are analyzed to explore differences between the techniques. Enhancements are suggested for each technique that would expand its modeling capabilities. Finally, types of systems are described that would be most appropriate for each technique.

Acknowledgements

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Major Michael McFarren stands out among all the many people who have helped make this an exciting and rewarding experience. He willingly shared his time, knowledge and equipment thereby greatly enhancing the quality of this research. I extend my most sincere gratitude to him. Don Monk and Mike McNeese have also been very helpful in getting this research off the ground by providing insight and references into human factors. I also thank Colonel William Marshak for his support this summer. Peter Huber for his help in developing the CPN models and Ron Laughrey for the chance to visit Boulder.

On a more personal note, I would like to thank my wife, Barbara, for her love and support and for packing up and moving to Ohio.

I. INTRODUCTION:

Simulation is an essential tool in research and analysis of systems. Much work has been done to expand the power of simulation tools to model more complex systems. Modeling complex systems which integrate human and machine performance is an ongoing activity at the Human Engineering Division of Armstrong Aerospace Medical Research Laboratory. Colored Petri Nets, first introduced as Condition/Event Nets by Carl Adam Petri (5), are seen as a possible alternative to standard discrete-event system simulation in certain situations by modelers in the laboratory (11). Thus, it was desired to evaluate colored petri nets vs task network modeling and determine which simulation tool would be most appropriate based on the type of system to be studied.

My background includes a Bachelor's in Computer Science and a Master's in Statistics. "Applying Queuing Theory to a Fixed Cycle Traffic Intersection" was the topic of my Master's Report. For the past two years I have been teaching a simulation class to computer science undergraduates at the University of Florida.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The objective was simply to evaluate the two techniques to determine what, if any, advantages one had over the other. From this evaluation appropriate areas of applications would be identified. The best way to evaluate two modeling techniques is to implement them on the same system. Therefore the short-term objectives were as follows:

1. Define the system -- the system needed to be complex enough to test the full capabilities of the modeling technique, yet, at the same time, it needed to be simple enough so that implementing the model was not overwhelming;
2. Implement and run the system in both techniques -- from this a one to one comparison could be made of the different features of the techniques;
3. Analyze the results -- a majority of the time the output is the goal

of the simulation. A variety of appropriate statistical methods would be applied to the results.

As we shall see only the first two short-term objectives were completely realized.

The analysis will be made on two levels: Concept and Application. The concept level is composed of the ideas underlying the techniques. The tools that make these ideas available to the user make up the application level.

The dividing line between these two levels is often hard to draw. One must resist the temptation to combine the levels into one, since a poor application could obscure any distinctive features of the underlying ideas.

The criteria used to evaluate the two techniques are

For Concept

1. Ease of learning,
2. Ease of use/implementation,
3. Power/Robustness of technique.

For Application

1. Ease of learning,
2. Ease of use/implementation,
3. Type, format and quality of information returned from simulation.

III. RESULTS:

Models

Two systems were chosen to test task networks and colored petri nets. The first is a simple task found in all business offices, producing letters. This was chosen as a straightforward example of a discrete-event stochastic system. In this office there are two bosses who write letters and there are two secretaries who type letters. Either secretary can type either bosses letters. When a letter is sent to the secretaries to be typed, but the secretaries are both busy, the letter waits in a basket until one of the secretaries is done. Fig. 1 shows an SADT representation of this model.

The features of this model that would make it appropriate are probabilistic times at each task, a necessary queue between the tasks, and appropriate response variables such as secretary utilization and letter wait time. The system is not multi-level, does not have any feedback loops, and

does not have enough activities to adequately explore system interaction.

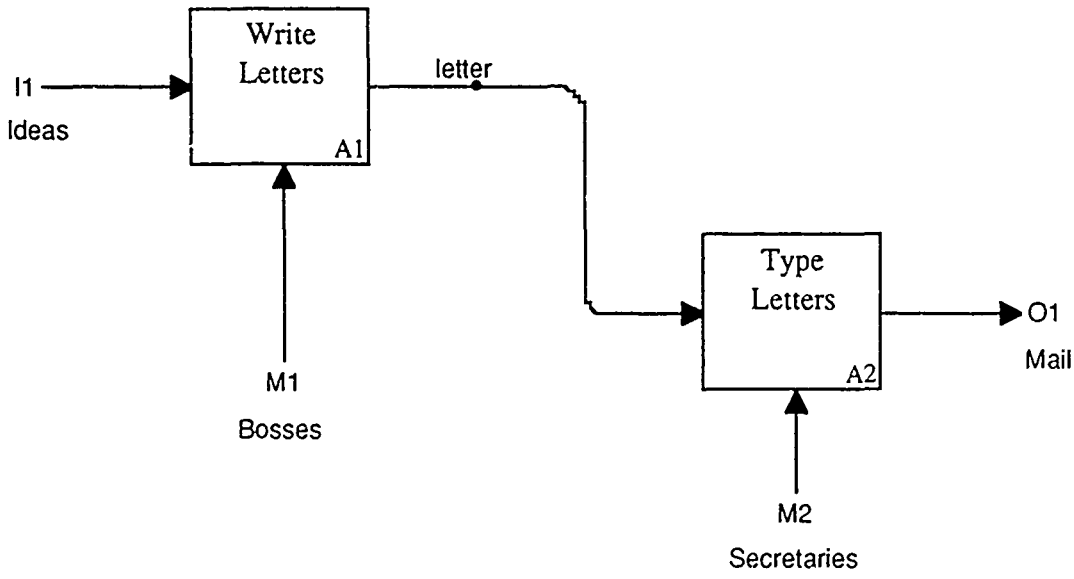


Fig. 1 Abbreviated SADT diagram of the office model

The second system is a student enrollment and registration. This model has all of the features of the Office model plus those that the office model does not have. There are nine activities: Make Tentative Schedule, Get Advisor's Approval, Check Closed Class List, Check In For Registration, Check Fines, Enroll In Classes, Pay Fees, Validate ID Card, and Pay Fines. This model will certainly explore the limits of the techniques better than the office model. Unfortunately, because of limited time this model is not implemented in Design/CPN. However, the knowledge gained from the Office model about colored petri nets and Design/CPN is sufficient to predict the major results of implementing the Registration model.

Analysis

Introduction to Colored Petri Nets

Task networks and queuing theory are extensively used ideas and, as such, there is a large amount of literature available. Therefore these ideas will not be reviewed here. Colored Petri Nets, on the other hand, are relatively new and a brief introduction is needed.

There are four primary parts to a colored petri net (CPN):

1. Places -- each place may hold tokens that it has received from other places via transitions. The places represent the states of the system and are drawn as ellipses.

2. Transitions -- each transition receives tokens from one or more places, possibly acts upon the tokens and then sends them to other places. The transitions represent the activities of the system and are drawn as rectangles.

3. Arcs -- arcs connect the places and transitions together and allow tokens to travel among them.

4. Tokens -- tokens move from place to place via transitions and along the arcs. Tokens may represent anything: people, information, goods, ideas, etc. In colored petri nets the tokens have 'colors' that can uniquely identify each token. The colors generally represent the type of object that is being moved. For example, in the office system, tokens might be 'boss1' and 'sec1' to represent the first boss and the first secretary, respectively.

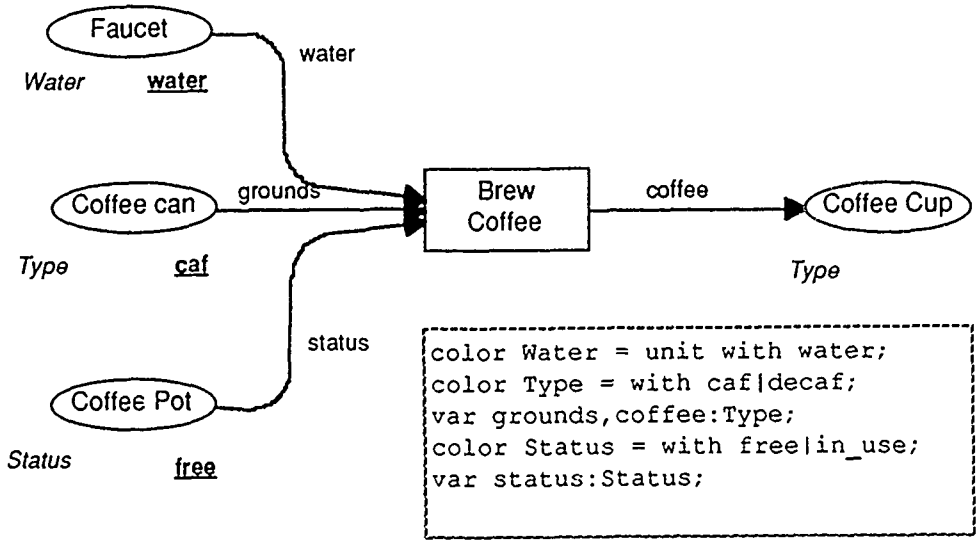


Fig 2. Colored Petri Net of Making Coffee System

Fig 6. shows a simple CPN for making coffee. The italicized words are the color types for places and the bold underlined words are the initial markings. The place Faucet initially holds one token colored 'water'. Coffee can holds regular ('caf') coffee grounds and the status of the Coffee Pot is 'free'. If at least one token resides at all three places then the transition Brew Coffee can fire. This would mean that the three tokens would move through Brew Coffee to Coffee Cup. In this case the three tokens would actually be consumed and a new token 'coffee' would be outputted to Coffee Cup. This token's value, either 'caf' or 'decaf', would, presumably, depend on the value of 'grounds'.

There are many other rules and features of CPN. Some of these will be discussed below as needed. For a deeper discussion see references 1-4. There are also other kinds of petri nets differing in how the basic petri net design is extended. Some examples are stochastic, timed, attributed, petri nets (STAPN) (6) and generalized, stochastic petri nets (12).

Analysis of Concept

There has been previous work that outlines the requirements that a modeling technique should have. For example,

"GOALS OF THE HUMAN PERFORMANCE MODELING LANGUAGE (HPML)

2.1 The objective of the HPML is to facilitate the development of models to predict human performance and workload in man-machine systems.
2.2 The HPML should be able to accommodate models based on empirical data and/or models based on theory.
2.3 The HPML should accommodate any
- Workload metric
- Performance metric
- Decision making model
2.4 The HPML should accommodate a variety of human information processing viewpoints, e.g.,
- Parallel processing
- single channel, sequential tasks
2.5 The HPML should be simple to comprehend.
2.6 The HPML should encourage, if not enforce, top-down modeling.
2.7 The HPML should support models for a wide variety of situations, e.g., from mission-level crew modeling to low-level interactions with equipment.
2.8 The HPML should be capable of modeling multi-person crews.
2.9 The HPML should operate in closed-loop and open-loop modes (i.e., where it can and cannot influence the (model of the) human's environment). (Valerie Gawron, Calspan Corp)"

With respect to such criteria both task network/queuing theory (TNQ) and colored petri nets (CPN) do well. Since the techniques are similar in their purpose their end result will also be similar.

This similarity comes across in learning the techniques. The basic idea of each is simple -- a network moving entities. The deeper parts of queuing theory may take longer to comprehend, but this is regardless of the technique used.

There is an initial difficulty in learning CPN ML, the language for programming CPN's, since it is a functional programming language. This difficulty is only because structured programming is the norm whereas functional programming is not.

Once the user is comfortable with both the theory and the network, then using each technique to model a system is straightforward. This is even more true when Structured Analysis and Design Techniques (SADT, also known as

$IDEF_0$) is the front-end. The difficult work of actually defining the system and creating the network is done in $IDEF_0$. After this the translation to TNQ and CPN can be easily done.

At this time neither TNQ's nor CPN's have a clear advantage in terms of the power of the technique. The local definition of power is the ability of the technique to model real-world systems.

For example, time is inseparable from systems in the real world and, therefore, a necessary part of any modeling technique. TNQ incorporates time in either a deterministic or probabilistic manner. CPN, on the other hand, does not incorporate a mechanism for time. The changes in states are considered to be 'out of time'. There are two crude ways to implement time in CPN's, neither of which is adequate. First, the firing of the transitions can be considered as measure of time. After all the enabled transitions fire, one unit of time can be considered to have passed. This is not realistic as it does not allow for transitions taking differing amounts of time. Second, each token can have a time counter as part of its color. Then a guard on the transitions would only allow them to fire when the timing is correct. This method quickly gives rise to the idea of a system clock and an event queue, two important features of TNQ's. Unfortunately, for the clock to communicate with each transition it must be connected by an arc. This soon becomes very unwieldy and not worth the trouble. A system clock must somehow be included, but not in this overly complex manner.

The stochastic (even chaotic) property of the real world can be modeled quite easily in TNQ's using random draws from appropriate probability distributions. Again, this could easily be included in CPN's but is not at the present time.

An important advantage of CPN's over TNQ's is the color for the tokens. Color is an abstract data type that provides another level of representation to the entities that pass through the system. TNQ's generally represent entities through counts. We may know that there are five letters waiting to be typed, but we do not know what the letters say or which boss they are

from. This information can easily be represented by the color in CPN. Further, the action to be taken at a transition can be dependent on the information. For example, suppose that only one secretary in the Office model has a classified security clearance. When a letter is sent to the secretaries the color of the letter could include the type, i.e. classified or unclassified. If it is classified, then only when the classified secretary is free can the letter be typed. Otherwise either secretary can type the letter. This distinction is much easier to implement in a CPN than in a TNQ because of the color.

Color provides more power for representing different aspects of a system or even different types of systems. For example, suppose a computer system is modeled and the tokens represent the programs sent by various users. Each program requires certain resources. One token carries the entire program as a color. When the token reaches the place representing the CPU the color is processed, as a list, for example, and the resources requested. When all the resources become available to the CPU the next transition is enabled and may fire.

Even more power is available to the user through the hierarchical inheritance feature of colors. Two colors can be created separately then combined into one new color which will inherit the properties of both original ones. For example, suppose the people in the Office model are listed in an employee database. The database structure could be defined as

```
color Name = string;                    color Position = string;
color Number = int;                     color Salary = int;
color Clearance = classified | unclassified;
color Employee = product Name * Position * Number
                         * Salary * Clearance;
color Employ_list = list of Employee;
```

Each level of Employ_list has all the attributes/colors of every level below it and all levels are accessible to the CPN in a very simple manner. The entire database can be sent from place to place with a token whose color is Employ_list. At some point it will be necessary to act upon an individual's

records. A new token can be created whose color is Employee and whose values come from the database. If the person's name and clearance are needed for another activity these can be copied onto a brand new token and sent to a different place. Therefore, color allows more abstractly complex systems to be modeled in a straightforward manner.

Both TNQ's and CPN's are mathematically analyzable. There is a good deal of literature on TNQ's dealing with their mathematical properties. Also, under certain restrictive conditions TNQ's can be of the class possessing a 'product-form' solution (15). For this class of networks, well-known and efficient solution algorithms exist (16).

CPN's can also be analyzed mathematically. The nets can be translated into colored petri matrices from which linear system invariants can be calculated, structural properties (which are known to imply behavioral properties) can be checked, and reduced nets derived (1). These abilities allow the user to understand the system in certain ways without actually simulating the system.

In summary, TNQ's have time and probability built into their theory which provides TNQ's with a major, but not uncorrectable, advantage over CPN's. On the other hand, the power and flexibility of color in CPN elevates this technique to a level of real-world representation that TNQ's can not have.

Analysis of Application

Concepts can be beautiful and powerful by themselves, but often they need to be applied to actual real-world problems to be useful. At this point the two applications, MicroSaint 3.2 and Design/CPN 1.1, will be analyzed for their respective advantages. In many ways it is unfair to compare the applications. MicroSaint is at least five years old, while Design/CPN just came out this year. But if this is kept in mind then an analysis can prove fruitful.

MicroSaint makes it very easy for the user to learn its product. The documentation is clear and easy to understand and includes a step by step

tutorial. The online help function makes the manual virtually unnecessary once the initial ideas are understood. The classes that MicroSaint offers are well managed and very informative. A wide variety of simple systems are built during the class that introduce most of the techniques that the everyday user will require. MicroSaint uses a language reminiscent of Pascal to define the model. Since Pascal is a very common language learning MicroSaint's structure is, again, very straightforward.

Alternatively, Design/CPN has taken a very long time to learn. The documentation is often unclear and incomplete and was certainly not meant as a tutorial. However, the telephone support service makes up for this by quickly and completely answering questions. Design/CPN uses CPN ML, which is an expanded version of Standard Meta Language which was created by the University of Edinburgh (9-10, 18), to program the system. Since SML is a functional programming language it is generally not used by the common modeler. Even the style of programming is much different than that of a structured programming language. This makes the startup time for using Design/CPN even longer. However, functional programming is necessary to take full advantage of the capabilities of CPN's. Fortunately, it is not necessary to learn the entire repertoire of ML and once the appropriate subset is identified learning CPN ML will be faster. The method for implementing CPN's is actually not very difficult. After a user bears the initial burden of learning the system, subsequent modelers drawing upon the experience will find it much easier to learn Design/CPN.

As mentioned before, using SADT to define the system under study greatly facilitates the use of both MicroSaint and Design/CPN. MicroSaint does not have a front-end facility that translates directly from SADT to MicroSaint. (Saint, the mainframe version and predecessor of MicroSaint, does have the "Generic Systems Analyst Workstation (GENSAW)" tool that does have SADT as a front-end analysis tool to define the system (17)). This lack is a minor disadvantage for MicroSaint, since it allows the user to build models piecemeal. One can certainly define the system using SADT and then use the structure to create the MicroSaint model, but the extra work involved

will discourage many people from this procedure.

Design/CPN does have an SADT package as a front-end tool called Design/IDEF (8). Design/IDEF was created by the same company who created Design/CPN. A system can be diagramed in Design/IDEF, the behavioral aspects added via CPN ML and then translated into Design/CPN. As a finished product this procedure will be very useful to modelers. As it is it needs a lot of work. There are some steps that are tedious, such as saving and loading text to create a global declaration node. Fortunately, these are only temporary until a new version of the package comes out.

In terms of application features that are of interest to the modeler, MicroSaint is more powerful than Design/CPN. This is a reflection of the concepts that the packages are built on. MicroSaint has a system clock, methods for generating task times from a variety of probability distributions, and strong control of which tasks are executed after the current one is complete. Unfortunately, Design/CPN has none of these.

MicroSaint has strong output facilities and even built in analysis tools. All of the standard information desired by modelers is available through the snapshot and trace facilities. MicroSaint has very fast execution, especially in the no display mode. The model can also be viewed in animation mode. Depending on the structure, parts of the model can be displayed on the screen; the entities are shown traveling from task to task and even building up in queues. Animation mode is very useful for debugging or demonstration purposes.

Because CPN ML is based on a functional programming language, output from Design/CPN is very poor, almost nonexistent. Every output statement must be explicitly stated by the user. All information must be converted to string before it can be sent to an output file which makes the statements very long and complicated. Since almost every use of simulation requires information from the system, poor output capabilities make Design/CPN a nonfunctional modeling tool until this lack is overcome. Also CPN ML is an interpretive language and many graphic fields updated at each step, including unnecessary ones. These two factors make Design/CPN a very slow

package to run; too slow for large simulations to be feasible.

In summary, MicroSaint is a much more capable tool for the everyday modeler at this time. But again, this advantage is solely because MicroSaint is more mature than Design/CPN.

IV. RECOMMENDATIONS

There are three areas which these recommendations will address. The first is recommendations on the enhancements which should be implemented in task network/queuing theory, colored petri nets and their respective applications. The second area is recommendations for appropriate uses for each technique. The final area is in the research that should be done to build on these results.

Enhancements

The changes that should be made in task network/queuing theory and colored petri nets would essentially make them mirror images of each other. Some authors have already discussed combining the two techniques to better model many systems (13). CPN's desperately need a system clock, probability functions and vastly improved output. Until these are incorporated, most real-world systems can not be modeled. These features are not unheard of in the petri net world. There are many articles dealing with stochastic timed petri nets in one form or another (e.g. 6,12). None of these include color as part of the net, but many do state the problems of complexity, etc. that color was designed to overcome.

One of the possible and valuable enhancements to Design/CPN is the ability of dynamic programming. Dynamic programming is already possible in ML and thus not too difficult for Design/CPN. The means for achieving this end would be the addition of another type called CPNET.

CPNET would be a sub-net. Variables would be declared of this type that could then be used in the main net. The sub-net must follow the hierarchy requirements that are in place now, but once these are met anything could be

done. For example, suppose that we had the net shown in Fig 4 designed to cook a meal.

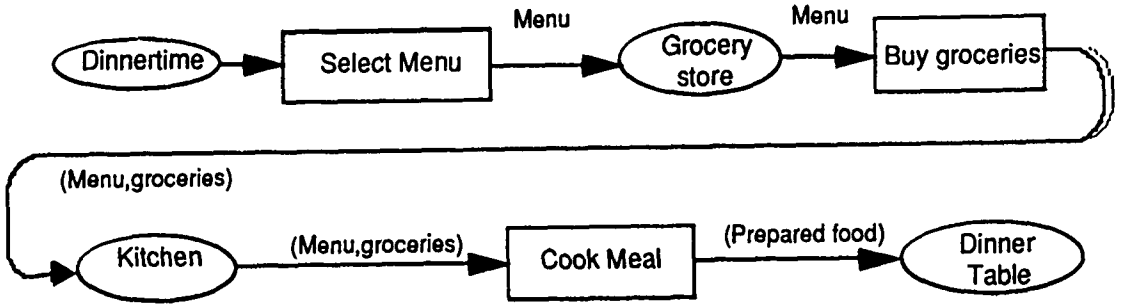


Fig 3. Colored Petri Net for Cooking a Meal.

In this model the transitions 'Buy groceries' and 'Cook Meal' would obviously be invocation transitions and, just as obviously, the actual structure of the sub-net would depend on the type of meal. Let the variable 'Menu' be a tuple defined as

```
color Attributes = string;
color Meal = list of Attributes;
color Recipe = CPNET;
color Ingredients = CPNET;
color Menu = product of Meal*Recipe*Ingredients;
var menu : Menu;
```

Ingredients and Recipe are both sub-nets, as noted by the color CPNET, that would be used in the next lowest level of 'Buy groceries' and 'Cook Meal', respectively. These sub-nets would include transitions such as 'go to dairy','go to deli', and 'go to meats' or 'boil', 'bake', and 'fry'.

If a brand new Meal were selected that did not have a Recipe and Ingredients net, then 'Select Menu' would recognize this fact. It would compare the attributes of the new Meal to the current Meals and select the closest Ingredients and Recipe modifying them as appropriate. For example, one night creamed corn might be served rather than green beans. The attributes for both could be [vegetable,hot,canned]. In this case no new

information is needed.

However, suppose that instead of baked potatoes, boiled potatoes were wanted and this was new to the model. If baked potatoes matched up the best with boiled potatoes the model would copy the baked potatoes recipe and ingredients to sub-nets for boiled potatoes. Keying on boiled the model would substitute the 'boil' transition for 'bake' in the Recipe sub-net. Again, this could all be done in the 'Select Menu' transition.

Now suppose that a poor substitution was made and the goal of a well-cooked meal was not met. The model would somehow identify possible problems, such as time of cooking, amount of spices, etc.. These could then be adjusted until it could make a well-cooked meal.

In summary, color is a powerful tool that can allow CPN's to better represent real-world systems, especially with the sub-net enhancements described above. If time and probability were incorporated into the theory then CPN's would be very formidable indeed.

To make TNQ's more versatile a data structure similar to the color found in CPN's is necessary. Techniques for handling color, such as decomposing and aggregating colors, should also be incorporated. This will probably be a more difficult enhancement to make than those for CPN's. The current data handling in tools like MicroSaint is not compatible with color. Therefore, the tools would need to be completely reworked. Otherwise, TNQ's and MicroSaint are very efficient now in modeling real-world systems.

Appropriate Uses

As has been mentioned, there are many disparities between TNQ's and CPN's and, thus, the type of systems that each should be used to model are quite different. TNQ's can model most of the traditional discrete-event stochastic systems of interest. CPN's on the other hand can not and should not at this time model systems that rely on time or probability. Although they can model systems with complex data structures, such as lists and databases, very easily, much easier than TNQ's.

Once the enhancements to TNQ's and CPN's that are recommended above

have been made then the two techniques will be essentially the same. There will be minor underlying differences, but the ability of the techniques to model systems will be equivalent. Each will be able to model any real-world system currently of interest.

Future Research

Most of the systems that are of interest today are based on the discrete-event stochastic simulation paradigm. As research is conducted in other areas such as decision-making processes new paradigms may arise. It is important that the simulation tools keep up with new thought. Therefore, modeling research should be closely coordinated with the theoretical research in these other areas. There has been discussion on the level at which time that needs to be incorporated into models. Many interesting systems may come from research on this topic.

V. CONCLUSIONS:

In conclusion, I feel that at this time task network/queuing theory is the technique to use when modeling real-world systems. The tool I would recommend to the everyday user is MicroSaint. Yet, in a year or two, when colored petri nets are expanded to include time and probability, and faster, more capable versions of Design/CPN are released, then I think that colored petri nets will become a very powerful modeling technique.

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FINAL REPORT

Degradation of the renal peritubular basement membrane in relation to toxic nephropathy of fuels of military interest.

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Abstract

Ultrastructural and biochemical aspects of damage to the kidney tubular epithelium have been studied in relation to nephrotoxic fuels and hydraulic fluids of military interest. Both routine and novel aspects of the onset and regeneration of tubular ultrastructural lesions have been correlated with biochemical mechanisms of tubular damage; in particular the peritubular basement membrane. Much of the proposed investigation has been derived from ongoing protocols underway at the Toxic Hazards Division of the Armstrong Aerospace Medical Research Laboratory, Wright Patterson Air Force Base. The results are applicable to basic science and medicine as well as applied to military toxicology.

Acknowledgements

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I. Introduction

Due to its unique structure and function the kidney is a critical target organ of great sensitivity to a wide variety of toxic substances including occupational-environmental substances of military interest. The Air Force and Navy have conducted studies including the nephrotoxic hazard of fuels, fire retardants and hydraulic fluids for more than 10 years using generally accepted routine procedures (Bruner, 1984; Bruner & Pitts, 1983; Kinkeod et al., 1987; Mattie et al, 1986, Norton et al., 1985; Norton & Mattie, 1987). Existing methods of renal toxicology and pathology in animals and humans are insensitive and far from definitive

(see below). Moreover, basic nephrotoxic mechanisms are not well understood. Current in vivo methods in toxicology suffer from a variety of well known scientific, economic and administrative problems. The development of in vitro methods with which to study toxic mechanisms and risk assessment is a high national priority in all fields of toxicology. Considerable gaps remain in present understanding of two aspects of tubular injury. (a) What is the biochemical-cellular basis for the irreversible disruption of tubular conduit structure and resultant permanent loss of the nephron? (b) How can this process be recognized as a predictive sublethal ultrastructural lesion in animal models, and how does tubular pathogenesis correlate with customary functional parameters routinely employed in clinical chemistry?

Several factors conspire to render the tubular epithelial cell population extremely sensitive to a wide variety of therapeutic and environmental-occupational agents. The transport function of the kidney epithelium requires very large amounts of energy as evidenced by prominent and numerous mitochondria; thus it is particularly sensitive to metabolic disruption of glycolysis or respiration. The kidney contains a high activity of drug metabolizing enzyme systems; these can produce reactive intermediates which cause macromolecular damage in various cell structures including membranes. The kidney receives 20% of the cardiac output which delivers large amounts of blood-borne toxicants. However, the most significant contribution to renal sensitivity is the result of the very function of the kidney: concentration. Because 99.5% of the water is reabsorbed, the potential concentrating effect or exposure to nonreabsorbed toxicants is 200 fold. Thus, the tubular epithelium is frequently the limiting organ of exposure to agents which only slightly damage other tissues. A major

methodological obstacle in the evaluation of renal toxicity is the large reserve of excess nephrons in the young mammal. Normal renal function can be maintained with only one half of one kidney remaining. Thus, 75% of nephrons must be destroyed before damage is systemically manifest.

II. Objectives of the research effort (preliminary results and proposed research)

A. Examination of kidney structural lesions caused by fuels and hydraulic fluids by scanning and transmission electron microscopy. Without compromising ongoing and planned protocols, kidneys from the identical rats exposed to nephrotoxic fuels and hydraulic fluids will be further examined using transmission and scanning electron microscopy. All lesions of glomerulus, tubule, vasculature and interstitium will be studied. Additional animals will also be exposed to compounds which prove to be of further interest. Resulting information on kidney toxicity of fuels and hydraulic fluids will be correlated with and provided to the general pool of information presently sought by the Air Force independent of this proposal. Conversely, the general toxicity data from ongoing and completed protocols will be of value to the novel basic information of this proposal.

B. Structural examination of the peritubular basement membrane in relation to tubular injury. In addition to all routine pathogenic lesions the degradation of the peritubular basement membrane (basal lamina) will be more closely examined by transmission and scanning electron microscopy. This phenomenon has been neglected in basic and applied renal pathology, however the lesion appears to be of

potential diagnostic value. Studies will attempt to correlate the ultrastructural appearance of the degraded peritubular basement membrane with tubular dysfunction and degeneration and systemic toxicology. It is hoped that this will contribute to a description of a diagnostic lesion by which a threshold of irreversible tubular damage and loss can be more accurately recognized and assessed at the ultra- structural level. Determination of the threshold of irreversible peritubular damage and nephron loss is fundamental to the estimation of a threshold of tolerable exposure.

C. Biochemical mechanisms of the enzymatic degradation of the peritubular basement membrane. The biochemical mechanisms of the injurious enzymatic degradation of the peritubular basal lamina will also be studied using the isolated perfused kidney. In the perfused organ the release of proteolytic and other degradative enzymes from the tubular epithelial cells will be characterized in relation to simultaneous tubular cell injury and basement membrane degradation. Release of degradative enzymes will be correlated with functional parameters of the preparation and ultrastructural examination. A description of the biochemical pathophysiologic mechanism of peritubular basement membrane degradation will contribute to an understanding of the mechanism of irreversible nephron loss after toxic exposure.

The novel basic and applied aspects of the present proposal will be published in the open literature of reputable journals of civilian professional societies (e.g., Lockwood and Bosmann, 1979a, 1979b; Hull and Lockwood, 1985). Accordingly, quality control will be of standards acceptable for ongoing research of the laboratory.

Continuing consultation will be sought from laboratory personnel; collaborative effort is welcome.

Preliminary results assuring the outcome of the proposed research have been developed by the applicant at the Aerospace Medical Research Laboratory at WPAFB while a guest under the summer faculty research program (administered under contract by Universal Energy Systems, Inc.). Additional background results using the isolated perfused kidney were developed independently in the laboratory of the principle investigator. Related research in renal toxicology by the applicant has been previously described (Lockwood and Bosmann 1979a, 1979b).

III. Approach

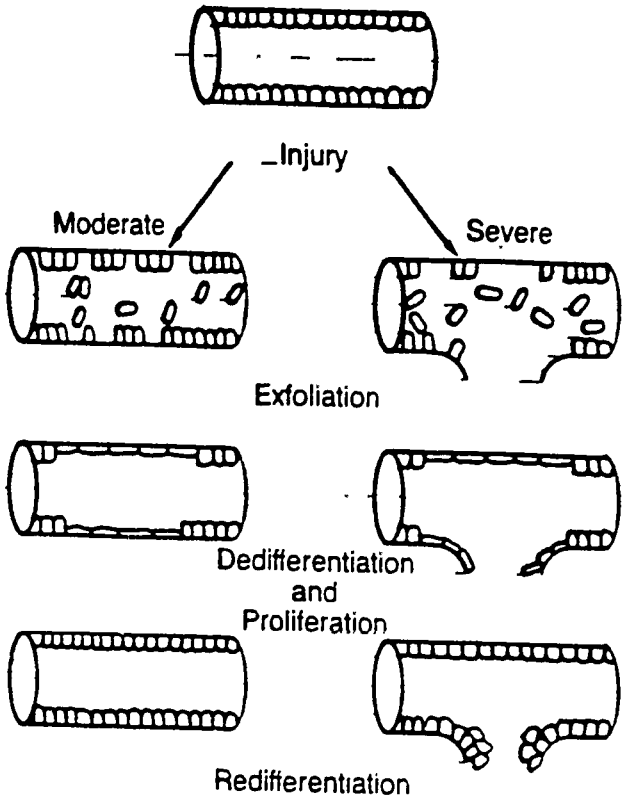


Fig.

A. Description of the structural sequence of events in the degradation of the peritubular basement membrane in relation to tubular injury and regeneration from nephrotoxins of military interest. The phenomena diagrammed here (see Fig. 1) have not been well described at the ultrastructural level. Preliminary results with electron microscopy have already demonstrated relevant features of the normal and degraded basement membrane. This electron microscopy will be continued this year at the Toxic Hazards Laboratory facility. Photographs already obtained cannot be reproduced here.

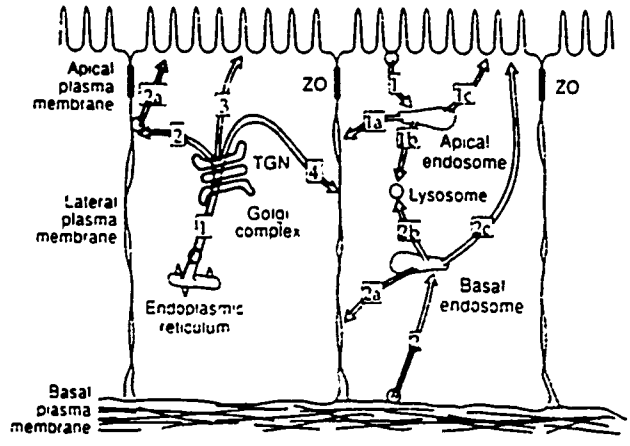
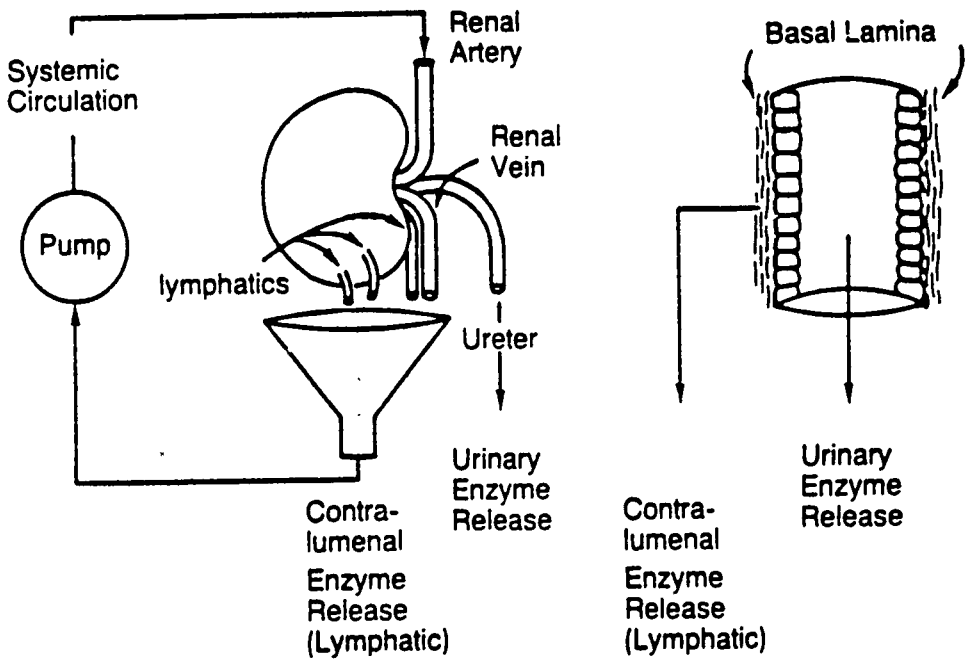
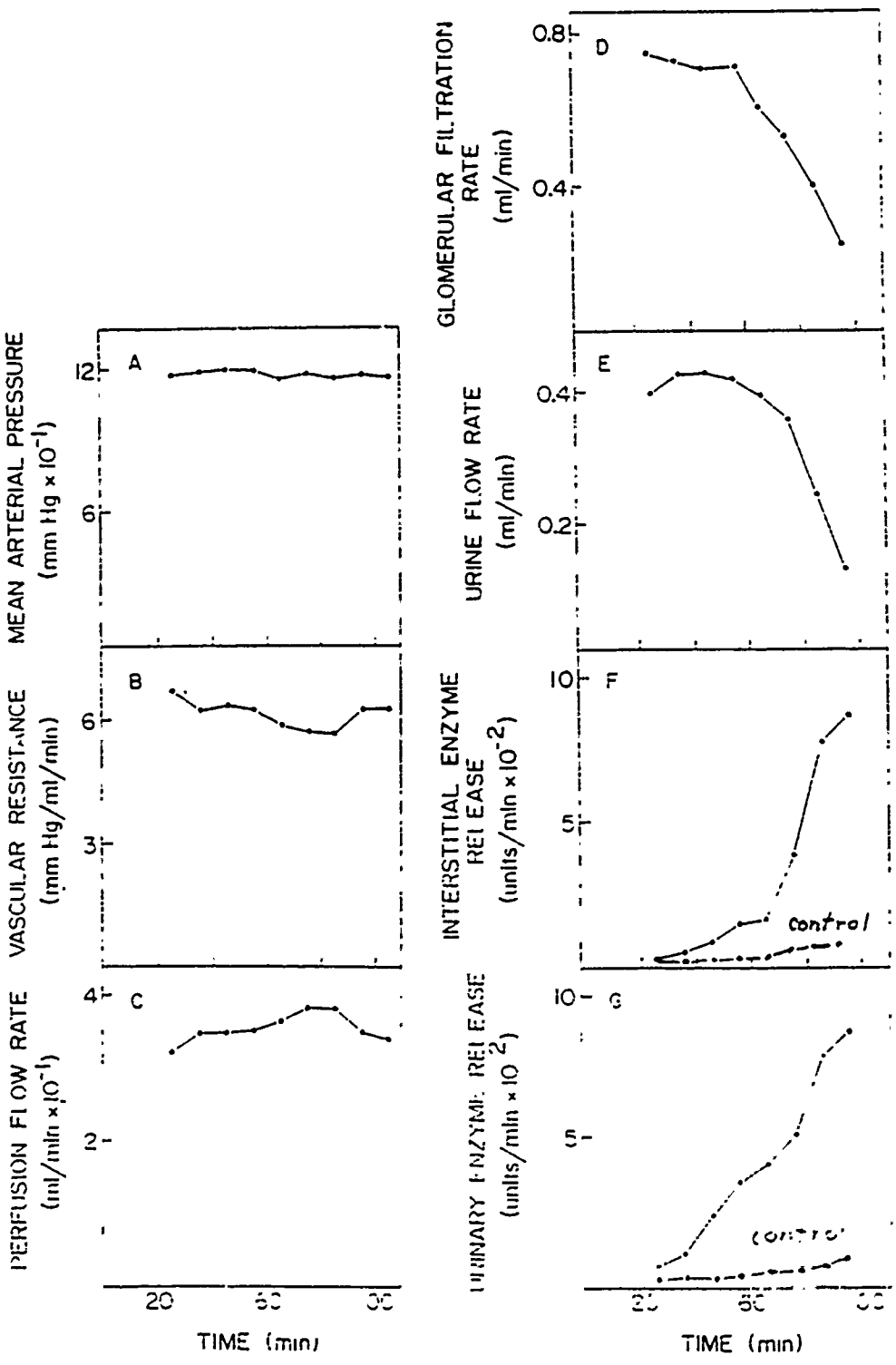


Fig. 2

B. Biochemical mechanisms of kidney damage from nephrotoxic compounds and the use of the isolated perfused kidney in in vitro toxicologic investigations. The use of the isolated perfused kidney to study the normal and pathogenic increased release of kidney degradative enzymes into urine and the renal interstitial space and lymphatic system is diagrammed (see Fig. 2-4 and Lockwood and Bosmann, 1979).

These diagrams summarize data indicating that there are two distinct lysosomal systems in kidney epithelial cells: the apical and basal. Our unpublished data from the isolated perfused kidney


Renal
Artery
Systemic
Circulation
Renal
Vein
Pump
lymphatics
Ureter
Urinary
Enzyme
Release
Contra-
lumenal
Enzyme
Release
(Lymphatic)
Basal Lamina
Urinary
Enzyme
Release
Contra-
lumenal
Enzyme
Release
(Lymphatic)


MEAN ARTERIAL PRESSURE (mm Hg × 10⁻¹)
VASCULAR RESISTANCE (mm Hg/ml/min)
PERFUSION FLOW RATE (ml/min × 10⁻¹)
GLOMERULAR FILTRATION RATE (ml/min)
URINE FLOW RATE (ml/min)
INTERSTITIAL ENZYME RELEASE (units/min × 10⁻²)
URINARY ENZYME RELEASE (units/min × 10⁻²)
A
B
C
D
E
F
G
control
control
TIME (min)
TIME (min)

(shown below) indicated that lysosomal degradative enzymes are normally released from the uninjured kidney only into the tubular lumen and urine. They are not normally released across the basal lamina. Hypothesis: after tubular cell injury we hypothesize that the normal system might work pathogenically: the massive extracell enzyme release might explain proteolytic and glycosidolytic degradation of the peritubular basal lamina to which cells normally adhere. If severe, such degradation prevents regeneration of a continuous cell monolayer after remaining cells proliferate following tubular injury. Thus far, we have simulated tubular cell toxic injury by perfusion of the kidney without its energy source, glucose. When the kidney becomes injured, tubular epithelial cells release their high amount of degradative enzymes from the basal portion of the cell where they can be rapidly detected in the circulating perfusate of the isolated perfused kidney preparation. This enzyme release presumably explains the degradation of the peritubular basement membrane observed approximately 1 day after tubular injury. This preparation might find application as an in vitro tool in toxicology with which to investigate compounds of military interest.

Preliminary results with scanning electron microscopy have demonstrated the feasibility of the elucidation of the diagrammed lesion.

IV. Recommendations

The objectives, approach and preliminary findings will be pursued at the Toxic Hazards Lab, WPAFB. Dayton, OH and in the laboratory of

the principle investigator at the School of Medicine, Wright State University. A research initiation proposal is being submitted.

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Heart Rate and Other Cardiovascular Parameters as Measures of Mental Effort

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Heart Rate and Other Cardiovascular Parameters as Measures of Mental Effort

by

Lewis M. Lutton

Abstract

The recognition and measurement of fluctuating levels of mental effort and fatigue in pilots is of great value to the Air Force. The testing of cockpit efficiency, the training of new pilots, the analysis of the multiple tasks required of pilots or the monitoring of pilots on long range missions all benefit from an awareness of the state of pilot alertness and mental stress. Since it is not possible to fully rely upon the pilot's subjective impressions of these parameters some physiological measures of them are desirable. This paper discusses the value of heart rate and heart rate variability as such measures.

In general, heart rate and heart rate variability are excellent qualitative measures of mental workload even though problems like individual variation, differential responses to different tasks and the roles of circadian and ultradian rhythms need to be investigated. Furthermore, these parameters need to be supplemented with other physiological parameters as is the goal of the PAT system used by the Ergonomics and Workload Branch of HEG at AAMRL.

Acknowledgements

I would like to thank the Armstrong Aerospace Medical Research Laboratory and the Air Force Office of Scientific Research for sponsoring this research. Universal Energy Systems and their cheerful staff must also be acknowledged for their ever ready support.

Special appreciation goes to Dr. Glenn Wilson of the Human Engineering Division for his interest in requesting the input of a physiologist to the work being done in his laboratories and for his continual support and encouragement throughout the summer. The help and insights of his laboratory associates, especially Kathy McCloskey and Iris Davis, have also been greatly appreciated.

Finally, I must thank my dear wife, Marti, for allowing me to wander off to Dayton, Ohio when I could have been home painting the house.

I. INTRODUCTION:

The Workload and Ergonomics Branch of the Human Engineering Division of AAMRL focuses on the measurement of mental effort as it relates to a variety of pilot tasks or states. Taking a broad approach to their responsibilities these reseachers monitor pilots in flight, flight simulators and the laboratory, where they also study civilian subjects. They measure performance levels on a wide range of workload assessment tasks which involve spatial orientation, linguistic and perceptual problems and they monitor the internal state of their subjects by a variety of physiological measures including heart rate, heart rate variability, electroencephalography, magnetoencephalography and eye blink.

The precise value of these physiological parameters for the measurement of mental workload, however, is still being investigated. Indeed, both the qualitative and the quantitative role of these measures is still unclear largely because of the many physiological factors which can affect them in the body. Consequently, it would seem wise for those who are trying to use these measures to gain as full an appreciation of their overall physiological background as possible.

For this reason I was invited to join the Workload and Ergonomcs Branch to give my input as a physiologist to the work which they are conducting. I am a broadly trained biologist with primary interests in the area of physiology and behavior. My research involves pharmacological influences on circadian rhythms and I teach courses in animal physiology, the biology of behavior and human exercise physiology. With this background I have developed a keen interest in the physiological aspects of human performance problems such as those with which the Workload and Ergonomics Branch is faced.

II. OBJECTIVES OF THE RESEARCH EFFORT

This paper is my assessment of the use of two of the aforementioned parameters, heart rate and heart rate variability, as measures of mental workload. Other physiological parameters such as respiratory rate, eye blink and evoked potentials in EEG's are discussed only briefly. The focus of the paper is upon an analysis of the physiological control of heart rate as it relates to mental workload and a discussion of the physiological bases of "mental workload".

The final goal of this paper is to use the ideas which are developed to suggest further research objectives for the Workload and Ergonomics Branch of HEG/AAMRL and for myself at Mercyhurst College. It is expected that the latter will be developed into a grant request for the AFOSR Research Initiation Program.

III. APPROACH

Since the primary goal of this effort was to evaluate the physiological nature of the orientation and assumptions of the research being done in the Workload and Ergonomics Branch of HEG, no actual experimental research was conducted. Rather, the ideas of this paper were developed through discussion with my colleagues in this research group and through extensive readings of primary and secondary research literature.

In addition, the members of our laboratory held a conference which was attended by eight internationally known researchers who study the psychophysiological assessment of mental workload, especially the utilization of heart rate and heart rate variability. Formal and informal discussions with the participants of this workshop also helped to develop the ideas presented in this paper.

Finally, so that I could gain a better understanding of the needs and requirements of the Workload and Ergonomics research group, I observed their experimental procedures and served as a preliminary subject for an experiment that was being conducted this summer. These activities have helped clarify my understanding of the requirements for any related research I conduct at Mercyhurst College.

IV: RESULTS: GENERAL BACKGROUND

The use of heart rate, HR, as a measure of physical activity is well established and relatively simple linear correlations between levels of HR and physical effort have been observed over a wide range of workloads (McArdle, Katch and Katch, 1986, p. 82). Numerous studies have also shown that HR can also be a valuable physiological indicator of mental workload. Although a quantitative relationship has not been established between HR and mental effort, some predictable qualitative results have been found (Wilson & O'Donnell, 1988).

Heart rate variability, HRV, which is the beat to beat fluctuation in heart rate and is also known as sinus arrhythmia, has been shown to fluctuate, generally decreasing with mental workload (Wilson & O'Donnell, 1988). Again, however, the precise nature of this relationship with respect to such factors as the level of response, the type of work which elicits a predictable response or why subjects respond differently has not been established.

There seem to be three major reasons why these parameters have not proved to be clearcut quantitative indicators of mental workload. The first reason is due to the complexity of HR control. The heart is controlled neurally, hormonally and by intrinsic mechanisms. The neural control involves both branches of the autonomic nervous system, ANS, the parasympathetic NS which decreases HR via the vagus nerve

and the sympathetic NS which increases HR and stroke volume. Another complicating factor is that the ANS typically responds to any type of stress (physical, emotional or mental) so that it is difficult to determine what is causing a change in ANS activity. In fact some stimuli cause an immediate increase in HR, the startle reflex (SR) while others course a immediate decrease, the orientation reflex (OR).

The second difficulty involves the variety of factors which are thought to influence HRV. HRV occurs at three broad frequency levels believed to be associated with temperature, blood pressure and respiratory rhythms, from low to high frequency, respectively. The latter two are most responsive to mental workload, and both of them seem to be influenced by a wide range of feedback mechanisms (Grossman, 1983). This predicament and the difficulties arising from the complexities of HR control will be discussed in detail later.

The third problem involves the fact that while it is easy to have a general conceptualization of mental workload it is not clear what kind of work is actually being done or what form of energy is being expended. Hence, it is not clear from a biological perspective with what physiological phenomena the changes in HR are being correlated. While a full physiological explanation of what is meant by mental workload is not possible at this time, the more we understand these processes the more we will be able to understand why HR varies as it does during mental "work". An attempt to elucidate the physiological components of mental workload is the subject of the next section

V. RESULTS: A BIOLOGICAL PERSPECTIVE ON MENTAL WORKLOAD

One of the primary matters in a discussion of the biological aspects of mental workload is deciding whether we are

dealing solely with those elements which produce the feelings of mental effort or whether other elements such as the automatic aspects of a mental task should also be included. Secondly, the notion of resource utilization and depletion is probably the best way to tie recent psychological models of mental work and fatigue (Heemstra, 1986) to potential physiological models.

Two questions can be asked regarding the biological resources for mental work: what are the resources which are being depleted and is there a relationship between mental work and metabolic work? These two concerns overlap since one of the resources may be ATP, the end product of cellular metabolism that is the primary source of energy for the biochemical activity of the body.

ATP itself, however, is an unlikely candidate since even though it is a major resource for physical work, physical fatigue actually results from a build up of lactic acid or the depletion of glycogen stores. Furthermore, during mental work no one has been able to measure local or general increases in oxygen consumption in the brain. The problem with these studies, however, is that the resting brain has a high metabolic rate and any increases in metabolism in a small part of the brain would not be large enough to be distinguished over noise levels. Indeed, recent studies using the PET Scan have shown that there are local increases in brain metabolism during mental activity (Phelps & Mazziotta, 1985). Consequently, it remains a possibility that metabolic processes are one of the limiting resources in mental work, either through the depletion of some substrate or the build up of a metabolite.

Other resources whose depletion may help limit mental effort relate to the potential energy that is stored in the neurons of the brain in the form of transmembrane ionic gradients (created through the use of ATP and leading to the establishment of the resting membrane potential) and the

neurotransmitter stored in the presynaptic vesicles. Indeed, nerve impulses may be propagated down or between neurons without the immediate expenditure of metabolic energy. However, studies have not shown either if there is a significant depletion of these resources during mental work or if cellular metabolism could replenish them quickly enough to keep them from being limiting factors leading to mental fatigue. It is of interest to note, however, that the classic Claude Bernard experiment indicates that neurotransmitter can be significantly depleted at neuromuscular junctions before the stimulated muscles are fatigued. Hence, it would appear that these potential energy resources may be limiting resources for mental work.

A fourth type of resource in the brain that may limit mental work is the neural circuits or, nerve cell assemblies that are thought to be at the root of brain function. Regarding resource depletion perhaps these neural units can be overloaded. If so what is meant by overloading and with what mental phenomena or processes are these units involved? Finally, it is also possible that the connections between critical neural units become overused, so they become a fifth resource that may limit mental work.

The most likely mental phenomenon with which these neural units are involved is the concept of "attention". Using a model of attention that seems to have wide acceptance, attention is said to have two separate components with separate neural circuitry, arousal and activation. The arousal "center" works more phasically and is closely tied to emotions, while the activation "center" works more tonically and is closely associated with motivation and is more directly involved with eliciting responses. Furthermore, it appears that attending and cognition to involve only one stimulus at a time. When people seem to be attending to more than one thing at a time they are probably sequentially time sharing the attention resource center in the brain. This is not to say, however, that our brains

cannot carry out other functions automatically at the same time.

Several other concepts important to the study of mental workload can be added to this model of attention. One is that the sensation of performing mental work seems to involve cognitive rather than automatic functioning. This does not mean that significant energy is not being expended by automatic functioning and if this energy utilization contributes to our sense of mental work and fatigue or if it should be included in the quantification of mental work is an open question. Secondly, the model suggests that there is a close relationship between cognition and emotion. Indeed, Heilman and Valenstein (1979) believe that to feel an emotion one must be in an appropriate cognitive state with an adequate degree of arousal, while Schwartz (1986) suggests that cognition and emotion are really two different qualities of the same phenomenon coining the word "cogmotion" just as "wavicle" represents the wave-particle duality of matter. If cognition and emotion are really two aspects of the same phenomenon, then there is some simplification in the use of heart rate as a measure of mental workload since one need no longer worry about the fact that heart rate responds to both of these phenomena.

In conclusion, if psychophysiologists are to gain a clear conceptualization of what they mean by mental workload, they must first decide whether it refers to any increase in brain function, cognitive and automatic, or if it should only refer to cognitive phenomena that elicit our sense of mental effort and fatigue. Having decided that, it should be easier to determine what brain resources are most significantly involved with mental work and how they limit mental functioning. One caveat, however, is that just as there are two primary forms of physical fatigue, short term lactic acid build up and long term glycogen depletion, so might there be more than one form of mental fatigue.

VI. RESULTS: THE CONTROL OF HEART RATE AND HEART RATE VARIABILITY

With this conceptualization of the biological aspects of what mental work may actually be, it is now necessary to look at the complexities of heart rate control before discussing the value of using heart rate as a measure of mental work. When studying HR control in relationship to mental workload the neurological control of the ANS is most important. However, it must not be forgotten that the hormonal and intrinsic mechanisms of control may also be present at any time and may be affecting the overall homeostatic state of the individual. Hence they may be important factors in the evaluation of HR and HRV, and they may help account for otherwise unexplained variation between tasks and between individuals.

In the control of HR both the PNS and the SNS of the ANS appear to be active at all times with the PNS predominating at low HR's and the SNS predominating at high HR's. a simple conceptualization of this relationship can be seen in Fig. 1. This diagram shows how at low HR's there can be a

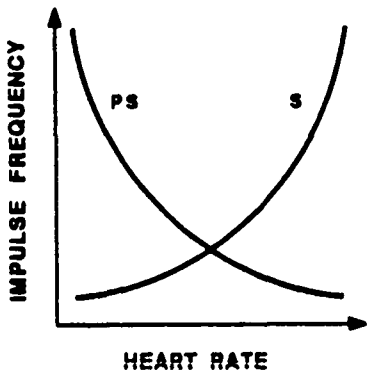


Fig. 1. A simple conceptualization of the firing rates of the parasympathetic (PS) and sympathetic (S) nervous system at different heart rates.

lot of vagal tone with little interferenc from the SNS, which appears to be the case when analyzing HRV.

Unfortunately, the situation is not this simple for a number of reasons. First of all, there are differences in the response time of the PNS and the SNS with the PNS being faster by 2 or 3 seconds. Secondly, and most importantly, is the fact that there are interactions between these two subsystems of the ANS. This can be seen histologically at the heart and the effects of this interaction can be seen when these two systems are stimulated and monitored simultaneously (Levy & Zieske, 1969). For example, these researchers have shown that the greater the sympathetic background activity the greater will be the depressant effect of any vagal activity. The converse is not true, however.

The typical view of how neurological control of HR occurs is that it is primarily the result of a number of simple reflexes through the cardiac center of the medullary brainstem. Such influences are important but there are also a number of higher brain centers which have strong influence on HR, the reticular activating system, the hypothalamus and the cortex. Neural activity in these areas of the CNS can either effect HR changes directly or modulate the gain on various autonomic reflexes such as those from the aortic and carotid baroreceptors.

One complexity of HR control that has proved to be particularly important for psychophysiologists studying mental workload is that even at rest there is beat to beat variability, the HRV briefly described earlier. This variation has been measured as both the variation in HR or in the interbeat interval, IBI. There are advantages to both, but it is essential to remember that HR and IBI are not linearly related.

On the other hand IBI is linearly related under simple laboratory situations to the frequency of vagal stimulation. It has been shown (Katona & Jih, 1975) that variation in IBI is directly proportional to the degree of vagal tone or parasympathetic control on the heart. However, even though HRV is established as a good measure of vagal tone, there may be more to HRV than vagal tone alone. Upon vagotomy HRV is greatly reduced but it can still be perceived (Levy et al., 1966) and oscillating sympathetic activity has also been observed (Langhorst et. al., 1983).

Under normal circumstances three primary frequency components of HRV can be seen when data is subjected to spectral analysis. The low frequency band is thought to be associated with temperature regulation, the middle frequency band with blood pressure regulation and the highest frequency band with respiration. It is the blood pressure and the respiration bands that seem to be most responsive to changes in mental workload so that understanding their etiology is of most value for the psychophysiologist.

To explain the origins of these rhythms both central and peripheral mechanisms seem important. Grossman (1983) has described four separate peripheral mechanisms the most accepted of which is that induced by carotid and aortic baroreceptor reflexes. During inhalation the decrease in thoracic pressure causes a decrease in blood pressure which through a decrease in sensory afferent firing from the baroreceptors inhibits vagal activity and causes an increase in HR.

Other studies have shown, however, that central factors are critical to HRV. For example Katona et al. (1970) noted that vagal activity was diminished or stopped during inhalation even if blood pressure was maintained at a high level. Furthermore, if the dogs with which they were experimenting were artificially ventilated, little change in vagal output was observed unless the animals attempted to

breathe on their own in which case there was an increase in variability. Finally, they observed that vagal cardiac responses occurred slightly before, not after respiratory changes occurred, which was also observed by Levy et al. (1966) and which suggests that a central mechanism is controlling the coordination between the cardiac and respiratory activity. Such a control center might be found in the reticular activating system where Langhorst et al. (1983) have found neurons whose activity reflect that of both the heart and the respiratory system. The adaptive value of such a system would be that blood pressure can be maintained more evenly if HR goes up just before or as inhalation occurs rather than waiting for feedback responses via the baroreceptors.

VII. RESULTS: HR AND HRV AS MEASURES OF MENTAL WORKLOAD

From the preceeding discussion it can be seen that the understanding of HR and HRV control and of what is meant by mental workload has many deficiencies. Consequently, it is unlikely that the use of HR and HRV can be used to rigorously quantify mental workload in the near future. However, there is no doubt that there is a substantial qualitative relationship between these parameters and any effort to help clarify this relationship will prove of value.

One of the most basic problems is the question of why HRV varies with mental workload. Is it in preparation for increased metabolic demands and is the orientation reflex in preparation for a lower metabolic rate to help the individual attend to some stimulus? Or are these relatively small changes in HR simply epiphenomena, the result of complex neural activity that have no adaptive significance per se? From a biological perspective this latter possibility is not as unlikely as it may seem. If a characteristic, like the presence of an EKG or HR variations

during mental work, should come about simply as a result of other more significant characteristics and they are not selected against, then they will persist even though they have no adaptive value in themselves.

Other problems need to be confronted by the researchers in this field. One of the most significant concerns is the variability between subjects, within subjects on different days (Wientjes, 1989) and between different types of mental tasks (Somsen et al., 1983). Another problem is the question of whether there are significant circadian or ultradian rhythms to HRV. This is a likely possibility since such rhythms have been shown to exist in resting HR and in performance tests. If such rhythms do exist they can add unwanted variability to the results of a study leading to the rejection of a valid hypothesis.

One of the major values of HR and HRV analysis for the study of mental workload and fatigue is the fact that they can be easily monitored under a wide range of circumstances: short term studies primarily geared towards the analysis of immediate reactions to stimuli (OR's of SR's), middle length studies like those conducted at HEG/AAMRL which assess the mental effort required by specific tasks and long term situations where subjects such as pilots can be monitored over periods of prolonged concentration.

In conclusion, while there are many difficulties to be faced, the use of HR and HRV for assessing mental workload seems to be well worth pursuing. For any measure to be good it must be sensitive to the task and to the differences between tasks and individuals. Psychophysiologists are still working on these challenges, but it has become clear that studies must not be confined to HR and HRV alone. Other windows on the response of the body to mental workload are needed. Some of these other techniques include recording evoked potentials, eye blink analysis, measuring respiration rate and tidal volume and seeking a non-invasive

measure of sympathetic activity. Although it appears that none of these techniques will work alone to provide a clear view of mental workload, a combination of them may prove to be successful. This is the approach which the Ergonomics and Workload Branch of HEG is presently pursuing.

VIII. RECOMMENDATIONS

Science is a fascinatingly self-correcting process and the Workload and Ergonomics researchers and their colleagues elsewhere are excellent at setting up intelligent and relatively efficient goals in a difficult field of study. Furthermore, while this research at HEG/AAMRL is oriented towards pilots and cockpit efficiency, the Air Force should not ignore the fact that it can also apply to pilot training and long distance performance, to radar operators and flight control experts on naval ships and to the general citizenry.

To pursue some of these other directions would require broadening the scope of the research at this lab to the investigation of more short term physiological responses, such as the OR and SR. (Apparently long term endurance monitoring of physiological variables is presently being done at Brooks AFB). If HEG cannot make such an adjustment without diminishing the work they are presently doing (since the short term tests are not strictly concerned with workload) the Air Force should consider setting up another laboratory to do so.

One recommendation for the researchers in this field is to take some time to try to determine what they mean by mental workload. The primary decision is whether it involves cognitive functioning alone or both cognitive and automatic functioning. If this can be decided, it may be easier to determine what biological resources are utilized during mental work which could then help establish what physiological parameters they could best study.

Since it is clear that no single physiological parameter will provide the full story about mental workload, it is encouraging that the researchers at HEG have developed the PATS system so as to be able to look at a multitude of parameters at one time. They should, however, add respiratory rate and tidal volume to their capabilities not only as an additional parameter but so as to be able to look for interdependence between those parameters and HR and HRV changes. They should also try to be able to add other physiological parameters to PATS, whenever any prove themselves to be of value. For instance, a parameter that would effectively measure sympathetic activity would be invaluable in the assessment of mental workload.

Finally, there should be concern that ultradian or circadian rhythm effects may be causing some of the variability in test results. These possibilities should either be controlled in future studies or tested for the significance of their influence on mental functioning and the physiological parameters involved. Since it is already known that there are circadian variations in HR it would be particularly valuable to investigate if the same is true for HRV. This final recommendation will be the foundation of the author's Research Initiation Program proposal.

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FINAL REPORT

Breakdown of Total Information Processing Time into During-Display and Post-Display Components for Serial and Spatially Distributed Visual Presentations

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Breakdown of Total Information Processing Time into During-Display and Post-Display Components for Serial and Spatially Distributed Visual Presentations

by

Ethel Matin

ABSTRACT

Information processing speed was compared in serial and spatially distributed visual displays, using performance measures that assess total information processing time and also allow separation of the time for processing while a display is visible from the further processing that occurs after it is removed. For all subjects, a significantly shorter total time was required with the serial display, which eliminated the need for saccadic eye movements to access the data. With both display types, all subjects were able to exercise some control over the distribution of the total processing time, allotting a relatively long during-display time and a correspondingly short post-display time under one set of instructions, and the converse under the other set. A high negative correlation, accounting for 80% of the response variance, was found between the two processing components, as would be expected if there is a tradeoff between them in accomplishing the total processing task.

Acknowledgements

It is my pleasure to thank Kenneth R.Boff, Director of the DEfTech Laboratory, and the members of his research team, Rebecca Donovan, Kristin Morton, Dieter Zirkler, and Sarah Swierenga. Together they created the stimulating, supportive environment that made my summer of research productive and enjoyable. I also thank Charles Bates, Director of Human Engineering at AAMRL, whose thoughtful "behind the scenes" encouragement has not gone unnoticed, and the Universal Energy Systems team, who carried out their mandate from the Air Force Systems Command and the Air Force Office of Scientific Research with skill and good humor. Finally, I thank my student at Long Island University, Karen Curto, who contributed important insights about the tradeoff between during-display and post-display processing time.

I. INTRODUCTION:

The DEfTech Laboratory at AAMRL is engaged in psychological research on designer use of human factors information and in research on human-computer interface concepts. Several years ago, as a guest of the Laboratory at the invitation of its director, Dr. Kenneth R. Boff, I contributed to the latter effort by proposing a new visual display technology characterized by the serial transmission of independent frames of visual information through a single display window. Dr. Boff and I planned a program of basic research on the scientific aspects of the proposed technology and initiated its implementation (Matin and Boff, 1988; Matin, Boff, and Donovan, 1987; Osgood, Boff, and Donovan, 1988). Moreover, we applied for a patent, which was awarded to the Air Force earlier this year (Matin and Boff, 1989).

This Spring, after my appointment as a Summer Faculty Research Fellow, Dr. Boff and I decided to utilize the ten week research period to explore a surprising phenomenon that had emerged in the course of our earlier work on serial displays. This phenomenon will be described later in the Introduction after a brief description of the serial display concept has been presented.

Essentially, a serial visual display presents chunks of information in rapid temporal succession at a single spatial location, thereby providing a multivariate channel for sequential transmission of visual information analogous to an auditory display. It offers three potential advantages as a design option:

1. Reduced display space requirements. In effect, a serial display with N frames occupies 1/N of the amount of display "real estate" that would be required with conventional, spatially distributed displays.

2. Increased rate of information transfer to the human operator. A higher rate is possible because the serial display eliminates the need for programming and executing the saccadic eye movements that are needed to scan an array of visual displays. For information that could be processed in rapid automatic mode, elimination of the saccadic overhead permits a substantial reduction in time to access and process visual information.

3. Designer control over information sampling sequences. In system control environments, operators monitor large numbers of displays to determine when they need to intervene in the computer-controlled processes they

are supervising. All of these displays must be sampled, some of them more often than others, depending on the bandwidth of the displayed information. With conventional, spatially distributed displays, the designer's task is to lay them out in a way that will encourage optimal monitoring sequences while minimizing the time to learn them. With an appropriately designed serial display, the optimal sequence can be built into the display itself.

For our research comparing serial and spatially distributed visual displays, we developed a measure called the "frame duration threshold". Briefly, this is the amount of time a frame must be exposed for a given level of performance (e.g., 90% correct). By computing the difference between the threshold for information presented in a spatially distributed array of frames and the threshold for serially presented frames, we obtain a measure of the overhead that can be attributed to the need for saccadic eye movements to access the data.

Early in the course of our work with the duration threshold measure, we found a very large saccadic overhead for a simple recall task. However, it seemed reasonable to expect a less dramatic overhead, if any, for tasks that require more complex processing. Accordingly, we decided to examine

digit addition for serial and spatially distributed displays of three digits. To our surprise, the saccadic overhead was not diminished by the additional complexity of the task.

A possible explanation for the surprising result was this: if subjects absorbed the information from the display and then added the three digits, the duration threshold might only be tapping the perceptual/encoding/ memory aspect of the processing, but not the further cognitive processing needed for the digit addition. If that is indeed the case, the serial/spatially distributed difference in processing speed might disappear if a performance measure that taps the total perceptual and cognitive processing time were used. The research performed during the ten week summer fellowship addressed this question.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The first objective of the research was to compare human information processing speed in serial and spatially distributed displays, using performance measures that permit separation of the processing time while a display is visible from the further processing that goes on after the display is removed.

The second objective was to study the effect of the subject's operating mode. The influence of this variable became apparent in the course of preliminary experiments, which showed that subjects could operate in two modes: emphasis on making the display time as short as possible with a longer post-display processing time, or emphasis on a minimal post-display time, with a correspondingly longer display time.

The third objective was to measure the magnitude of the correlation between during-display and post-display information processing times for the serial and spatially distributed display conditions. These correlations, which are expected to be negative if there is a tradeoff between display duration and post-display response time, will allow assessment of the proportion of variance that can be attributed to this tradeoff.

III. METHOD

The three research objectives were accomplished with the following experiment, which was run on four subjects.

a. Apparatus. The experiment was run with an IBM XT microcomputer equipped with an enhanced graphics adapter

card, a data acquisition card, a standard IBM keyboard, and an enhanced color display. The computer presented stimuli, recorded responses, and stored the data on disk for later statistical analysis. It was programmed in BASIC with 8088 assembly language routines for display timing, synchronization with the 60 Hz display raster, and measurement of post-display response times.

b. Subject's Task. In order to ensure a reliable response time measure that was not complicated by the subject's ability to type on the keyboard, the digit addition task from our exploratory study was modified to permit a binary response - Odd if the sum of the digits was Odd, Even otherwise. For this modified task, subjects could get the correct answer either by adding the digits and checking to see if the sum was odd, or by counting the number of odd digits, and making an Odd response if this number was odd. Subjects were asked to use the latter method, and all reported that they did so. The keys "V" and "M" were used to enter the Even and Odd responses, respectively.

For all experimental treatments, subjects initiated a trial by looking in the center of a small window on the monitor (the fixation window), and hitting one of the two response keys, keeping their two index fingers on the keys, ready

for the binary Odd/Even response at the end of the trial. For the serial condition, which eliminated the need for saccadic eye movements, the 3 digits were then presented as individual frames in temporal succession in the fixation window for the frame duration selected for the trial. Feedback - the subject's response (O or E) and the correct response (O or E) were presented together for 800 ms just above the fixation window. As soon as the feedback disappeared the subject was free to initiate the next trial. For the spatially distributed condition, there were two windows centered vertically on the screen and separated horizontally by 11 deg (visual angle). For this condition, the first digit was presented in the left (fixation) window. The subject then made a saccade to the right window to get the second digit, and then a second saccade to the left window, where the third digit was presented.

At the beginning of the run, the subject was informed whether the Serial or Spatially Distributed condition was in effect, and whether the operating mode was short display time or short post-display time. In the first half of the run, the computer "tracked" the frame duration threshold for the conditions of that run (i.e., it measured the frame duration for 85% correct performance). This usually required 50-60 trials. Immediately thereafter, the subject

received another 40 trials, for all of which the frame duration was fixed at the duration threshold. Post-display response time (which could be negative if the response occurred before the end of the display) was measured on each trial in both parts of the run. However, this measure was only analyzed for the 40 trials at the fixed duration. In short, for the purposes of the present analysis, each experimental run yielded three numbers: the duration threshold, the mean post-display time for the 40 trials that were run at the threshold, and the % correct responses for those trials. Subjects were encouraged to look at their results, which were printed at the end of each run, in order to learn to discriminate between the two operating modes (short display time or short post-display response time). Each block of trials took approximately 5-7 minutes and subjects rested and relaxed between the four blocks that constituted the experimental session.

c. Experimental Design. A 2 X 2 factorial design was used. Factor 1 was the display condition (serial or spatially distributed) and Factor 2 was subject operating mode (emphasis on short display time or emphasis on short post-display time). Each of the four experimental treatments was run as a block in every experimental session to control for improvements in performance as the experiment progressed.

In addition, the position of a given treatment within the four block session was counterbalanced across sessions to control for possible performance differences within a session. Each block of trials yielded a duration threshold measure (frame duration for 85% correct performance), a mean post-display response time measure for 40 trials run at the threshold, and a %correct response measure for the 40 trials. If the latter was not within 5% of the 85% target, the data for the block were discarded. Additional sessions were then run as needed until 8 blocks of data were available for each experimental treatment. This design feature ensured that subjects were operating close to the 85% correct criterion for all data used in the analysis. All subjects practiced the procedures for several weeks before formal data collection began.

IV. RESULTS

a. The experimental findings relevant to objectives 1 and 2 are summarized in Figure 1, which is presented on the next page. For each of the four subjects, Figure 1 shows the effect of the two experimental factors - display method (Serial - SER, or Spatially Distributed - SPA) and subject's operating mode (emphasis on short display time or emphasis on short post-display time). The height of a bar represents total processing time (onset of the display to

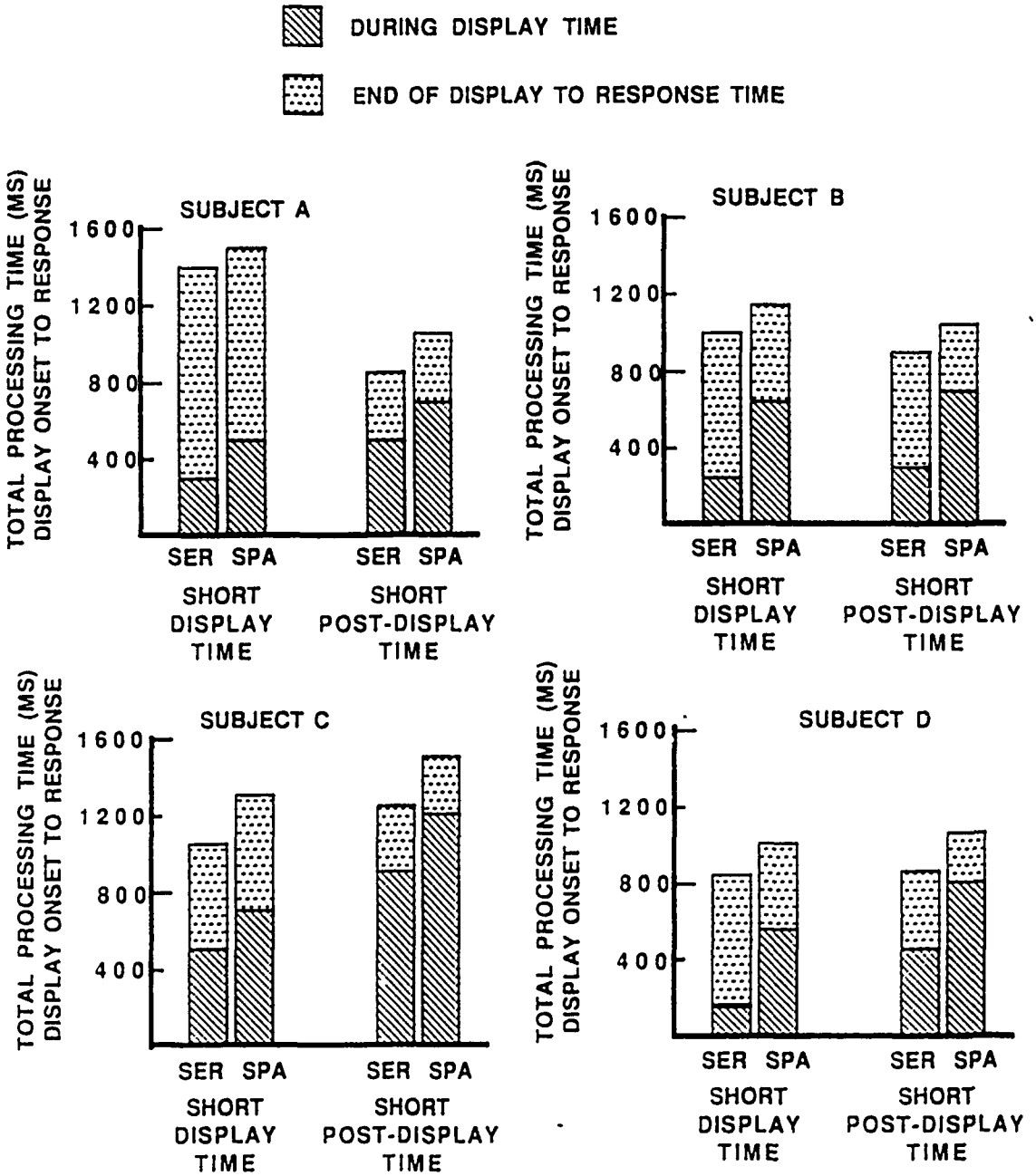


Fig. 1. Information processing time for serial (SER) and spatially distributed (SPA) displays, with 3 frames per display. The data are shown separately for short display time and short post-display time operating modes. Total processing time is broken down into two components: during display time and end of display to response time.

the subject's response). In addition, each bar shows the breakdown of the processing time into two components: display duration for the three frames (the subject's 85% duration threshold X 3), and time from the end of the display to the response.

From the results shown in Figure 1 and the associated statistical analysis, which is presented in Table 1 on the next page, the findings with respect to objectives 1 and 2 can be summarized as follows:

The serial method resulted in a faster total processing time for both the short display time mode and the short post-display time mode. Averaged across all subjects and all treatments, the time saving was 194 ms, for an overhead of 97 ms/saccade (note that the three frames were accessed with two saccades).

All subjects were able to exert some control over the distribution of the total processing time: In the short display time mode, the duration threshold was short and the time from the end of the display to the response was correspondingly long. The converse was true in the short post-display time mode. However, for three of the four subjects (D is the only exception), total processing time

was significantly less for one of the two operating modes for both the serial and the spatially distributed displays.

Table 1

Analysis of Variance for the Total Processing Time

	Subject			
Source	A	B	C	D
Display Type				
F	25.47	23.14	21.88	166.55
(p)	(.0001)	(.0001)	(.0001)	(.0001)
Operating Mode				
F	254.81	9.58	16.20	.19
(p)	(.0001)	(.0044)	(.0004)	(.6634)
Interaction				
F	3.70	.03	.75	.11
(p)	(.0646)	(.8537)	(.3947	(.7412)

b. The third objective of the study was measurement of the correlation between during-display and post-display information processing times. Table 4 on the next page shows these correlations along with r^2, the proportion of explained variance, and p, the probability of chance occurrence.

For all subjects and for both the serial and the spatially distributed display conditions, the correlations are negative, as would be expected if there is a tradeoff between during-display processing and post-display

Table 4

Correlation between the Duration Threshold and Post-Display Response Time Measures for Serial and Spatially Distributed Displays

Display		Subject A	B	C	D
Serial					
	r	-.9366	-.8248	-.9026	-.8976
	r^2	.8771	.6802	.8146	.8056
	(p)	(.0001)	.0001	(.0001)	(.0001)
Spatially Distributed					
	r	-.8849	-.8582	-.8760	-.9248
	r^2	.7830	.7366	.7674	.8552
	(p)	.0001	.0001	.0001	.0001

times to achieve the 85% correct performance. Moreover, they are remarkably high in absolute value, accounting for 79.4% of variance for the serial display and 78.6% for the spatially distributed display (averages of r^2 over the four subjects).

V. RECOMMENDATIONS

The findings of the present study need to be generalized beyond the three frame digit processing task, and the implications of the tradeoff between during-display and post-display processing that was discovered in the course of the research needs to be explored. Specifically, the following lines of further research would be useful from both the basic and applied points of view:

a. Examine the effects of number of the frames (and therefore the number of saccades) on total information processing time. If each saccade adds an overhead of approximately 100 ms to the total processing time, we would expect an increase in the serial display's speed advantage as the number of frames increases. In addition to its basic significance in the study of eye movements and information processing, data on the effect of the number of frames is important from the viewpoint of engineering tradeoffs in design applications.

b. Examine the effect of the type of information processing task, using the total information processing time measure. In our earlier published work (Matin and Boff, 1988), we hypothesized that the speed advantage of the serial display format would be found with automatic but not with controlled processing. The total processing time measure and the technique for breaking it down into two components that was developed in the present study provides a useful new tool to approach this question.

c. Examine the effect of the distribution of the response time between the during-display and post-display components on overall processing efficiency.

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Final Report

Dr. Randy Beth Pollack

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FINAL REPORT

Kinematic Mappings Between the EXOS Handmaster Exoskeleton[1] and the Utah/MIT Dexterous Robot Hand

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Contract No:	F49620-88-C-0053

Harness Belt Task

by

Joseph Szucs (Summer Faculty Research Fellow)

Vincent Dimicelli (Graduate Student Research Fellow)

ABSTRACT

A new version of the Harness Belt Option of the Articulated Total Body (ATB) model [3,8,9] has been constructed. This new model minimizes the total elastic potential energy of the harness system under the following constraints: (1) the component of the belt tension force that is perpendicular to the body surface is balanced by the deflection force exerted by the body due to deformation; (2) the friction force created by the belt tension force is not smaller in magnitude than the component of the belt tension force that is tangential to the body surface. It was found by us that the old version of the Harness Belt Option does not minimize any function, it only imposes constraints (1) and (2) with the difference that in (2) it stipulates that the friction force is not smaller in magnitude than those two components of the tension force that are tangent to the body surface and are parallel and perpendicular to the belt line. These constraints are physically wrong and do not determine the motion of the harnesses uniquely. This causes the failure of the old model.

We have started computer implementation by writing two new subroutines.

ACKNOWLEDGMENTS

We wish to thank the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems is also thanked for its administrative assistance.

We would like to thank the employees of the Harry G. Armstrong Aerospace Medical Research Laboratory for creating a friendly environment that made our work enjoyable. Dr. Ints Kaleps, our technical focal point, discussed the model with us several times. He pointed out that the potential energy is a special case of the least squares method suggested by us. We would like to thank him for his support. We would also like to thank Ms. Louise Obergefell and Mrs. Annette Rizer for answering numerous questions concerning the ATB model. They both, especially Louise, were very helpful. We also thank Chief Lasniey for his technical assistance. Finally, we would like to mention Dr. Chi Ming Tang, who answered our questions concerning the graphics of the ATB model.

I. INTRODUCTION

The Summer Faculty Research Fellow, Dr. Joseph Szucs has been interested in various areas of mathematics and physics. He published several papers in the fields of functional analysis, ergodic theory (commutative or not), abstract algebra, cybernetics, and measure theory. His recent interest lies in the mathematical problems of biology. He has recently submitted two papers on population genetics. His formal education is in mathematics, physics, and computer science.

The Graduate Student Research Fellow, Vincent Dimicell, has his formal education in mechanical engineering (B. S.) and applied mathematics (M. S). He is facile in the computer language FORTRAN.

Dr. Szucs knows a good portion of the theory of dynamical systems because of his work in functional analysis (von Neumann algebras). One of his results has several applications in the theory of noncommutative dynamical systems and quantum statistical mechanics.

The research task Dr. Szucs and Mr. Dimicell have been assigned to was the review of the Harness Belt Option of ATB [3,8,9] and correction of modeling and programming errors. (The Harness Belt Option only works in some trivial cases with rigged data.) The modeling part requires knowledge of theoretical mechanics and mathematics. The programming part requires the ability of planning large subroutines and coordinating them.

The varied background of Mr. Dimicell contributed to his selection by Dr. Szucs. The manifold backgrounds of Dr. Szucs and Mr. Dimicell contributed to

their assignment as participants of the Summer Faculty Research Program/ Graduate Student Research Program.

II. OBJECTIVES OF THE RESEARCH EFFORT

The ATB model together with the Harness Belt Option, describes what happens to ejected pilots and crash victims [3,8,9]. A harness is a system of belts tied together and fastened to the seat. The ATB model describes what happens to a passenger or several passengers in a crash or during ejection from an airplane. Besides this model, actual manikin tests have been performed during which the force and torque is measured at several places of the body. These tests are very expensive and it is difficult to follow the motion of the harness. It is therefore desirable to construct a mathematical model and study the outcome of ejection or crash by solving the equations of motion exactly or numerically on a computer. Exact solutions are not known nor are they expected even if the entire body is considered rigid without a harness[1, p.146]. If the body is assumed to consist of several ellipsoids or hyperellipsoids coupled together, the equations of motion can be written and solved numerically on a computer, provided that no harness is considered. If the affects of a harness are also taken into account, even the exact Newtonian equations of motion are not known. Nevertheless, one may attempt to describe the motion of the body-harness system by programming the computer to calculate the location of the center of mass and the Euler angles at any time t at the end of the first time interval, using the initial data of the body segments and the harness. The harness is assumed to have no mass and it moves with the body segments. The tension caused by this motion of the harness makes the harness move a little in relation to the body. In its new position, the harness exerts forces and torques on the body segments. These

forces and torques, together with the other forces and torques are the input for calculating the motion of the body in the next time interval. This process can be repeated as many times as needed. The Harness Belt Model is supposed to describe the infinitely fast motion of the harness at the end of each time interval.

The Harness Belt Option currently used, works only in simple cases or when the initial data are tuned. The objective of the research was review of the model and the accompanying program and the identification of modeling and programming errors.

Our objective had to be modified drastically when we found a fundamental error in the Harness Belt Model (see section IV). This error meant that we had to construct a completely new model and had to write the corresponding subroutines. This task could not be done in the amount of time provided.

III. MATHEMATICAL ERROR IN THE HARNESS BELT MODEL

The formulas on p. 7-17 of the Advanced Harness-Belt Restraint System [3] all checked O. K. (allowing approximation) except the first formula on p. 17.

Using the abbreviated notation $x = e_x^1 FB_y$, the formula at the top of p. 17 is equivalent to the following:

$$\delta|x| = (\delta x)(\text{sign}x).$$

The correct formula for $\delta|x|$ is as follows:

If $|\delta x| < |x|$ and $\text{sign}x \neq \text{sign}(\delta x)$, then

$$\delta|x| = (2x + \delta x)(\text{sign}(\delta x)).$$

If not ($\delta|x| \neq |x|$, $\text{sign}x \neq \text{sign}(x)$), then

$\delta|x| = (\delta x)\text{sign}x$.

Note that the last formula has been used in all cases in the existing model.

In our new model (see section VI) we avoid the above mathematical error by using $\delta x^{**}2$ instead of $\delta|x|$. This has to be done because $\delta|x|$ is not homogeneous linear in x.

IV. PHYSICAL ERROR IN THE HARNESS BELT MODEL

The fourth and fifth displayed equations on p. 12 (the first two constraint equations) of the Advanced Harness-Belt Restraint System [3] contain a physical contradiction. To illuminate this, we describe a thought experiment in the next paragraph.

Let us place a sanding disk on a table with the sandpaper down. Suppose the disk is pushed to the table by a force perpendicular to the table. Denote the magnitude of this force by F. Let us assume that the friction coefficient μ is independent of the direction of motion of the disk on the table. Then, independently of direction, a force parallel to the table and of magnitude larger than μF is needed to move the disk. This is what we think would actually happen if we were to perform the experiment. Suppose two forces parallel to the table and perpendicular to each other act on the disk. Assume that they have the same magnitude which is equal to $(3\mu/4)F$. Then the resultant force has magnitude $(3\sqrt{2}/4)\mu F > \mu F$. Therefore, the forces would move the disk. Let us now see what the Advanced Harness-Belt Restraint System predicts. This model imposes two independent constraints on the components of the force acting on the disk. Namely, the disk will not move according to the model if the magnitudes of the

perpendicular components are both smaller than μF. Since these conditions are clearly met in our case (the components have magnitude $(3\mu/4)F < \mu F$) the disk would not move according to the model.

Our recommendation is that the two constraint equations be replaced by one equation imposing a condition on the magnitude of the component of the belt force which is tangential to the ellipsoid in question. The magnitude of the friction coefficient in an arbitrary direction can be calculated from μ_1 and μ_2 by considering the ellipse with half-axes of lengths μ_1 and μ_2 (see section VI).

There is possibly another physical error in the model. However, this error can be corrected easily by altering the definition of the force deflection function. The current model does not make any distinction between static and dynamic force deflections. We cannot tell whether this distinction is taken care of by inputing the dynamic force deflection function or not.

V. LOGICAL ERROR IN THE HARNESS BELT MODEL

The current harness-belt model postulates two independent friction constraints and one deflection constraint. The friction constraints are inequalities and the deflection constraint is an equation. These constraints are required at each point that is not anchored and is on a body segment. The number of constraints is correct but two-thirds of them are inequalities. Therefore, there is a continuum of solutions. In spite of this, the corresponding program calculates the coordinates of the reference points. It seems that the calculation is achieved by converting the inequalities into equations. It is possible that the inequalities are converted into equations for only those points where the constraint inequalities are not satisfied and the other points are

ignored. The problem with this approach is that after the program is run and the new coordinates of the reference points are calculated, the inequalities at the points where they were satisfied may be violated.

VI. A NEW MODEL FOR THE HARNESS-BELT RESTRAINT SYSTEM

We propose that the locations of the belt reference points be calculated by minimizing the total elastic potential energy of the harness system under the following conditions: (1) the component of the belt tension force that is perpendicular to the body surface is balanced by the dynamic deflection force exerted by the accelerating body due to deformation; (2) the friction force created by the belt tension force is not smaller in magnitude than the component of the belt tension force that is tangential to the body surface. If a reference point is not on a body ellipsoid or the tension force of the belt at the point is directed away from the body ellipsoid, then the above two conditions are replaced by three conditions stipulating that the belt tension forces are balanced at the point under consideration.

We use an approximation quadratic in the perturbation coordinates for the potential energy of the harness system and linear approximations for the constraints. Therefore, we face a quadratic programming problem [2]. We are going to use the notation of [3]. To avoid sub- and superscripts, we use linear writing and indicate superscripts and subscripts in parantheses. First we list the superscripts separated from subscripts by a semi-colon.

For the calculation of the objective function we need an approximate expression for $|x+d|$ up to quadratic terms in d, where x and d are vectors of the same dimension. We obtain:

$$|x+d| = (x^2 + 2xd + d^2)^{1/2}$$

$$= |x|(1 + 2xd/|x|^2 + d^2/|x|^2)^{1/2}$$

$$= |x|[1 + xd/|x|^2 + d^2/|x|^2 - (1/2)(xd)^2/|x|^4+...]$$

$$= |x| + xd/|x| + d^2/2|x| - (xd)^2/2|x|^3.$$

We want to calculate the work done if a belt segment is stretched from its unstretched length B to length b. This work is equal to

$$\int_B^b f_b(t/B - 1)dt$$

where f_b is the stress-strain function of the belt segment. If the belt is first stretched to length $|x|=b$ and then to length $|x + d|$, where x and d are 3-dimensional vectors, then the change in the above work is

$$\int_{|x|}^{|x+d|} f_b(t/B - 1)dt.$$

We calculate this integral up to terms quadratic in |d|. It is then sufficient to use a linear approximation for f_b. We have

$f_b(t/b - 1) = f_b(b/B-1) + f_b'(b/B - 1)(t - b)/B + ..$

Then

$$|x| + xd/|x| + d^2/2|x| - (xd)^2/2|x|^3$$

$$\int_{|x|} [f_b(t/B - 1) + f_b'(b/B - 1)(t - b)/B]dt.$$

$$= f_b(b/B - 1)[xd/|x| + d^2/(2|x|) - (xd)^2/(2|x|^3)] +$$

$$f_b'(b/B - 1)(xd/|x| + d^2/(2|x|) - (xd)^2/(2|x|^3))(b - |x|)/B + ...$$

The objective function is the sum of the above expression over all intervals defined by adjacent reference points. Let us denote the endpoints of such an

interval by X and Y; let c = fb(b/B - 1//b and c' = fb'(b/B - 1)/(2Bb**2). Then x = X - Y, o = |X - Y|, d = d(X - Y) = dX - dY and the contribution to the objective function, of the interval with endpoints X, Y is

c(X - Y)(dX - dY) + c(dX - dY)**2/2 + [(X - Y)(dX - dY)]**2[c' - c/(2b**2)]

= c$\sum_j$[(X(j) - Y(j))dX(j) + (Y(j) - X(j))dY(j)]

+ (c/2)$\sum_j$[(dX(j))**2 - 2dX(j)dY(j) + (dY(j))**2]

+ [c' - c/(2b**2)]($\sum_j$[(X(j) - Y(j))dX(j)]**2

+ 2[$\sum_j$(X(j) - Y(j)dX(j)]·[$\sum_j$(Y(j) - X(j))dY(j)]

+ $\sum_j$[(Y(j) - X(j)dY(j)]**2

=c$\sum_j$[(X(j) - Y(j))dX(j) +(Y(j) - X(j))dY(j)]

+ (c/2)$\sum_j$[(dX(j))**2 - 2dX(j)dY(j) + (dY(j))**2]

+ [c' - c/(2b**2)]·$\sum_j$[[(X(j) - Y(j)dX(j)]**2

+ [Y(j) - X(j)dY(j)]**2]

+ 2 $\sum_{j<j'}$ (X(j) - Y(j))(Y(j') - X(j'))dX(j)dY(j')

+ $\sum_{j=j'}$ (X(j) - Y(j))(X(j') - Y(j'))(dX(j)dX(j') + dY(j)dY(j')))

= dX(j)(c[X(j) - Y(j)]) + dY(j)(c[Y(j) - X(j)])

+ (dX(j))**2((c/2 + (c' - c/(2b**2))(X(j) - Y(j))**2)

+ (dY(j))**2((c/2 + (c' - c/(2b**2))(Y(j) - Y(j))**2)

+(for j=j'dX(j)dX(j')((c'- c/(2b**2))(X(j) - Y(j))(X(j') - Y(j')))

+(for j=j'dY(j)dY(j')((c'- c/(2b**2))(Y(j) - X(j))(Y(j') - X(j')))

+(for j=j'dX(j)dY(j')((c'- c/(2b**2))(X(j) - Y(j))(Y(j') - X(j')))

+dX(j)dY(j')(-c/2 -(c'- c/(2b**2))(X(j) - Y(j))**2)

Now let us re-index the reference points X(1),...., X(N) in such a way that all points have exactly one index m and the first p points are exactly the points that are not anchored. We denote the point with this new index by ξ(m). The symbol ν(m) denotes the index of any point that is a neighbor of the point with index m. The quadratic programming has 3p variables. They are the dX(j), (j=1,2,3), where

X(j) is not an anchor point. Let us denote the jth coordinate of the perturbation of the mth point by x(3(m - 1) + j). Since the last side of the chain of equalities for the contribution of the interval (X, Y) is invariant under interchanging X and Y, the coefficients g and H of the objective function

$$(g^T x + (x^T H x)/2)/2$$

are:

$$g(3(m-1)+j) = \sum_{k(m)} c[X(j;m) - X(j;k(m))]$$

$$H(3(m-1)+j,\ 3(m-1)+j) =$$

$$\sum_{k(m)} (c + [2c - c/b^{**}2][X(j;m)-X(j;k(m))]^{**}2$$

$$H(3(m-1)+j,\ 3k(m)-1)+j) =$$

$$-c + [c/b^{**}2 - 2c][X(j;m)-X(j;k(m))]^{**}2$$

$$H(3(m-1)+j,\ 3k(m)-1)+j') =$$

$$[-2c + c/b^{**}2][X(j;m)-X(j;k(m))][X(j';m)-X(j';k(m))]$$

If j=j', then

$$H(3(m-1)+j,\ 3(m-1)+j') =$$

$$\sum_{k(m)} [2c - c/b^{**}2][X(j;m)-X(j;k(m))][X(j';m)-X(j';k(m))]$$

The remaining H are zero. Note that H(3(m - 1) + j, 3(k(m) - 1) + j) may not be zero only if k(m) is the index of a point that is not anchored.

To generate the linear constraints of the above quadratic objective function let us consider any point with index K (K=1,...,p) and any neighboring point (anchored or not) with index k. Let e(K) denote the matrix of the local basis vectors at the point with index K. The symbol U(Kk) will denote the unit vector from point K to point k and similar indexing will be used for b, c and B. Define

$$A(K;k) = [D(Kk)/B(Kk)]e(K)\cdot U(Kk)U(Kk)\cdot[[b'(Kk)/B(Kk)$$

$$- c/B(Kk)]].$$

where the dot denotes transpose. A(K;k) is a 3x3 matrix.

we have

$$\delta FR(1;K) = e(1;K)\delta FB(Kk) - \sum_j [A(1,j;Kk)\delta\xi(j,k) - A(1,j;Kk)\delta\xi(j,K)].$$

If point $\xi(K)$ is on an ellipsoid and $FR(3,K) < 0$, then we generate an equation and an inequality. The equation is

$$\delta FR(3;K) + \xi D'(\varphi K)\delta\varphi(K) = -FR(3;K) - \xi D\varphi;K),$$

where $\delta\varphi(K) = \sum_j L(j;K)\delta\xi(j;K)$

with $L(j;K) = (\varphi(K)r(K)\cdot/(r(K))^{**}2 -$

$$r(K))((E(K)r(K)\cdot/(r(K)\cdot E(K)r(K))^{**}(3/2))D(K)\delta\xi(K).$$

The inequality is

$$-FR(1;K) + \delta FR(1;K))^{**}2 + (FR(2;K) + \delta FR(2;K))^{**}2 \le$$

$$[(\mu(1)(FR(1;K) + \delta FR(1;K))]^{**}2 + [\mu(2)(FR(2;K) + \delta FR(2;K))]^{**}2)\cdot$$

$$(FR(3;K) + \delta FR(3;K))^{**}2.$$

If we only keep the terms linear in $\delta FR(1;K)$, we arrive at

$$\sum_j a(1;K)\delta FR(1;K) \ge (1/2)\{[(FR(1;K)^{**}2 + (FR(2;K))^{**}2]^{**}2 -$$

$$[(\mu(1)FR(1;K))^{**}2 + (\mu(2)FR(2;K))^{**}2](FR(3;K))^{**}2)$$

where

$$a(3;K) = FR(3;K)[(\mu(1)FR(1;K))^{**}2 + (\mu(2)FR(2;K))^{**}2]$$

$$a(2;K) = (\mu(2)FR(3;K))^{**}2 - 2[FR(1;K)^{**}2 + (FR(2;K))^{**}2])FR(2;K)$$

$$a(1;K) = (\mu(1)FR(3;K))^{**}2 - 2[FR(1;K)^{**}2 + (FR(2;K))^{**}2])FR(1;K).$$

If we now use the identity $FR(K) = e(1,K)\sum_{k(K)} FB(Kk(K))$, we obtain the entries of a and b in the constraint equations $A(1)x = b$ and the constraint inequalities $A(2)x \le b$. They are

$$a(m, 3(m-1)+j) = -\sum_{k(m)} A(3, j; m, k(m)) + \xi D'(\varphi m))L(j;m)$$

$$a(m, 3(k(m)-1)+j) = A(3, j; m, k(m))$$

$$b(m) = -\xi D(\varphi(m)) - FR(3;m)$$

for an equation. For an inequality,

$a(m, 3(m-1)+j) = -\sum_{l} Q(l;m) \sum_{k(m)} A(l, j; m, k(m))$

$a(m, 3(m-1)+j) = -\sum_{l} Q(l;m) A(l, j; m, k(m))$

$b(m) = (1/2)\{[(FR(1;K)**2 + (FR(2;K))**2]**2 -$

$[(\mu(1)FR(1;K))**2 + (\mu(2)FR(2;K))**2](FR(3;K))**2\}.$

All other a's are equal to zero. Here the index m of the point ξ indexes the equation and the inequality. In the computer implementation, the index of the inequality has to be different from that of the equation.

If the point $\xi(m)$ is not on any body ellipsoid or if $FR(3;m) \geq 0$, then three equations express the condition that the resultant of the belt forces at $\xi(m)$ is zero. These equations are

$\sum_{k(m)} e(l;m) FB(m,k(m)) + FR(l;m) = 0, \quad (l=1,2,3)$

Using $A(k)$, we obtain:

$\sum_{k(m)} \sum_{j} A(l,j;m,k(m)) [d\xi(j,k(m)) - d\xi(j,m)] = -FR(l;K), \quad (l = 1,2,3)$

The indices of the three equations will be (m,1), (m,2) and (m,3). We have

$a(m,l;3(m-1)+j) = -\sum_{k(m)} A(l,j;m,k(m));$

$a(m,l;3(k(m)-1)+j) = A(l,j;m,k(m));$

$b(m,l) = -FR(l;m) \quad (l = 1,2,3).$

A few words are in order about the objective function. First of all, the objective function is certainly positive semidefinite or definite. It is sufficient to see this for each term seperately. We know that $fb(b/B-1) \geq 0$ and $fb'(b/B-1) \geq 0$. On the other hand,

$(x*a)**2/|x| - (x*a)**2/(2|x|**3) \geq 0,$

since this inequality multiplied by $2|x|**3$ gives

$(a**2)(x**2) - (x*a)**2 \geq 0$

which is the Cauchy inequality. The nonnegativity of the term containing fb'(b/B-1) is obvious. If both fb(b/B-1) and fb'(b/B-1) are nonzero (which is expected if b > B), then the contribution is zero if and only if

(d**2)(x**2) - (xd)**2 + (xd)**2 = (d**2)(x**2) = 0.

Since x = X - Y ≠ 0, this implies that d=0. Therefore, the objective function is positive definite if for at least one interval of neighboring points, fb(b/B-1) ≠ 0 and fb'(b/B-1) ≠ 0.

VII. COMPUTER IMPLEMENTATION OF NEW MODEL

(1) We have eliminated the portion concerning DELMAX from the subroutine HPTURB since we do not need any convergence criterion for the new harness model.

(2) The subroutine HSETC has been rewritten to calculate the arrays A, Q, L needed to find the coefficients of the quadratic programming.

(3) A new subroutine, HINDEX, has been added. It converts the old indices of the belt reference points to the new indices and vice versa. It also finds the indices of the neighbors of each point. The new indices are needed to number the variables of the quadratic prgramming. The neighbors are needed for the sums in the coefficient formulas.

(4) Still another subroutine, HCOEF, has been added to calculate the coefficients of the quadratic programming.

VII. RECOMMENDATIONS

a. Guidance for implementation. The computer implementation of the new model is not completed. The old subroutine HBPLAY has to be changed to find the index of the body ellipsoid on which a given reference point is situated. If the point is not on any body ellipsoid, then the index is zero. This index is called KKS in HSETC and HCOEF. In the old model points cannot move from one body segment to the other. We have eliminated this restriction in the model.

The subroutine HBELT has to be changed to accomodate the new model. The forces and torques at each reference point are found in HBELT. The implementation of the model has to be tested.

b. Follow-on research. (1) It may turn out that the quadratic and linear approximations used in the quadratic programming are not sufficiently accurate. In this case the quadratic programming could be repeated with the perturbed data obtained in the preceding quadratic programming. This iteration could be continued until the outputs of two consecutive quadratic programmings are closer to each other than a small positive number. Another way of overcoming inaccurate approximation is the replacement of quadratic programming with convex programming [2].

(2) In case the proposed objective function does not yield good results, other objective functions can be explored. Candidates for objective function are the total length of the harness and the total quadratic perturbation of reference points. Of course, any quadratic or convex function with cleverly chosen inputs can serve as an objective function.

(3) The current model and the proposed new model also, approximate each belt with a broken line. This approximation can be made more accurate if the curvature of the pertinent body ellipsoid is taken intgo consideration betgween two neignooring belt points. A similar phisical model is described in [7, p. 62-64].

(4) The old and the proposed new models both use one kind of friction coefficent (two of them). In reality there are two different friction coefficients, a static and a kinetic one [7, p. 61 and 64]. They are of the same order of magnituge but different. In the constraints of the quadratic programming, the static coefficients nave to be used. If the inequality constraints of the quadratic programming are violated, the belt system will move relative to the body and the kinetic friction coefficients have to be used. During the sliding motion of the belt, energy is dissipated. It is desirable to determine whether this energvy loss is significant or not.

(5) The quadratic programming calculates the position of reference points at the end of each time interval. Since the quadratic programming is not an approximation of an existing continuous model but rather it is an a priori discrete "model", it is not clear whether the harness can move continuously from one discrete position to the next one as calculated by the quadratic programming.

(6) Better approximation can be obtained if the width of the belt is taken into consideration.

(7) It is desirable to include rate dependent functions in the new code, as is done in the old one.

(8) The new model could be extended to handle hyperellipsoids as body segments.

(9) Both the old and new models approximate the belt line by polygons. This is a geometric approximation only and not a discrete approximation of a continuous model describing the simultaneous continuous motion of the body-harness system. Such a model would probably contain coupled ordinary and partial differential equations. The partial equations are needed because one has to keep track of which point on a belt coincides with a given point on the body. The construction of such a model seems to be a difficult if not impossible task.

(10) It is plausible that the motion of the body-harness system is chaotic. (Concerning the theory of chaos, cf. [6, 10].) The real-life experiments with manikins show that very similar initial conditions may lead to drastically different outcomes [11]. The paper [5] suggests that chaos can be present in situations that are similar to the body-harness system and are much simpler at the same time. It is desirable to test for chaos. Such tests are described in [6]. If it is found that chaos is present, the chaotic behavior should be separated from the nonchaotic one. The chaotic part could be handled by methods of the theory of chaos. The probability density of outcomes rather than exact outcomes can be described [10].

c. Miscellanea. (1) It is desirable that a complete list of all variables used in the computer program be made. This list should contain a dictionary translating the names of variables in the model into their names in the program and vice versa.

(2) An advanced version of the ATB model would probably require a more advanced computer with larger memory than the Perkin Elmer computer currently used.

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FINAL REPORT

Harness Belt Task

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Kinematic Mappings Between the EXOS Handmaster Exoskeleton[1] and the Utah/MIT Dexterous Robot Hand

by

Michael M. Stanisic

ABSTRACT

Part of the overall Robotic Telepresence effort at the Harry G. Armstrong Aerospace Medical Research Lab (AAMRL) is controlling the fingers of the Utah/MIT Dexterous Robot Hand so that they will emulate a human's finger motions, providing a direct human-in-the-loop kinematic control of the robotic fingers. The focus of this effort was to develop the required kinematic mapping (the mathematical transformation), allowing the Dexterous Robot Hand the ability to grasp and position objects (such as tools) with its finger tips under the telepresence control of a human operator. The developed mapping of arbitrary human finger tip motion to robotic finger tip motion was successfully implemented.

[1] The EXOS Handmaster Exoskeleton was originally developed by Arthur D. Little Inc. and is now referred to commercially as the EXOS

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Finally, I also would like to thank the Air Force Office of Scientific Research for their generous support during the summer and the Universal Energy Systems, Inc. for their considerate administration of the program.

I. INTRODUCTION:

Robotic Telepresence is a highly advanced concept of teleoperation. Teleoperation provides for human operator commands which generate robotic motions through the use of either controlling joysticks or master/slave systems. Teleoperator control is frequently used in atomic research laboratories and in deep sea explorations, where the environment is hazardous to human life. In its more advanced forms, teleoperated systems can provide "force reflection" back to the human operator. With force reflection, the operator is capable of sensing forces acting upon the robot, and is thus capable of controlling the forces that the robot imparts to the environment.

A main drawback of teleoperated systems is that a high degree of expertise is required on the part of the operator. The operator must mentally map the motion of the controlling joystick into the motion of the robot. This is a problem similar to that encountered by the operators of heavy machinery such as backhoes and cranes. Observation of a backhoe operator reveals a "musician-like" quality in the way that the operator's fingers are moved and coordinated in order to produce a specific type of motion of the hoe. Although not as dramatic, the teleoperator of a robot needs a similar type of coordination. Learning this coordination of movements between the controlling joystick and the robot, requires a considerable amount of training and practice. When the problem of force reflection is added, the teleoperator of the robot becomes a highly trained person.

Telepresence is an advanced form of teleoperation, which allows the operator to command a robot which directly emulates the operator's motion, and in addition allows the operator a natural awareness of the sensory capabilities of

the robot. Telepresence control of a robot should allow the operator to see, hear and feel exactly what the robot sees, hears and feels. To achieve telepresence control, the robot must posses a full set of sensory capabilities, and a system must exist which allows these sensations to be realized by the operator. The telepresence effort in the Air Force is currently in its initial stages.

In order to achieve telepresence at the level of motion emulation (the emphasis of this summer's work), mapping of the motions should no longer be performed by the operator in his mind, but rather by an autonomous system, capable of directly mapping human operator motions into identical robot motions. The controlling joystick would thus be removed from the teleoperation system. Also, the burden of coordinating the robot would be transferred from the mind of the operator to the machinery of a computer, allowing the operator a greater mental awareness of the task at hand and eliminating the necessity of training the operator in using controlling joysticks. In more advanced forms of telepresence, the operator also "feels" the forces acting upon the robot, directly in the manner in which they are encountered by the robot. To the Air Force, telepresence control of robots would be useful in Air Base Operations such as flight line aircraft servicing in biologically, chemically or radioactively contaminated environments. In these situations, a human in a protected environment can safely and simply command the motion of the robot in the hazardous environment.

In the Robotics Laboratory of the Biological Acoustics Branch at AAMRL/WPAFB, the concept of robotics telepresence is being brought into practice with a variety of upper body exoskeletons used to control two, six degree-of-freedom (DOF) robots, and left/right hand exoskeletons (EXOS) used to

control sixteen DOF left/right robot hands (known as the UTAH/MIT Dexterous Robot Hands). My personal research interests have been .in the kinematics, dynamics and design of machinery and robots. With these skills I was chosen to assist the Robotics Group with the kinematic portion of telepresence control of the Dexterous Robot Hands.

II. OBJECTIVE OF THE RESEARCH EFFORT:

The basic goal of the research effort was to develop a computer algorithm for controlling the fingers of the Dexterous Robot Hand directly, with human-in-the-loop control. Of primary concern was mapping an operator's finger motions directly into the motion of the Dexterous Robot Hand's fingers. The available equipment was the Dexterous Robot Hand, an EXOS Handmaster (to be worn by the human operator) and an IBM AT connected to a VME bus with a Motorola 68000 cpu and a three bit shared memory window.

III. METHOD OF SOLUTION:

In mapping the operator's hand motion into the Dexterous Robot Hand's motion we concerned ourselves primarily with finger and thumb tip positions. As of this date, the mapping algorithm has been successfully implemented. In developing the mapping, the problem was divided into four steps.

Step 1:

The portion of the EXOS Handmaster mounted to a human's finger was modeled as a series of three, four-bar linkages, with one of the relative joint angles in each four-bar linkage representing the relative angular displacement of two segments of a finger. A reading obtained from the Hall effect sensor provides the input joint angle to each four-bar linkage model.

With this angle we then computed the relative angular displacement between two finger segments, which is represented by the output joint angle of the four-bar linkage model. The solution for this output joint angle was in closed-form.

In the case of the thumb, the EXOS Handmaster was modelled as a five-bar linkage serially connected to a four-bar linkage. Two sensor readings provide two angle inputs to the five-bar linkage. With these two angles we computed the relative angular displacement that occurs between the first two thumb segments, which were represented by two output joint angles of the five-bar linkage. The solution for these two output joint angles was also in closed-form.

Step 2:

We developed simple kinematic models of the human's fingers and thumb. Knowing the relative angular positions of the segments of the human's fingers and thumb (from the EXOS Handmaster) we used these kinematic models to compute positions of the human finger tips and thumb tip.

Step 3:

We developed the forward and inverse kinematic equations of motion for the fingers of the UTAH/MIT Dexterous Robot Hand. A portion of the inverse kinematic equations had a closed-form solution, while another portion required a numerical solution.

Step 4:

Finally, we developed the forward and inverse kinematic equations of the Dexterous Robot Hand's thumb. Again, a portion of the inverse kinematic equations had a closed-form solution, while another portion required a numerical solution.

Steps 1 and 2 were used to determine the human's finger and thumb tip positions. These were used as inputs to steps 3 and 4 in order to produce the same finger and thumb tip positions for the Dexterous Robot Hand. The kinematic control equations developed in Steps 1-4 are not presented in this report due to their length and complexity of derivation. They have been submitted to Lt. Ammon K. Wright (Biological Acoustics Branch of the AAMRL at WPAFB) in a publishable form, and will be organized by Lt. Wright into a technical paper for review and possible presentation at the 1990 IEEE Conference on Robotics and Automation (Cincinnati, OH.)

IV. RESULTS

The developed kinematic mapping was encoded into C, (a computer programming language) and then implemented into the controlling software of the Dexterous Robot Hand controller. Telepresence control of finger and thumb tip positions was achieved with considerable accuracy. By allowing for a size scaling factor, a reasonable posture emulation was also achieved. Without photographs, it is difficult to display our results. A visual demonstration of the telepresence control of the Dexterous Robot Hand will soon be developed at the Lab.

V. RECOMMENDATIONS AND FUTURE WORK:

a.) When the EXOS Handmaster is mounted to the human operator's hand, there are dimensions which must be measured and entered into the control algorithm. These dimensions depend upon the size of the operator's hand, and the way in which the EXOS Handmaster was mounted. Measuring these values can be a tedious and slow process. Although it is not critical that these dimen-

sions are exact, reasonably accurate values must be used for reasonably good performance. With repeated use, an operator will tend to mount the EXOS Handmaster in a similar fashion each time it is worn. For this reason, a computer data file can be constructed for each operator, and with every mounting of the EXOS Handmaster, these dimensions may be saved in the file. After many mountings, it will no longer be necessary to remeasure these dimensions. Instead an average value from the data file may be used.

b.) The algorithm provided to Lt. Wright will achieve an accurate duplication of finger tip and thumb tip positions between the operator and the Dexterous Robot Hand. Due to variations between the size of the operator's hand and the rather large size of the Dexterous Robot Hand, when finger tip positions are controlled, hand posture may not be well duplicated. For this reason a scaling factor (which indicates the relative size of the Dexterous Robot Hand to the operator's hand) of the finger tip positions may be included the algorithm.

c.) With the algorithm developed in this project, it is now possible for a human operator wearing the EXOS Handmaster to directly control the finger and thumb tips of the Dexterous Robot Hand. Thus it is possible to grasp various objects with the finger tips of the Dexterous Robot Hand. After the grasp has been achieved, the question remains whether the joint torques and grasping forces have values which correspond to a stable grasp, i.e can the object be lifted without falling out of the grasp of the Dexterous Robot Hand. For this we must study the static force balance of the Dexterous Robot Hand grasping the tool and optimize the grasping forces, in a fashion similar to that described by Kerr and Roth (1986). It is recommended as an additional project, that such a stable grasping algorithm be computerized and implemented.

d.) Authors such as Kerr and Roth (1986), Salisbury and Craig (1981), have studied finger tip grasps and manipulations. These are suitable for situations

requiring a high degree of manipulability (such as rolling a tool within the fingertips). However there are situations where manipulability is less important and a good "lock" on the tool is more important (such as a firm grasp on the handle of a hammer). These are referred to as "power grasps". Power grasps are necessary when doing heavy work. It is recommended that the kinematics and stability of power grasps be studied, and a computer algorithm be developed so as to allow the Dexterous Robot Hand to achieve stable power grasps in order that it be capable of performing significant work with more powerful tools.

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FINAL REPORT

ARTICULATED TOTAL BODY (ATB) VIEW PROGRAM WITH HARNESS-BELT IMPLEMENTATION

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ARTICULATED TOTAL BODY (ATB) VIEW PROGRAM WITH HARNESS-BELT IMPLEMENTATION

BY

Chi-Ming Tang

ABSTRACT

The Articulated Total Body (ATB) model is currently being used by the AAMRL to study the human body biomechanics in various dynamic environments. The VIEW program provides a graphical representation of the simulation output from the ATB model. The original graphics output of the VIEW program was developed to suit the early ATB model version, that is, the graphics output of the human body is represented by the ellipsoids without the harness belt attached. The new version of the VIEW program has the capability to depict harness belts as well as the body segments.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems must be mentioned for their concern and help to me in all administrative and directional aspects of this program.

My experience was rewarding and enriching because of many different influences. I would like to express my deepest appreciation to Annette Rizer for her valuable knowledge, suggestions, and support during the development of this research project. Finally, I wish to thank Dr. Ints Kaleps, chief of AAMRL/BBM, who provided me the research opportunity with his support and an enjoyable working environment during my research effort.

I. INTRODUCTION

The Articulated Total Body (ATB) model is currently being used by the Armstrong Aerospace Medical Research Laboratory (AAMRL) to study the human body biomechanics in various dynamic environments. The ATB model is a coupled rigid body dynamics computer simulation program which outputs the linear and angular position of each object at user defined time intervals. The output data is used by the VIEW graphics program to plot the projected image of the objects on the viewing plane. Over the course of several years, a number of changes and additions have been made to the ATB program, but the corresponding modifications, needed to show all of the ATB elements, have not been made to the VIEW program.

The basic graphics elements used in program VIEW are ellipsoids and polygons. These basic elements correspond to the ellipsoids and contact planes used in the ATB model program. The original configuration, however, is only able to depict an ellipsoid for each body segment without displaying, especially, any harness-belt system.

The concept of the harness-belt was introduced and added to the ATB model in 1975. Any implementation regarding the harness-belt system should be essential to complete the simulation. Therefore, the new feature developed for this research program shall have a capability to depict the harness-belt system for each simulation of the ATB model.

In compliance with these specific needs, a number of modifications and six additional subroutines have been made to the VIEW program. The details of this implementation will be discussed in section III and an example will be given in section IV.

II. OBJECTIVES AND APPROACH

The objective of the research effort was to modify the VIEW program to depict harness belts. The original VIEW program source code contains 1717 lines of FORTRAN code, consisting of a main program and 36 subroutines and functions (Ref. 4). A harness consists of from one to several belts (Figure 1). Each belt is defined as a set of straight line segments connecting reference points. The reference points, P_k, are selected from a prescribed set of points by an algorithm (Ref. 1).

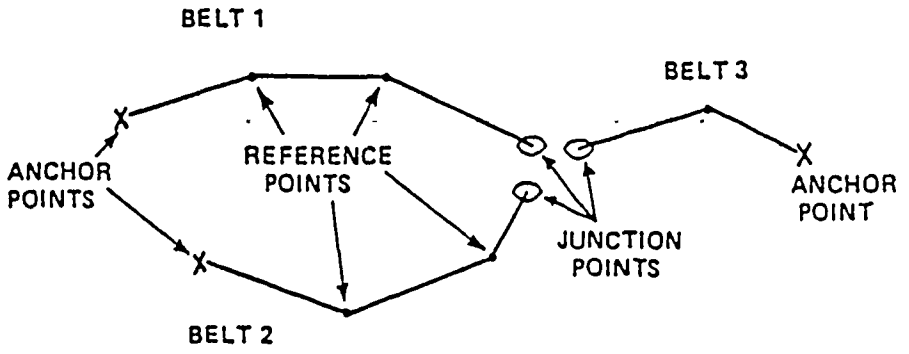


Figure 1 Belt Harness Model

The graphics algorithm of the harness belts can be divided into two portions for discussion. The first portion is the line segments between two consecutive reference points on the same ellipsoid. Each line segment will be depicted by the connection of a sequence of the surface points on the ellipsoid. The surface points are generated by the following procedure:

Based on the signs of the third coordinates of two reference points, use the first and second coordinates to set up a grid mesh (on the local ellipsoid reference system). For example, let the third coordinate be the z-coordinate and if the signs of the z-coordinates are the same, then create a grid square mesh in the xy-plane by using these two consecutive reference points as the

opposite diagonal corners of this grid square mesh. After the diagonal of this grid square mesh has been divided into equal NSTEP subintervals, the z values will be calculated by the ellipsoid equation at these ending points of the subintervals.

If it is not the case described above, that is, two z-coordinate values are different signs and two y-coordinate values are the same sign, then the grid square mesh will be in the xz-plane. If it is not the cases described above, then the grid square mesh will be in the yz-plane.

The second portion is the line segment between two different ellipsoids or two different segments. Each line segment is represented by a series of short vectors, even though one long vector could be plotted. That is, the connection of the tips of these short vectors will be the line segment between these two reference points.

In order to give the harness belts width the fact that the surface of an ellipsoid is a differentiable surface is used. Thus, the gradient vector exists at each point on the surface of the ellipsoid. Also, the gradient vector, if not zero vector, is perpendicular to the surface at that point. Referring to the Figure 2 below, let vector **DVEC** be the directional vector between two points P_1 and P_2 on the ellipsoid, the vector **GVEC** be the gradient vector of the ellipsoid at the point P_1, the vector **WVEC** be the width vector of the harness belt at the point P_1. If the points P_1 and P_2 are sufficiently close, these three vectors, **DVEC**, **GVEC**, and **WVEC**, are mutually perpendicular to each other, approximately. Therefore, the width of the harness belts can be calculated as follows:

(1) find the vector **WVEC** by using the cross product of two vectors **DVEC** and **GVEC**,

(2) find the unit vector in the direction WVEC.

The final width vector of the harness belt is the vector of length WFAC in the direction of **WVEC**. The real coefficient width factor WFAC is to be determined by the user.

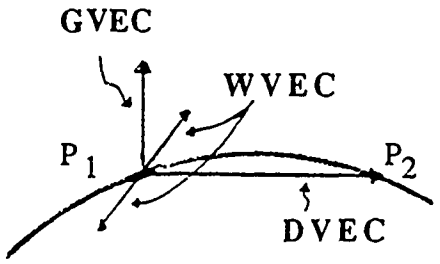


Figure 2 Geometric Figure among the Vectors **DVEC**, **GVEC**, and **WVEC**

III. MODIFICATIONS AND NEW SUBROUTINES

To fit the specific needs of the harness-belt system, a number of modifications and new subroutines have been made to the VIEW program. In order to distinguish from the original program code, these changes are labeled by the name "HARN" in columns 77-80 on each program code.

(A) Modifications:

(1) New Common Block: "HRNESS"

In order to implement the harness belt's option, a new common block named HRNESS was added to the related main program and subroutines such as: MAIN, BLDIEH, HARBLT, HARELP, HTRANS,

HWDTVA, HWDTVE, INPUT, AND PRJBLT. The descriptions of the variables in this new commom block HRNESS are as followed:

NHRNSS	--	Number of harness-belt systems.
NBLTPH(5)	--	Number of belts per harness.
NPTSPB(20)	--	Number of points per belt.
IBAR(5,100)	--	Array of indicators containing KS, KE, and others for each point.
BAR(15,100)	--	Coordinates of reference point in the local reference frame.
NL(2,100)	--	Pointers to the IBAR and the other array for each point in play.
NPTPLY(20)	--	Number of points in play per belt.
NSTEP	--	The number of steps between two reference points.
NTOBLT	--	The total number of belts in play.
WFAC	--	The scale factor for the width of the harness belts.

(2) Extended Dimension Declarations

The dimension of some variable arrays on the common blocks have been extended to fit the needs of the harness-belt system. Note that each belt occupies one dimension array, the maximum number of belts is 20. The following is a list of these changes in alphabetical order.

Variable Name	Original Dimension Declaration	New Dimension Declaration
A	(3,3,30)	(3,3,40)
CONVEC	(2,4,90)	(2,4,110)
D	(3,3,90)	(3,3,110)

ICOLOR	91	111
IE	(90,90)	(110,110)
NIE	90	110
NPPP	90	110
POS	(2,90)	(2,110)
SEGLP	(3,90)	(3,110)
SEGLPO	(3,90)	(3,110)
SIGN	90	110

Also, the dimension of the variable array D1 in the subroutine POLYD was extended from 810 to 990, that is, D1(990).

(3) Input Data File on Logical Unit Number 1:

This file is a data file generated by the ATB model simulation program. In order to input the information regarding the harness, the \subroutine named UNIT1 of the ATB program has been changed so that it can fulfill the need. The common block HRNESS is included in the common block section at the beginning of the subroutine code.

In the ATB program, the coordinates of each reference point on the harness belt are defined in the principal coordinate system. There is a difficulty to define the variable DPMI, the direction cosine matrix from the local reference frame to the principal reference frame, at an anchor point on the VIEW program. Thus, the subroutine UNIT1 shall include the procedure that changes each reference point on the harness into the local reference frame instead of the principal coordinate system.

The additional information passed to the data file stored on logical unit number 1 is:

NHRNSS, NBLTPH(5), NPTSPB(20), IBAR(5,100), BAR(15,100), NL(2,100), and NPTPLY(20).

The detailed information regarding these variables can be referred to in section "(1) New Common Block: HRNESS".

(4) Input Data file on Logical Unit Number 5:

This file is so called the input control file read from FORTRAN unit number 5. Two new record sets, RECORD 10.1 and RECORD 12.2 are included in the input control file of the new VIEW program. These records are described below:

(a) RECORD 10.1

Variable List : ICOLOR(92-111)
Format : (3(5X,I5))
Discussion : This three record set contains 20 intege numbers that define the colors for the belts. These colors are used for the multicolor plotter and the multicolor graphics terminal. The detailed description can be referred to in Reference 5.

Variable	Description
ICOLOR(111)	Color numbers for belts. Position 92 of ICOLOR represents the color number for belt1, position 93 represents belt 2, etc.

(b) RECORD 12.2

Variable List : NSTEP, IHA IN(2), WFAC
Format : 3I2,F10.2
Discussion : This record defines the variables used in the

harness.

Variable	Description
NSTEP	The number of plot vectors used in plotting the belts. Subroutine HARELP, the belt line generation routine, divides each belt in NSTEP pieces between any two consecutive reference points. The limitation is between 1 and 99, inclusive.
IHARN(2)	IHARN(1) = 0, draw the belt with width. IHARN(1) = 1, draw the belt without width. IHARN(2) = 0, draw the belt with hidden algorithm. IHARN(2) = 1, draw the belt without hidden algorithm.
WFAC	The width scale factor for the belt.

(5) Subroutine BLDIEH

The subroutine BUILDIE has been modified to fit the needs for harness-belt. Also, the name is changed to BLDIEH for the purpose of distinguishing from the previous name. However, the basic principle to build the IE and NIE arrays is the same as before. Before, the algorithm checked the segments (ellipsoids) and the polygons. Now, the subroutine will also check with the belts. The principle is that the belt would not hide any part of the body, but the body might hide a part of the belt. Also, the belts would not hide each other.

(6) Subroutine HYDE

The original purpose of HYDE is to determine if a point is hidden by an ellipsoid. Referring to the Appendix A, "Hidden Line Problem Between Two Ellipsoids", of the Reference 4, we can claim that if the magnitude of the vector **M** is close to zero, the points on each ellipsoid are very close together, and it is not necessary to hide the point on the surface of the ellipsoid with respect to the ellipsoid itself. The tolerance value for **P** • **M** is 10^{-9} for the case N = IELP. If a part of the belt is on the surface of an ellipsoid and this region of the surface is not blocked by the ellipsoid itself, the other ellipsoid, or any polygon, this part of the belt is displayed. The new tolerance value for **P** • **M** is 10^{-3} instead of 10^{-9}. That is, if **P** • **M** < 10^{-3}, then the point of the belt is not hidden.

(B) New Subroutines

There are six new subroutines being attached to the original VIEW program. Their names are called HARBLT, HARELP, HTRANS, HWDTVA, HWDTVE, and PRJBLT. The following is the description of the purpose of each subroutine.

(1) Subroutine HARBLT

This subroutine is called by the MAIN program to set up arrays containing belt data for the subroutine PNTPLT to plot.

(2) Subroutine HARELP

The purpose of HARELP is to generate the XYZC, XYZL, and XYZR

arrays of the belt points between two reference points. These points are used by subroutine HARBLT to plot the belt.

(3) Subroutine HTRANS

The purpose of HTRANS is to transform the input vector **R** in the local reference frame into the inertial reference frame vector **P**.

(4) Subrcutine HWDTVA

The purpose of HWDTVA is to find the width vector **WVEC** at point **p2** for the case of two different segments or two different ellipsoids. The point **p2** is assumed to be a point on the ellipsoid of the segment number KS with semiaxes coefficients array **AA**.

(5) Subroutine HWDTVE

The purpose of HWDTVE is to find the width vector **WVEC** of the harness belt at the point **P1**. The point **P1** is on the ellipsoid that has the semiaxes coefficients array **AA**.

(6) Subroutine PRJBLT

PRJBLT is called by the MAIN program to project the harness belts onto the projection plane.

IV. SAMPLE INPUT AND OUTPUT

This section provides an example of the visual data display of an ATB model simulation. The output of the simulation from ATB program consists of two harness belts. Its input control file and output graphics file plot show as follows.

(A) Sample Input Control File

The following table is an example of the input control file. This file consists of the information on the following records:

Record Number	Variable List
1.0	DEVFLG
2.0	NFRME
3.0	ID(1-10)
4.0	STIME, DTIME, ETIME
5.0	IDEBUG(1-4)
6.0	NFAST, NPREM, NISG
6.1	IREMOV(1)
7.0	NSP
8.0	ICOLOR(1-30)
9.0	ICOLOR(31-90)
10.0	ICOLOR(91)
10.1	ICOLOR(92-111)
11.0	NSTEPS(1-17)
12.0	NSTEPS(31-46)
12.2	NSTEP, IHARN(1-2), WFAC
13.0	INT, SFACTR
14.0	OFSETX, OFSETY
15.0	VP(1-3), RA(1-3), IVP, ICODE
16.0	XMIN, XMAX

For further information on these variables, see Reference 5. The corresponding data information is given below Table 1.

Table 1 Sample Input Control File

```
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890
1
 0
(blank line)
0.0000   0.0100  0.0000
1111
 0 116
 7
 0
         2        2        2        2        2        2        2        2
         2        2        2        2        2        2        2        2
         2        2        2        2        2        2        2        2
         2        2        2        2        2        2        1        1
         1        1        3        1        1        3        3        1
         1        3        3        1        1        1        1        1
         1        1        1        1        1        1        1        1
         1        1        1        1        1        1        1        1
         1        1        1        1        1        1        1        1
         1        1        1        1        1        1        1        1
         1        1
         4
         2        2        2        2        2        2        2        2
         2        2        2        2        2        2        2        2
         2        2        2        2
 8 5 8 3 5 5 3 3 5 3 3 3 3 3 31010
20202020202020202020202020202020202020202020202020202020
20 1 0       0.50
  4         110.0
       5.5        5.5
    1000.0   1000.0     -20.0       10.0        0.0        0.0         16          2
      0.00      11.00
```

(B) Sample Output Graphics File Plot

The graphics outputs of the Table 1 are shown on Figures 3-5 at the viewpoints which are located at (1000.0, 1000.0, -20.0), (1000.0, -1000.0, -20.0), and (1000.0, 10.0, -20.0), respectively, in the IVP segment number coorinate system.

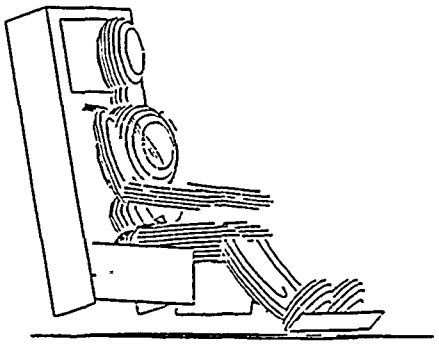


Figure 3 Sample Graphics with Viewpoint (1000, 1000, -20).

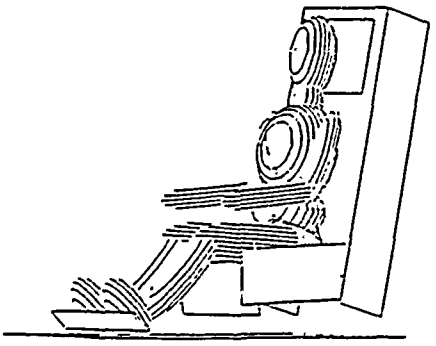


Figure 4 Sample Graphics with Viewpoint (1000, -1000, -20)

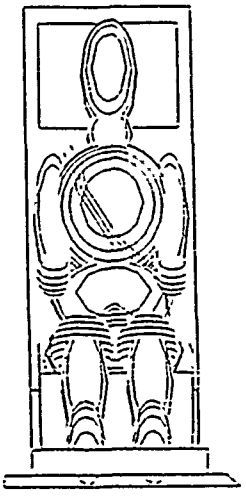


Figure 5 Sample Graphics with Viewpoint (1000, 10, -20)

V. RECOMMENDATIONS

The revised VIEW program with harness option generates a nice visual data display for the users of the ATB model program. Still, the program has not yet reached its final development nor fulfilled its complete potiential. The "finishing touches" should address the following implementations.

(A) If the line segment between the anchor point and the first reference point penetrates the reference ellipsoid, we shall find the supplement point on the ellipsoid such that the line segment between the anchor point and the supplement point will be tangent to the ellipsoid. Otherwise, a partial part of the belt will not display. The reason is that these points of this part are not the surface point of the ellipsoid. When applying the hidden algorithm, these points will be hidden and so they will not be plotted.

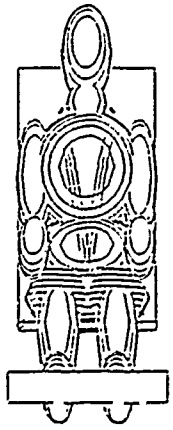


Figure 6 Example of Five Harness Belts

Figure 6 above is an example which shows this symptom. This example consists of five harness belts. The parts of the belts between the anchor points (behind the neck of the body) and the reference points on the upper corners of

segment number 3 are not plotted. One possible algorithm can be developed as follows. Let the supplement point described above refer to a tangent point. The tangent point may be found by the following formula:

Minimize

$$D = (x - x_2)^2 + (y - y_2)^2 + (z - z_2)^2$$

subject to the constraints

$$(x_1)(x)/a^2 + (x_2)(x)/b^2 + (x_3)(x)/c^2 = 1$$

$$(x/a)^2 + (y/b)^2 + (z/c)^2 = 1$$

where

the point (x_1, y_1, z_1) is the anchor point,

the point (x_2, y_2, z_2) is the first reference point on the ellipsoid in play,

The point (x, y, z) is the required tangent point.

However, the analytical solution of the minimization of equation D with two constraints is very difficult to solve because one of the constraints is nonlinear. Thus, the numerical solution shall be introduced here.

(B) Over the past several years, a number of changes and additions have been made to the ATB program. Some of these changes are discussed below.

(1) The latest ATB model allows additional ellipsoids for each segment instead of only allowing one ellipsoid per each segment. Modifications of the VIEW and ATB programs to show additional ellipsoids as defined by the ATB program are essential and important for the simulation. Figure 7 below is a good example to show the drawback of the old VIEW program. This example is the same as the input data files described on the previous section IV

except the viewpoint is located at (1000, 10, -20) instead of (10, 1000, -20). The number 1 segment contains two ellipsoids, number 1 and number 23. However, the VIEW program does not have the capability to draw the second ellipsoid on the same segment, therefore, the part of the harness belt is misplotted on the top portion of the number 1 ellipsoid.

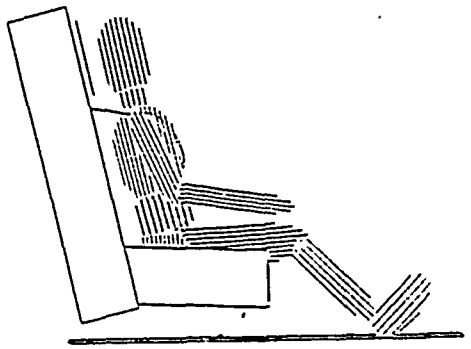


Figure 7 Sample Graphics Output with Viewpoint (10, 1000, -20)

(2) In order to improve the modeling of corners and other geometries the option to use hyperellipsoids as contact surfaces rather than standard ellipsoids was added to the ATB model. This option was originally developed for General Motors Corporation and has been incorporated into the ATB model with their permission. Again, the VIEW program does not have a capability to depict this graphics option.

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FINAL REPORT

INSIGHTS INTO HUMAN FACTORS IN AVIATION WITH EMPHASIS ON NON-CANONICAL FLOW FIELDS

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INSIGHTS INTO HUMAN FACTORS IN AVIATION WITH EMPHASIS ON NON-CANONICAL FLOW FIELDS

by

Ebo Tei, Ph.D.

ABSTRACT

In accordance with the summer research goals and objectives that were arrived at, research effort during the 10-week period was concentrated in two areas: (a) developing a broader perspective of the various research activities pertaining to human factors in aviation that were of interest to the US Air Force; (b) designing a preliminary experiment to explore the perceptual factors that may operate during low-altitude flights in non-rigid environments. Both these objectives were achieved.

ACKNOWLEDGMENTS

I wish to thank the Air Force Systems Command, the Air Force Office of Scientific Research, and the Armstrong Aerospace Medical Research Laboratory for sponsoring this summer research program. Appreciation should also be extended to Universal Energy Systems for their help during the summer pre-visit and for administratively making the whole program possible.

I wish to personally thank the following: Dr. Rik Warren, my technical focal point, for his friendship, patience, and understanding in guiding me through solutions of the problems I encountered, and for helping me become familiar with the field of Aviation Psychology; Dr. Don Topmiller for his friendship and for sharing his vast experience in the field of human factors with me, and also making his library resources available to me; Bob Todd for his kindness and putting up with me while developing the computer program; Lawrence Wolpert, Kim Reardon, and Willie Hallford for making this a memorable summer; and finally, Dr. Mike Vidulich and Captain M. Lee Fracker for taking time out to share their research ideas and activities with me. To all of you, your help was greatly appreciated.

I. INTRODUCTION:

Interest in this AFOSR summer faculty research program stemmed from the recent effort of the Department of Defense (DOD) to encourage Historically Black Universities and Colleges (HBCUs) to participate in research efforts pertaining to defense. Traditionally, HBCUs have not participated in such research activities. However, in the past year, the DOD started a program of minority set-asides. This involved a pool of money that HBCUs could compete for through well-developed, defense-related research proposals. The major problem was developing a proposal that was meaningful and technically sound to DOD.

My background is Experimental Psychology with specialization in Perception, Human Performance, and Information Processing. My teaching and research experience have dealt with human factors issues. I knew the Air Force was interested in human factors in aviation, yet when I sat down to develop a proposal in response to the DOD program mentioned above, I realized that I did not have any knowledge about which specific human factors problems were of interest to the Air Force. I also did not have any background in doing research specifically related to aviation. Needless to say it was going to be very difficult or impossible to develop a meaningful proposal.

It was just about this time that I received the brochure on the AFOSR summer faculty research program. It became immediately apparent that I should take advantage of the opportunities offered by the program before attempting to develop any DOD proposals. Subsequent investigations

revealed that the Human Engineering Division of the Armstrong Aerospace Medical Research Laboratory (AAMRL) offered the best environment to achieve the necessary background that I needed. My summer experience has confirmed this. AAMRL/HEF is a researcher's paradise. Besides working with my technical focal point on visual perceptual factors in low altitude flights, I have had the opportunity to interact with those working in the areas of workload and situation awareness.

II. OBJECTIVES OF THE RESEARCH EFFORT

In consultation with the technical focal point, three goals for the summer were arrived at. Given my lack of background in the aviation psychology area, the first two goals were designed to broaden my perspective in topics dealing with human factors in aviation, especially as they relate to low altitude flights. The main objectives were:

1. To acquaint myself as much as possible with the various research activities going on in the Human Engineering Division of the Armstrong Aerospace Medical Research Laboratory.
2. Review seminal books , published articles, and technical reports dealing with both the role of perceptual variables in low altitude flight and those dealing with human factors in general.
3. Design an experiment to explore the perceptual factors that may operate during low-altitude flights in non-rigid environments--that is, environments involving non-canonical flow fields.

III. REALIZING OBJECTIVES AND THEIR RESULTS

a. First objective

To achive the first objective, effort was centered on the following activities:

1. Tours of the various human factors research labiratories within the Division to gain insight into the kinds of research activties going on in the labs.
2. Visit and engage in technical dicussions with the other scientists on their ongoing research activities.
3. Attend lectures/seminars dealing with research activities and preliminary findigs on ongoing research within the branch.
4. When feasible, arrange to attend meetings of working committees dealing with technical and research related matters.

All of these activities were very successfully carried out. Research labs that could be toured with no restrictions or not requiring clearance were visited. Other scientists were very willing to share their ideas and their current research activities in the technical discussions that I had with them. Several lectures were attended including those on the preliminary studies done with the data glove. Committee meetings attended included technical discussions on the Pilot Associates' Program and Cockpit Automation Technology Program. As a result of these activities, a greater insight has been gained and a broader perspective has been developed concerning the various human factors problems that are of interest to the

Air Force. This new insight will definately play a major role in the future development of proposals to be submitted to DOD.

b. Second objective.

To achieve the second objective a reading list was developed in consultation with the technical focal point.When possible, library searches were done to track down books or journals. Several seminal books, technical reports,and journal articles were read and discussed with the technical focal point. Most of these readings dealt with human factors in low altitude flights. In addition, I critically reviewed articles on egomotion and motion perception that had been submitted for publication in journals and a book on egomotion. The books, journal articles, and the technical reports (especially those coming out of the Ohio State University Aviation Psychology lab) have further enlightened me on the field of aviation psychology. This experience has definately enriched my background in this area.

c. Third objective.

To achieve the third objective, a preliminary experiment was designed to explore the perceptual factors that may operate during low-altitude flights in non-rigid environments.

Rationale.

The rationale of the experiment stems from the observation that sometimes pilots may fly missions over non-rigid terrains. For examle, search and

rescue missions by helicopter pilots on the sea. The waves provide a non-rigid environment. Another instance may be a helicopter flying over a wheatfield. The downwash of the rotating blades create a non-rigid environment. The flow fields in these non-rigid environments are here referred to as "non-canonical" flow fields as opposed to those in rigid, stable environments. The question of interest was discovering the perceptual factors or variables that operate under conditions of non-canonical flow fields, and the extent to which these variables lead to the detection of changes in altitude without flying into the sea or ground.

Simulation

To simulate a non-rigid environment (like the dip in a wheatfield from the downwash of a helicopter or the troughs of adjacent waves) an equation with simple mathematical properties was used. The equation was based on the 'witch of agnesi', which in symbol form, is:

$$z = \frac{a^3}{a^2 + y^2 + x^2}$$

The equation generates a surface with a bump, where

a = size of the bump

and uses the following directions/dimensions:

x : forward

y : lateral

z : height

On a flat ground, z = 0. When "a" is a negative number however, the equation

generates a dimple on the ground. In this experiment, "a" assumed a negative value.

For purposes of this preliminary experiment, two categories of displays were generated on a Silicon Graphics IRIS 4-D computer. In one category, the ground surface over which the subject travelled had a checker-board texture, and the other category had wire-form polygons with no texture. For each display in either category, there were three motion combinations that could be generated:

a. The "self" travelling over a stationary dimple--"self-motion-only"
b. The dimple travelling with the "self" stationary--"dimple-motion-only"
c. Both the "self" and "dimple" travelling--"co-motion."

For "self" and "dimple" motions, the motion could either be forward or backward. But for "co-motion," both the "self" and the "dimple" travelled in the same direction--both moving forward or backward at the same time. A fourth type of motion in which the self and the dimple travelled in opposite directions was not incorporated into the design because of some complexities of relative motion which have not been resolved yet. Table 1 shows the different types of motions.

Design

The proposed experiment was a 6 x 3 x 2 within-subject design, with 6 motion combinations, 3 levels of exposure duration, and 2 levels of display-type (checkered surface vs. wire frame). Each condition in Table 1 had an exposure duration of 1, 2, or 3 seconds. Out of the six possible motion combinations in the table, only the first two involving self-motion were

designated as displays that portrayed a "rigid" surface , the other four as

Table 1

Self-motion and Dimple-motion Combinations

	Self	Dimple	Motion	Surface
1.	+	0	self-motion	rigid
2.	-	0	self-motion	rigid
3.	0	+	dimple-motion	non-rigid
4.	0	-	dimple-motion	non-rigid
5.	+	+	co-motion	non-rigid
6.	-	-	co-motion	non-rigid

+ = forward motion

- = backward motion

0 = stationary

"non-rigid." Whenever there was dimple movement, the display was designated as portraying a "non-rigid" surface. To counteract this imbalance, the number of instances involving "rigid" surfaces was doubled. So there was a total of 24 conditions altogether: 4 involving "rigid" environment (with 3 different exposure durations for each), and 4 involving "non-rigid"

enviroment (with 3 different exposure durations for each). These 24 conditions were randomly sequenced with the constraint that no two successive trials were made up of conditions from the same category of motion. For example, if trial 10 involved "forward" self-motion at 3-sec exposure duration, then trial 11 could not also be "forward" self-motion with 1 or 2-sec exposure duration. However, trial 11 could either be "backward" self-motion at any of the three exposure durations, or any of the other conditions involving dimple movement. There were four such random sequences created to form one order of presentation for a total of 96 trials. Two such orders of presentation were created. Each order of 96 trials formed an experimental session that lasted about 12 minutes.

Procedure

On each trial of the experiment, the task of the subject was simply to view the display and then indicate whether a particular display depicted a "rigid" or "non-rigid" surface. The subject's judgment was indicated by pushing one of two mouse buttons marked R for "rigid" and NR for "non-rigid". On each trial, the display stayed on for a designated exposure duration. At the end of the presentation, the subject was prompted by the computer to indicate which surface was presented. The subject had 5 seconds wihin which to respond, so speed of response was not of the essence. Following the response, the subject was again prompted by the computer to give a rating of confidence in the response using a three-point scale. The subject was required to push one of three butons also clearly marked 1, 2, and 3. The subject had 5 seconds to do this. Immediately after giving both the

response and the confidence rating, the computer prompted the subject to get ready for the next trial.

It was hypothesized that subjects should generally tend to see "rigid" surfaces when display involved only self-motion, and should generally see the "non-rigid" surfaces when there was dimple movement.

Results

While a fully complete design experiment is in place, a full study could not be run as a result of lack of time. A complete simulated run through of the experiment was done to determine whether the computer program worked the way it was designed to. The last two weeks of the summer research period were spent in debugging the program, setting the parameters of the various variables, and testing the various displays to see whether they achieved their effect.

IV. RECOMMENDATIONS

The experiment described above was designed with a Silicon Graphics IRIS 4-D computer. Because there are no such facilities im my home institution, the technical focal point and I have agreed to collaborate to run the initial experiments and all its later phases here at AAMRL/HEF. Non-canonical flow fields are of major theoretical interest to the technical focal point. So while no direct recommendations of the applications of the experiment can be made at this time, it is hoped that findings of future studies to be made could be applied to simulator design and also incorporated into training schedules.

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FINAL REPORT

EFFECTS OF DATA ERROR ON PROBLEM-SOLVING HEURISTICS

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EFFECTS OF DATA ERROR ON PROBLEM-SOLVING HEURISTICS

by

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and

David R. Harper

ABSTRACT

The effects of two levels of system failure on scientists' and pilots' problem-solving heuristics using the Wason 2-4-6 rule induction task were assessed. Results indicated that most subjects preferred to test their hypotheses by examining evidence which would confirm their ideas. Subjects given system failure conditions were less likely to solve the task and used significantly more tests and test replications. Furthermore, the heuristics used to solve the task in the current study were very similar to those used in earlier studies which had utilized undergraduate subject pools, demonstrating that advanced education and scientific experience does not necessarily change problem-solving styles.

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We are especially grateful to Mr. Gary B. Reid, research sponsor, for providing his inspirational and technical support. Without the cooperation of Dr. Mike Vidulich, Mr. Mark Crabtree, Ms. Sharon Dorsey, Mr. Raymundo Marcelo, and Mr. Mike Ewry we wouldn't have been able to begin data collection as quickly as we did. Furthermore, we truly appreciated the assistance of all the AAMRL employees who volunteered as subjects, showed interest in the research, and provided valuable input for future research objectives.

I. INTRODUCTION

Little is known about how knowledge of the possibility of the random occurrence of system failure affects an operator's problem-solving processes. If a pilot is aware that an oil pressure warning light has malfunctioned occasionally, indicating a problem when none actually existed (a false positive reading), what effect might that knowledge have on evaluation of the current engine condition? Would the pilot be more or less likely to check other instruments which could rule out the possibility of an engine failure? On the other hand, if the oil pressure warning light did not always come on when there actually was a problem (a false negative reading), would this knowledge change the pilot's evaluative procedures?

AAMRL at Wright-Patterson Air Force Base is concerned with decision processess under high workload conditions. Special attention is directed towards cognitive functioning during stressed conditions. Therefore, information about the problem-solving heuristics and biases employed by pilots and scientists working under system failure conditions is of interest.

Our research interests have been in the areas of problem-solving and decision-making. Dr. Walker's work investigating confirmation bias under system failure and Mr. Harper's work investigating decision processess under uncertainty

contributed to our assignment to the AAMRL/HEG.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Most experimental tasks designed to study problem-solving processes, such as how hypotheses are discovered and tested (i.e., Wason, 1960; Mynatt, Doherty & Tweney, 1977, 1978; Tweney et al., 1980), provide subjects with an ideal, error-free testing environment. In reality, completely error-free data are rarely available. Yet, theoretical and practical inferences are routinely made based on data which contain varying degrees of error. One type of data error, system failure, "occurs when an indicator fails unpredictably, but gives no sign of its malfunction" (Doherty & Sullivan, 1986, p. 4). Data which are a function of random system failure can contain false positive and/or false negative observations. How system failure affects hypothesis-testing heuristics is, therefore, an important factor in extending our knowledge of problem-solving processes from the experimental laboratory to more realistic situations.

Our assignment as participants in the 1989 Summer Faculty/Graduate Student Research Program (SFRP/GSRP) was to conduct a pilot study to investigate how subjects test hypotheses and make decisions under system failure conditions. The research design was based in part on the results of previous studies of confirmation bias and system failure conducted by Dr. Walker at Bowling Green State University, Bowling Green, OH. Furthermore, since most problem-solving research has been conducted using undergraduate university

students, it was decided that using active researchers would provide an alternative subject population which might be expected to differ in problem-solving skills.

II. PILOT STUDY:

a. According to Popper (1959), rational scientific inquiry should involve active attempts to gather evidence which might disconfirm or rule-out, rather than confirm or support, proposed hypotheses. The accumulation of supporting evidence can never insure that a hypothesis is correct, but a hypothesis can be eliminated by one instance of disconfirmatory evidence. For instance, consider the hypothesis, "All pilots are male". If 100 pilot records were checked and all were found to be male, the evidence would support, but not prove, the hypothesis. If one female pilot's record were found, the evidence would disprove the hypothesis.

While disconfirmation might be the logically correct method for assessing hypotheses, many studies of hypothesis-testing using error-free data have shown that most subjects prefer to examine evidence which was predicted by the hypothesis to occur, a "positive test (+test) strategy" (Klayman and Ha, 1987, p. 213). The +test strategy is most likely to confirm or provide supporting evidence for the subject's working hypothesis (Wason, 1960; Mynatt, Doherty & Tweney, 1977, 1978; Tweney, et al., 1980). Examining evidence which is not predicted by the hypothesis to occur (a negative test (-test) strategy) is most likely to disconfirm or rule-out

the subject's working hypothesis. It has also been shown that the +test strategy is a useful heuristic for both hypothesis development and establishing data reliability before attempting disconfirmation (Mitroff, 1974; Mynatt, Doherty & Tweney, 1977, 1978; Tweney, Doherty & Mynatt, 1981; Klayman & Ha, 1984; Tweney, 1985). Thus, both confirmatory and disconfirmatory strategies are appropriate heuristics for developing and testing hypotheses.

Three recent studies attempted to explore the effects of system failure on the use and effectiveness of positive and negative test strategies in hypothesis-testing situations. In one study, Kern (1982) compared the effects of both system failure and measurement error on hypothesis testing in an "artificial universe" study. (Measurement error is a partial component of input data or feedback, e.g. miscalibration or misreading of a measuring instrument, affecting the reliablility of the results.) Subjects were asked to discover the location of a north/south boundary line dividing an unexplored planet by dropping imaginary creatures from a space ship onto the planet's surface. On one side of the boundary line the creatures would live and on the other side they would die. Information on whether each dropped creature lived or died was sent back to the subject and contained either no error, measurement error in the predictor data (the location of the drop was not precise), system failure (20% of the survival feedback was wrong), or both measurement and system failure. Subjects given system

failure could check the survival feedback on a limited basis to see if the data were correct. The results of Kern's study demonstrated that subjects given measurement error behaved similarly to subjects given no error. Subjects given system failure were reluctant to change their hypotheses when disconfirmed by the data and more likely to replicate only disconfirming trials.

Gorman (1986) used a group problem-solving task based on a card game called "Eleusis" to assess how warning subjects that system failure might occur affected hypothesis testing. The Eleusis task involved having subjects play individual cards to discover sequencing rules about card order, such as 'Alternating red and black'. Groups of four subjects were assigned to one of three types of strategy instructions: confirmatory (emphasizing +tests), disconfirmatory (emphasizing -tests), or no strategy. In an earlier study using Eleusis, Gorman et al. (1984) had demonstrated that groups shown how to disconfirm their hypotheses using a -test strategy performed significantly better than groups either shown how to confirm hypotheses using a +test strategy or given no strategy instructions. All groups were told that "On anywhere from 0 to 20 percent of the trials, the feedback you receive will be inaccurate" (Gorman, 1986, p. 89), though none of the feedback actually contained error. Gorman (1986) found that knowing that the data might contain error severely disrupted performance on the task, even for subjects shown the -test strategy. All groups

Subjects were asked to state the rule when they felt sure they knew what it was, based on the outcomes of their tests. If the first rule announcement was wrong, they could continue to make more tests and rule announcements. Walker found that subjects in both system failure conditions (informed and uninformed) used a significantly higher number of tests and had substantially decreased solving rates compared to subjects in the no error conditions. This finding indicated that feedback error seriously disrupted task performance. Walker also found that only subjects who had been informed that system failure might occur and who received actual error repeated tests. Thus, hypothesis-testing heuristics were only affected by the test results and not by the knowledge of a potential system failure.

While both Kern and Gorman found that subjects who knew about the presence or possibility of system failure tended to ignore disconfirming data and/or preferred to replicate only disconfirming trials, several methodological difficulties were present which could have affected interpretation of the results. Kern did not distinguish between the psychological effects on hypothesis testing of knowing that system failure might occur and the effects of actual system failure. Gorman focused on group problem-solving, used a possible (0-20%) rather than an absolute system failure condition, did not include an actual system failure condition and did not have a no-error instructional condition. Walker allowed subjects to query the experimenter as many

spent a significant amount of time replicating tests to check for error. In addition, subjects appeared to use the knowledge of error possibility to classify potentially useful disconfirmatory evidence as error.

Based in part on the results of the Kern and Gorman studies, Walker (1987) compared the effects on hypothesis-testing heuristics of informing or not informing subjects that system failure might occur under actual system failure and no error conditions. A computerized version of the Wason (1960) 2-4-6 rule discovery problem was implemented for the study. Various versions of the task (using error-free feedback conditions) had frequently been used to evaluate the roles of positive (potentially confirmatory) and negative (potentially disconfirmatory) test strategies involved in hypothesis testing under various instructional conditions (Tweney et al., 1980; Walker & Tweney, 1983; Walker, 1985, 1986; Gorman & Gorman, 1984; Klayman & Ha, 1985; Tukey, 1986). The Wason task involves having subjects attempt to discover the general number-sequencing rule, "three ascending numbers," when given the sequence, "2-4-6", as a positive instance of the rule. Subjects test their ideas about the rule by trying additional sequences to discover what fits and does not fit the general rule.

Under the system failure conditions in the Walker (1987) study, subjects were either informed or not informed that feedback about their tests might not always be reliable.

times as they wished concerning their rule guesses. This method allowed subjects an opportunity to eliminate possible hypotheses by announcing rules rather than by conducting disconfirming tests. The Walker study used a constant system failure rate of 20%, which may have been too high to allow subjects to develop a well-confirmed hypothesis before beginning disconfirmation. Furthermore, the results of the Walker, Gorman, and Kern studies were based on undergraduate subject samples. As previously noted, this type of subject population might not have been indicative of how experienced observers would handle the possiblity of system failure.

b. One purpose of the current study was to compare the Walker (1987) system failure rate of 20% to a decreased failure rate of 10% and to explore the possibility that a hypothesis must be well-confirmed before an error-detecting heuristic, such as test replication, is employed. A second purpose of the study was to limit subjects to only one rule announcement to insure that they depended on the test results for hypothesis disconfirmation. A third purpose of the current study was to explore the possibility that active researchers who are familiar with data unreliability would have developed appropriate heuristics for dealing with such a problem.

c. Subjects. Thirty subjects (AAMRL/HEG employee volunteers) participated in the study. The sample included ten Ph.D.'s, nine M.S. and M.A.'s., and eight B.S. and B.A.'s. Five subjects were between 20 and 30 years of age; 16 be-

tween 31 and 40 years of age; five between 41 and 50 years of age; and four between 51 and 60 years of age. There were 24 males and 6 females. All subjects were actively involved in research at the time of the experiment. Areas of research interests included biology; physics; chemistry; anthropology; experimental and human factors psychology; and biomedical, industrial, systems, and electrical engineering.

Procedure. The experiment compared two levels of system failure (10% and 20%) to a no error condition. Ten subjects were randomly assigned to each of the following separate groups: (1) No Error; (2) 10% Error; and (3) 20% Error. For all groups, rule testing was accomplished by a computer entry of three-digits (e.g., 1, 3, 5) which the computer compared to a general number-sequencing rule, three ascending numbers. For the two Error conditions (10% and 20%), the programs also included a subroutine which was randomly activated for either 10% or 20% of the data entries. The subroutine reversed the outcome of a data entry so that a sequence that actually fit the "ascending numbers" rule was responded to as not fitting and vice-versa.

All subjects completed a demographic questionnaire and read the task instructions before starting keyboard entry of their numeric tests. The instructions included the sequence "2, 4, 6" as an example that fit the rule and for the error conditions, a highlighted warning about the possibility of incorrect computer responses.

After each three-digit keyboard entry, the subject was instructed to indicate whether he or she thought the test would fit the rule. For instance, if a subject's first hypothesis was "Even numbers" and the sequence "8, 10, 12" was entered, the screen displayed the question: "Do you think 8, 10, 12 will fit the rule?" After indicating what he or she expected the test outcome to be, the actual test outcome was displayed as: (1) "That sequence fits the rule" or (2) "That sequence does not fit the rule".

When a subject was ready to announce the rule, he or she summoned the experimenter, who told them whether or not the rule announcement was correct and debriefed them concerning the research objectives.

d. Results. Six out of ten (60.0%) subjects in the No Error condition were able to solve the task, compared to three out of ten (30.0%) subjects in the 10% error condition and two out of ten (20.0%) subjects in the 20% error condition. The difference in solving rates between the no error and error conditions was significant ((1, N = 30) = 4.8, $p<.05$). The mean number of tests conducted by subjects in the No Error condition was 13.7, compared to 33.7 trials for the 10% Error condition and 35.0 for the 20% Error condition. The difference in the number of tests conducted between the no error and error conditions was also significant ($t = -2.891$, $p<.01$).

Three measures were used to indicate problem solving heuris-

tics -- test repetition, test result expectations, and confirmation / disconfirmation. For all conditions, the outcome of each non-error trial was categorized as confirmatory, disconfirmatory, or unclassifiable by comparing what test outcome the subject expected ("Yes", "No", or "Unsure") to the actual outcome. A trial outcome was categorized as confirmatory if the subject's expected outcome matched the actual outcome, disconfirmatory if the expected outcome did not match the actual outcome, and unclassifiable if the expected outcome was "Unsure".

The mean number of repeated tests in the no error condition (0.6) was lower than the two error conditions, 10.8 and 16.7 respectively. The difference between the no error and error conditions was significant ($t = -2.657$, $p = .015$). There was also a significant effect of condition on the number of individuals who repeated tests in each condition ((2, N = 30) = 7.2, $p = .02$). Only 20.0% of the No Error subjects repeated tests, compared to 50.0% of the 10% Error subjects and 80.0% of the 20% Error subjects.

An analysis of variance among the three conditions indicated no significant difference in the percentages of total trials of subjects' "Yes" responses ($F(2,27) = .5927$, NS). Subjects in the No Error condition indicated that they expected 45.5% of the total trials to fit the rule. Subjects in the 10% Error condition expected 55.7% to fit and subjects in the 20% Error condition expected 41.6% of the trials to fit.

The percentages of "No" responses among the three conditions were also not significantly different ($F(2,27) = .525$, NS). Subjects in the No Error condition indicated that they did not expect 21.5% of the total trials to fit the rule. Subjects in the 10% Error condition did not expect 13.7% to fit and subjects in the 20% Error condition did not expect 19.9% of the trials to fit. Similarly, the percentages of "Unsure" responses among the conditions were not significantly different ($F(2,27) = .113$, NS). Subjects in the No Error condition were unsure of 33.0% of the trials. Subjects in the 10% Error condition were unsure of 30.6% of the trials and subjects in the 20% Error condition were unsure of 37.8% of the trials.

The mean percentages of total test outcomes categorized as confirmatory were compared across the three conditions. The mean percentage of confirmatory trials in the No Error condition was 52.4% as compared to 35.0% of the trials in the 10% Error condition and 51.4% of the trials in the 20% Error condition. An analysis of variance indicated no significant difference in the mean percentages of confirmatory trials among the conditions ($F(3,27) = 1.252$, NS). The mean percentage of disconfirmatory trials in the No Error condition (14.6%) was similar to the mean percentages of disconfirmatory trials for the 10% Error condition (20.3%) and the 20% Error condition (26.8%). An analysis of variance indicated no significant difference in the mean percentages of disconfirmatory trials among the three

conditions (F (2,27) = 1.707, NS).

e. Discussion. Results of the experiment were very similar to those reported by Walker (1987) in which task performance was seriously disrupted by system failure. The percentage (20.0%) of solvers in the 20% Error condition was only slightly greater than the percentage (15.0%) of solvers in Walker's error condition. The difference between the mean number of trials for the No Error (13.7) and the 20% Error (35) conditions was also similar to the Walker findings, in which subjects in the no error condition used an average of 14.5 trials compared to 28.85 trials in the error condition. As in the 1987 study, only 20.0% of the no error subjects in the current study repeated tests compared to 80.0% of the error subjects. Similar results were also found for the percentages of subjects' expected test outcomes and amounts of confirmation and disconfirmation. Thus, as Walker found earlier, system failure affected hypothesis-testing heuristics by increasing overall testing and repetition, but not attempts to disconfirm hypotheses. The similarity of results for the two studies indicates that the difference in educational background and research experience between the two samples was not a major factor affecting subjects' ability to solve the problem or the heuristics involved.

IV. RECOMMENDATIONS

a. The similarity of the pilot study results using a scientifically sophisticated sample as compared with the Walker (1987) undergraduate sample is quite surprising. However,

it should be noted that many subjects, after hearing about the purpose of the study during debriefing, commented that they were conceptually aware of how hypotheses should be tested and eliminated using disconfirmation. Why the strategy was not usually implemented during the task is not clear. It may be that the task is not seen as analagous to scientific experimentation. Since it was not possible using the pilot study's data collection technique of direct keyboard entry of sequences and expectations to thoroughly analyze what subjects were thinking, the question must remain unaswered. It is recommended that future investigations of the effect of system failure on hypothesis-testing should, therefore, include prompts during the task to motivate the subjects to keep track of their ideas and a comprehensive protocol analysis study in which subjects "think aloud" while solving the task.

b. Since the Wason 2-4-6 task has repeatedly shown confirmation bias, development of several new experimental tasks involving the same type of hypothesis-testing technique and combined with system failure is imperative. Several investigators (Gorman & Gorman, 1984; Klayman & Ha, 1989) have used numerical and nonnumerical domain variations of the original 2-4-6 task with similar results. The question that has not been answered by these versions of the task is whether or not the effects of system failure will differentially affect problem-solving heuristics across numeric and nonnumeric domains.

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FINAL REPORT

Software Development to Support Data Collection and Analysis of Cognitive Task Analysis Studies

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Contract No:	F49620-88-C-0053

Software Development to Support Data Collection and Analysis of Cognitive Task Analysis Studies

by

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ABSTRACT

The Air Force Human Resources Laboratory (AFHRL) is engaged in a series of projects to capture expert knowledge and strategies to facilitate training of novices and to hasten the acquisition of expert level skills. The summer project has developed software to automate and simplify the capture and analysis of Precursor / Action / Result / Interpretation (PARI) data (both text and graphics) by the AFHRL personnel. An existing standard software package (dBASEIII+) has been modified, significant extensions made to the available knowledge base concerning aspects of this package and new software modules created and tested to achieve this automation. A hypertext system was evaluated to permit the analysis of data across problem domains. The project has demonstrated the utility of dBASE III+ as a file management system for complex file interactions in a portable computer environment.

Work in applying expert system strategies for automating the analysis stage and in the hypertext based extraction of common themes will lead to further increases in the productivity of AFHRL researchers.

Acknowledgements

We wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this project. We are grateful to the personnel of the Air Force Human Resources Laboratory at Brooks Air Force Base, and particularly the support personnel of the Basic Job Skills Project, for their warm acceptance of visiting academics and their willingness to share their limited resources with us during our too brief stay. Special mention is appropriate of the efforts of the Air Force personnel in direct contact with us, in particular Lt. Col. David Brown for his excellent administrative efforts on our behalf, Capt. Pat Johnson for his knowledge of available resources and his exemplary efforts to provide us with those resources needed to maintain our activities, SSgt. Dennis Collins for his willingness to use his technical skills and humor to ease a trying period. Civilian employees of the project have also been very forgiving of the imposition of new personnel on scarce resources. We wish to thank Dr. Sherrie Gott for allowing us the opportunity to apply our capabilities to this project, Drs. Robert Pokorny and Ellen Parker for fully sharing the ambitions and current difficulties of their projects which enabled us to better contribute our skills, to Dr. Paul Nichols, for his good natured acceptance of our presence and significant non-quantifiable contributions, and to Kathy Suggs for her secretarial and personal assistance.

I. Introduction:

High quality maintenance is critical to the modern aircraft system. This maintenance is performed by personnel of varying levels of expe ience and expertise using sophisticated test equipment. As weapons and support systems have become more complex, a larger share of the routine support procedures have been automated. This has tremendously reduced the ratio of service to air time for new systems. One negative effect of this automation has been the loss of opportunities for the development and extension of skills by maintenance personnel.

Learning opportunities for apprentices have also disappeared as routine tasks have become automated. Increased complexity and improved system reliability have simultaneously increased possible sources of malfunction while decreasing practice opportunities for fault isolation. This has lead to a great increase in the time required to achieve expert status. Personnel spend a significant part of their tour becoming skilled in tasks for which their is a high commercial demand. Attrition of the most skilled personnel to private industry and DOD contractors, loss of expensively acquired knowledge and increased maintenance costs due to lower quality and less efficiently performed work are some of the side effects of this shift toward automation.

The Basic Job Skills (BJS) program represents an effort by the Air Force to overcome the negative effects of increased

automation on maintenance personnel. One segment of the BJS program involves the capture of interactions among experts in structured problem solving activities using a Precursor / Action / Result / Interpretation (PARI) methodology to generate insights into trainable skills. These insights are used to generate computer based tutors to improve the functioning levels of less experienced personnel.

A goal of the BJS program is to: 1) identify common job skills within and across advanced weapons systems to increase mental adaptiveness and skill generality among maintenance personnel while 2) accelerating the rate of skill development with training that is tuned to problem solving skills based on the principles of apprenticeship learning (Gott, 1988). Our task this summer has been to develop software which will aid in the identification, classification, and subsequent analysis of these expert skills in the context of PARI cognitive task analysis studies.

The methodology to achieve this is linked to identifying the components of expert problem solving. At present this involves a series of sessions where expertise can be captured through transcriptions and sketches as it is being demonstrated. After the initial problem is solved a series of rehash sessions are used to explore alternate solution avenues that were considered but rejected by the expert. The expert's solution paths are subsequently evaluated by independent experts. These sessions result in a large number of transcribed responses by multiple experts to

varied problems, i.e., PARI records. These records serve as the raw material to be used to study problem solving expertise leading to improved training in the skill areas reviewed.

Presently, the PARI records are generated manually. A researcher interacts with an expert dyad and records responses to problem solving scenarios. Subsequently, the PARI data are standardized and distilled where appropriate (still manually). Among the outcomes of this is a series of statements made up of common actions and equipment components being acted upon. For example, common actions would include swapping cards, reseating cards, taking DC voltage measurements, and so forth. The standardized actions and equipment objects provide the basis for groupings and other compilations across problems that occur in subsequent PARI data analysis.

II. Objectives:

Our summer task was to produce software to support the data gathering and analytic tasks. The software to be developed needed to provide easy data gathering during the interview sessions. Once the data had been gathered, the system had to be robust enough to protect the original data and the extracted information. It needed to provide opportunities for the application of expert system-based automated analyses and hypertext extraction techniques. User training or satisfactory prompting mechanisms were seen as necessary. During subsequent discussions with AFHRL

personnel, an interest was expressed in having software developed which would be appropriate for a study of the misconceptions of novices. A further request was made that an interface to the file manipulation software be created which would permit the user to enter data using a common word processing software (i.e.; PC-WRITE).

III. Restrictions:

In the delivered software, PARI data is entered directly into the software during the protocol sessions and then analysed within the framework of an automated file management structure. The software needed to be usable in the field and developed within the resources and restrictions available to the AFHRL personnel.

The majority of the data gathering is done using experts located at active air bases. The software system was developed to operate within the restrictions imposed by the use of available Air Force portable computer systems. At present, the only approved portable computer is the Zenith 248 operating under MS-DOS, using a 80C86 microprocessor, no numeric co-processor, an internal twenty megabyte hard drive and single micro-floppy of 720 kilobyte capacity.

IV: General Software Issues

There were several motivations and restrictions shaping the development of the software. The development team agreed that a commercial data base package would avoid many of the problems associated with maintaining custom developed

software. The research team needed a robust file handling package able to provide integral word processing capabilities. In addition, the software had to be developed within an MS-DOS environment and not require the use of extended memory features. The package must minimize the likelihood that the users would be able to damage data or file components accidentally. The software data entry modules, at least, would need to be operable within the restrictions of a portable computer environment. The users requested that data analysis be facilitated by the ability to easily transit from one set of problems to another, using commonalities in the data to provide linkages across the various files.

Given the above interests and restrictions, the development team chose to work with Ashton Tate's dBASEIII+ and the programming language Turbo-Pascal. DBASEIII+ is a highly regarded and commonly used data base file management system. The Pascal language is commonly taught in academic computer training programs and thus has a large established base of programmers. It was felt that support for the maintenance of the software produced would be readily available. Many of the special features requested by the research team could be implemented using special programs and modules written using Turbo-Pascal.

Given the anticipated size of the various data elements, it was decided that the best alternative was to use the memo fields available under dBASEIII+. The choice of memo fields

over the other alternates available was dictated by the integral word processing capability, the capacity of memo fields for storing data, and the ease with which memo fields can be manipulated within dBASE.

A difficulty with using memo fields is that the published material did not provide information concerning how the 512 byte storage segments are linked within the memo field. Several of the software extensions being planned would require that new memo fields be created independently of the dBASE software. To achieve this it was necessary to determine the linking technique used by dBASEIII+ examining the addressing structure used by the database designers to associate the individual 512 byte memory segments into the larger memo fields. Once this information had been extracted from an analysis of the hexadecimal code structure used in the header areas, it was possible to develop and manipulate memo fields usable by dBASEIII+ in any external program module. The exact procedure to achieve this will be published later.

V. Graphic issues:

Each of the PARI sessions has the problem solver sketching the components and their relationships as well as discussing them as part of the solution process. Text capture and subsequent manipulation within a commercial data base is routine. Graphics capture for subsequent analysis and manipulation is much less routine. The AFHRL research team wished to be able to record the expert's

sketches as they were created, link them to the associated text, and then be able to extract them without limitation when needed.

The research team also desired that the software for this operation be simple to use, able to function within the portable environment, and not interfere with the data gathering environment. The integration of text and drawings for analysis has required that the graphics software be rigorous enough for use in the field, easily mastered by either the subject matter expert or the members of the research team, and provide for easy capture of data. A further goal for the graphics software was that the files be either compact in size initially or easily compressible to facilitate storage and subsequent display.

The development team had planned to construct a set of graphic icons which could be used to reproduce the expert's drawings. Such a set of standardized elements would have permitted additional levels of analysis by the users and potentially by searching procedures.

The development team did not attempt this aspect of the project because of the anticipated time required for satisfactory resolution. As a temporary solution a simple drawing package has been adapted to the user's requirements. It produces files which integrate well with present text file handling procedures and which are compressable to permit up to forty images to be stored on the data disk. This package provides sufficient flexibility to achieve all of the elements of drawings which the

research team has observed. Either the expert or a member of the research team may use the chosen package for entering the graphic information. Pilot testing of this package has indicated that the software can be quickly learned (approx. 15 min. for the least competent subjects). Data compression software for the files produced by the graphics package have been outlined for coding if requested by the research team. For subsequent analysis the graphics images may be displayed in the portable environment or on systems available to the researchers in their office environment.

VI. Software produced:

The software has been designed so that a variety of manipulations and abstractions of the PARI data can be performed within the software package. Provision is also made to add new data items associated with the original problem.

Specifically, processes are available to the analyst in the software for general editing functions, for navigation through the data fields, and for maintaining an overview of project data. Additional processes are available to enter raw data steps, alternatives to the Result and Interpretation elements of the raw data steps, alternatives to Action elements, and alternatives to Precursor elements. All of the entries are linked with the originating problem and have provision for abstraction across several problems

and categories.

Critical to the success of the software package is that a robust file management system be used to maintain the very complex interplay of the components of the transcriptions and their interpretations. While an independent set of code could have been used for this, the research team felt that the advantages to such an individualized package were outweighed by the significantly inceased costs of maintenance to the system. Ashton-Tate's dBASEIII+ was chosen because of the availability of support personnel, widespread documentation of most functions, and its ability to meet the anticipated file management requirements implicit in this project.

By using external modules written in Pascal, the functions required of the software could be achieved or made to run significantly faster than would be the case if dBASEIII+'s routines were used. We have arbitrarily accepted the microfloppy capacity as the target maximum for data generated by the software during the typical PARI session.

A hypertext-based concept structure has been examined to automatically create linkages between the various problem steps sharing similar action statements. If implemented, this structure will enable the analyst to automatically associate and subsequently examine all records relevant to a given concept and associated by one or more critical terms selected by the analyst or drawn from pre-entered word lists.

It is important that the file generation and manipulation

be essentially transparent to the research team.
Significant effort was directed to producing file interfaces which require little or no user knowledge of the system. Because of the request for data entry through an external word processor, a great deal of programming effort has also been directed toward protecting the data files during the initial collection period.

VII. Present status:

At present the file manipulation software has been completed and tested. The data collection procedures can be achieved using either the internally provided data collection software or through the use of the external word processing software. Graphics items can be entered by the expert at the time of the PARI session or subsequently transcribed from the expert's notes by a member of the research team. Under the control of the researcher, all of the PARI sessions or any subset of the sessions can be displayed completely, or any specific step or stage within a step over all or over any subset of all of the problems may be displayed.
The entire data gathering, analysis and presentation package has been demonstrated on the portable computer system described in the body of this text. It has also been demonstrated on a more powerful machine available to the researchers within their office environment.
Software to use in a misconception analysis has not been

independently developed. A software package from the MaxThink Corporation was identified and presented to a member of the research group which had the capabilities requested for the misconception analysis. There was insufficent time to develop software specifically for this aspect of the research interest. The senior member of the development team would encourage the development of this software.

The hypertext software was prototyped at the screen interface level. There was insufficent time to further develop this aspect of the project. Both members of the development team strongly encourage further work in this area.

The graphics package could be extended to permit automated searching and analysis procedures. Development work is being conducted on analyzing images and abstracting the result for further manipulation. In addition, file compression programs may be created which will reduce storage requirements for the graphics files. Further development could be directed toward either the creation and subsequent use of a set of icons for the graphics segment of the data gathering / analysis period, or more sophisticated software could be developed for abstracting and analyzing images and components of images already stored using the graphics capability provided in the project software. Both members of the development team strongly encourage further work in this area.

The analysis process of the data shows promise of

benefiting from automation using available expert system development techniques. A continuation of the summer project examining the analytical techniques with the goal of automation is encouraged by both members of the research team.

VIII. Recommendations:

The software developed during this session provides the research team with the ability to enter data using either a highly structured set of prompts and fields or a word processor with a predefined file organization. Once the text material has been entered, a series of dBASEIII+ programs capture the text components, organize the entries into a series of files for management purposes, provide linkages to similar components in other problems, and allow for subsequent analysis at the researcher's specification. Opportunities to print or display needed information are provided with screen prompts.

At present, the software is provided with a copy of dBASEIII+ under the control of the senior researcher. The users will need to purchase a copy of dBASEIII+ from the Ashton-Tate organization prior to applying the software. It is the recommendation of both authors that observations of the data collection process done at Eglin Air Force Base, Florida be provided to facilitate any additional changes in the software which will assist data collection in the future. Further, support for implementation of the

hypertext data management system and for development efforts in applying automated classification software to the raw data being collected will result in improved efficiency for data analysis.

The graphics portion of the data is presently managed seperately from the text. Integration of the two would simplify the analysis of PARI data. This integration will require the purchase and implementation of "windows" oriented display software and hardware (graphics card, monitor, mouse, internal memory) able to support this capability. The senior author suggests that this option be explored by the members of the analysis team for their use.

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Final Report

Computer-based training for complex, dynamic tasks.

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Computer-based training for complex, dynamic tasks.

by

Kevin B. Bennett

ABSTRACT

For a variety of reasons the Air Force is interested in improving the efficiency and effectiveness of programs for training personnel to perform complex, dynamic tasks such as those found in the command and control environment. This report describes a research and development (R&D) program that was initiated to investigate issues in computer-based training for dynamic tasks. R&D completed during the USAF SFRP includes the design, implementation, and initial analysis of two experiments and the continued development of a part-task trainer. This development includes the design of an integral display, an animated functional mimic, an enhanced learning environment, and the incorporation of the part-task trainer into a "windowing" environment. The report also briefly describes proposed R&D for the AFOSR RIP.

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I. INTRODUCTION:

Personnel training is a major concern to the Air Force and it is clear that discovering principles of instructional system design and instructional strategies to improve the effectiveness of training programs would be a benefit to the Air Force. One alternative that has the potential to make the training process more effective and less costly than the standard approach to instruction is computer-based training. (Kearsley, 1987; Polson and Richardson, 1988; Psotka, Massey, and Mutter, 1988). One particular type of training system has an especially pronounced potential to improve the cost-effectiveness of Air Force training programs: those that incorporate mathematical simulations that replicate the behavior of complex systems and/or scenarios. This is especially crucial to the Air Force because there is the expectation of inordinately high losses during first time combat engagements. There are obvious difficulties in attempting to train personnel for these situations, including cost and safety factors. With learning environments that simulate critical situational factors that characterize combat engagements, Air Force personnel could be provided with hands-on experience that is otherwise not available: the trainee could make the inevitable mistakes with a computer simulation rather than an adversary. Thus, personnel could be trained to a higher state of readiness, thereby reducing loss of life and equipment.

During my appointment in the United States Air Force Summer Faculty Research Program (USAF SFRP) I worked at Air Force Human Resources Laboratory (AFHRL) Ground Operations Branch under the Logistics and Human Factors Division at Wright-Patterson Air Force Base (LRG). One of the primary concerns of this laboratory is to develop training simulations for the areas of command and control, logistics, and air defense. At a high conceptual level these domains are

characterized by a number of issues, but the single most characteristic feature is that they are dynamic in nature. Dynamic domains of application are distinct from other domains by the fact that the goals to be accomplished are constantly changing as a function of the operational context. Unfortunately, most of the research on computer-based training has been conducted with static environments and there is a pronounced need to investigate computer-based training in dynamic environments.

II. OBJECTIVES OF THE RESEARCH EFFORT:

During the ten-week USAF SFRP appointment I continued working on a research and development (R&D) program which was initiated to investigate issues that will contribute to the effectiveness of compuber-based training in dynamic environments. This R&D program is of direct interest to the AFHRL/LRG laboratory, and the Air Force in general, because of the similarities between and the domain of application being investigated (process control -- a nuclear power plant) and the domains of interest to the AFHRL/LRG (command and control, logistics, and air defense). The high-level abstract properties are exactly the same: both are dynamic, event-driven worlds, where individuals control complex systems with a large number of highly interactive parts, where individuals must collect and evaluate large amounts of potentially noisy data, where individuals must monitor highly automated subsystems, where individuals must effect and revise operational procedures, and where there are serious consequences for performance errors.

For several years now myself and my colleagues have been involved in an R&D program investigating issues in computer-based training in the domain of process control, and, in particular, for a task that has proved to be difficult for operators in a nuclear power plant to perform: the manual control of feedwater. A high-fidelity, part-task trainer that runs on a Sun Engineering Workstation has been developed to

simulate a portion of the power plant and this part-task trainer is used as an experimental testbed for the evaluation of instructional aids and strategies. During the USAF SFRP appointment my goals were to continue development work on the part-task trainer and to conduct experiments on the training aids that were developed.

III. RESEARCH AND DEVELOPMENT PROGRAM

The research and development program for training personnel on the manual control of feedwater task is based on a rigorous approach to the design of computerized instructional environments. This program is divided into roughly four phases, and each will be described briefly. Phase I consisted of a cognitive task analysis of the manual control of feedwater task, the development of the mathematical models that simulate the behavior of the steam generator, and the development of graphic displays (Roth, Woods, & Gallagher, 1986; Bennett, Roth, & Woods, 1986). The goal of Phase II is the evaluation of the graphic displays that were developed in Phase I, as well as the development and evaluation of additional enhancements to the part-task trainer. The goal of Phase III is the evaluation of part-task trainer design and instructional strategies that will effectively teach the learner to complete the entire start-up task, rather than the narrowly-defined manual control of feedwater task. The goal of Phase IV is to design and evaluate an intelligent tutoring system for this dynamic environment. Both the R&D that was accomplished during the USAF SFRP and the R&D that is being proposed for the AFOSR RIP falls in Phases II and III. The experiments that were completed, and the development that was completed will be described in greater detail.

USAF SFRP EXPERIMENT

The predictor and the steam/feed flow displays that were developed in Phase I of the R&D program were evaluated in a preliminary experiment during the USAF SFRP. The goal of the study was to evaluate the extent to which the predictor display and the steam/feed flow meters assisted the learner in completing the manual control of feedwater task (relative to the baseline that operators have in the real-world version of the task). The part-task trainer was used in the experiment and the interface was similar to Figure 2. The predictor display appears in the top trend display and the steam/feed flow meter appears below, in the lower trend display. Although the displays appear together in Figure 2, during the experiment the subjects saw the baseline condition, the baseline plus the predictor, and the baseline plus the steam/feed flow meter seperately during twenty segment time intervals of an experimental session.

Method

Subjects. Six volunteers (two females, four males) from a government research laboratory participated in the experiment.

Stimuli. A part-task simulator was developed on a Sun-4 Microsystem Workstation. The part-task simulator was designed to reflect those aspects of the real-world task that make it difficult. The underlying model replicates the dynamics of a single nuclear power plant steam generator with high functional fidelity through the use of differential equations. The simulation incorporates the influence of a number of factors on the actual steam generator level, including steam flow, feed flow, rate of power, and temperature. The steam generator dynamics can be adjusted to represent a wide variety of existing steam generators.

The rate of feedwater was under the control of the subject, whose task was to adjust this rate (by pressing one of two keys on the keyboard) with the goal of

keeping the actual steam generator level between the upper and lower trip set points. The level of the reactor power and the temperature of the feedwater remained fixed throughout the experiment. The starting position of the steam generator level was randomly determined between 30 and 70%. Changes to steam flow were introduced, under program control, to produce the primary challenges to the subject. The steam flow rate was altered in two ways on all trials: 1) continuous adjustments, and 2) disturbances. Continuous adjustments refers to the fact that the rate of steam flow was constantly changing due to contributions from three sine waves and a ramp. The second variation produced in steam flow did not happen continuously, and instead was a result of random changes, or disturbances, in steam flow. The number and size of these disturbances varied as a function of the time of the experimental trial, which could last for as long as 5 minutes. The effect of the programmed changes in the rate of steam flow was to produce an experimental trial that constantly changed and became progressively more difficult as the trial progressed. The primary dependent measure was the time before the upper or lower trip set points were surpassed.

The interface to the part-task trainer varied as a function of the experimental condition. The baseline condition (conditions approximating the information that operators have in the real world) consisted of two "trend" displays that were constantly present in the interface. The value of the variables, on a scale of 0 to 100%, are plotted on the vertical axis and trial time, on a scale of 0 to 5 minutes, is plotted on the horizontal axis. The displays consisted of two major sections: a current value for each variable, and a history of the values for these variables across the trial. The present value of a variable is illustrated by a diamond in the small rectangle on the right-hand side of the displays, as illustrated in Figure 2. Historical information

about the changes in those variables is shown in the larger rectangle to the left of this, containing lines and fills and covering the majority of each display. The displays were updated with information from the model every 2 seconds and random noise ranging from -2% to +2% was added to the value of each variable displayed (these changes were not added to the model, only displayed to the subject). In the upper trend display (the "level" trend) the actual value of the steam generator level was always shown, along with horizontal red lines indicating the upper and lower trip set points (77% and 20%, respectively). In the lower trend display (the "flow" trend) the reactor power was always shown. Together, this information approximates that which nuclear power plant operators actually have in the real world and constitutes the experimental baseline condition.

This baseline condition was augmented with two additional pieces of information: the predictor display and the steam/feed flow display. For the steam/feed flow condition the rate of steam flow and rate of feedwater flow were obtained from the model and displayed in the flow trend (the lower trend display). For the predictor display condition a predicted value for steam generator level was displayed in the level trend display (the upper trend display). Although Figure 2 illustrates both the steam/feed flow and the predictor display together, the subjects in the experiment never saw both pieces of information at the same time.

Apparatus. All experimental events were controlled by a general purpose laboratory computer (Sun Microsystem 4-110 Workstation). Subjects were seated in an enclosed experimental room and a video monitor (16" color with 1152 by 900 resolution) was used to present experimental prompts and to record user responses.

Procedure. The experiment was conducted during a two-week period with one experimental session per day (lasting one hour), for a total of ten sessions. In all

sessions the user's task was to increase or decrease the rate of feedflow by pressing the appropriate key on the keyboard. Each trial could last up to 5 minutes and ended either when 5 minutes had elapsed or when the steam generator level surpassed either the upper or the lower trip set point. In each session the user experienced all three experimental conditions: the baseline, the baseline plus the predictor display, and the baseline plus the steam/feed flow display. The order of these conditions was randomly determined and the conditions were switched at twenty minute intervals, producing the same amount of experience with each display. In summary, the experimental design contained 4 independent variables (starting position of steam generator level, day of experimental session, type of trial, and display condition) and one primary dependent variable (time-on-task, or latency). Latency was recorded at 1/100 second accuracy.

Results

Since the experiment was completed on Aug. 11 a simple data analysis has been performed. The latency scores for starting position of steam generator level and the type of trial were averaged, examined, and since no noticeable differences were evident these variables were not considered in the analysis. Three latency scores (one for each display condition) were computed for each subject during each experimental session, for a total of thirty scores per individual. A 3 x 10 repeated measures ANOVA was performed on these scores. The main effects of day $F(9,45)=4.62$, $p<0.000$ and display, $F(2,10)=12.18$, $p<0.002$ were highly significant, while the interaction between display and day $F(18,90)=1.69$, $p<0.055$, was nearly significant. The means for the three displays are illustrated in Figure 5. A one-tailed t-test indicated that the differences between the predictor display and the baseline (obtained difference = 29.27) and the steam/feed flow display and the baseline

(obtained difference = 35.45) were significant (critical difference at p<.05=13.91 sec), while the difference between the predictor and the steam/feed flow displays (6.18 sec) was not significant. It is clear that both the steam/feed flow meter and the predictor display enhanced performance, relative to the baseline condition, at the manual control of feedwater task.

USAF SFRP DEVELOPMENT

During the USAF SFRF appointment I also completed several enhancements to the the part-task trainer. This developmental work included the translation of the interface software to run in a windowing environment, the development of two additional graphic displays, and the development of an initial enhanced learning environment. This development work will be described in greater detail.

Animated Functional Mimic

The first enhancement to be described is an animated "functional mimic." This graphic display is represented in Figure 1 (due to the monochromatic limitations of the word processing system only the outline has been shown). This display incorporates both flow rates and colors which change as a function of the system state, and was designed in the spirit of the STEAMER project (Hollan, Hutchins, and Weitzman, 1987). It is designed to provide a "simultaneous graphic explanation" of the complex process that the student is learning to control. The animated functional mimic is a high level conceptualization of the subsystems in the nuclear power plant that are critical in the manual control of feedwater task. The connections between these subsystems are animated and color-coded to illustrate the flow of resources between them. For example, if the user increased the rate of feedflow this would be visibly illustrated by a change in the speed of the animation in the secondary loop between the turbine housing and the steam generator. This would be followed by a

shrink effect in the steam generator, illustrated by a momentary decrease and then a gradual rise in the graphic fill inside the representation of the steam generator. The animated functional mimic should provide users with a high-level conceptual explanation of the manual control of feedwater task.

The Enhanced Learning Environment

One of the major enhancements to the part-task trainer was changing the interface to work in the "windowing" environment on the Sun Workstation. This was desirable because the windowing environment contains pre-defined, high-level interface conventions (e.g., menus) and multi-tasking capabilities. Using these tools an enhanced learning environment for the manual control of feedwater task was created which allows critical task variables to be changed interactively. The revised interface to the part-task trainer is illustrated in Figure 2: the displays appear in a pop-up window with three buttons (Run, Init, Quit). Selected model parameters can be changed if the user clicks on the Init button (see Figure 3).

The enhanced learning environment is an attempt to provide the learner with graphic representations and explicit tools that will make it easier for he/she to learn the manual control of feedwater task. The enhanced learning environment will utilize the graphic displays that were the focus of Phases I and II. The trainee will have the capability to actively explore a domain, and in some cases to perform learning experiments. For example, the student will have the capability to make successive simulation runs while systematically varying a single variable (e.g., feedwater temperature), or successive simulation runs to observe the interaction effect between two variables. The trainee will also have the capability to vary the rate at which the simulation runs ("step-by-step" or "fast- forward") and to "rewind" the simulation to return to the point at which control of the system was lost, and attempt an

alternative control strategy.

Integral Display

An additional display that was developed during the AFOSR SFRP is an object display that integrates information from four variables (steam flow, feedwater flow, steam generator level, and predicted steam generator level) into a single graphical object (see Figure 4). This display was designed was designed to portray the data relationships that should be communicated to the user. In the manual control of feedwater task the important relationships are the difference between actual and predicted SGL, and the difference between steam flow and feedwater flow. To effectively complete the task the user must consider these differences to determine the optimal control strategy and the integral display communicates this information to the user. The values for actual and predicted SGL are plotted on the Y-axis; the steam flow rate and the feedwater flow rate are plotted on the X axis. Where the values for the 4 variables intersect in the display grid a rectangular shape is drawn, and the sides of the rectangle are color-coded to reflect the relative values of the four variables. Both the size of the rectangle and its position within the display grid change as a function of the underlying variables. For example, if the steam flow and feed flow were equal the result would be a very small rectangle. If the rate of feed flow was increased the rectangle would become elongated in both axes (but predominantly in the X-axis), and would begin migrating upward in the Y axis. This display integrates important variables and their relationships in a single integrated graphic object and makes the relationship between these variables graphically explicit.

IV. RECOMMENDATIONS: PROPOSED RESEARCH FOR THE AFOSR RIP

Research is proposed for the AFOSR RIP in each of the three development areas just described. The major technical problems in each area were solved during the USAF SFRP, and I have two graduate students who are interested in one of the areas for a thesis topic. Thus, although the proposed research is ambitious, I believe that significant work in all three areas will be accomplished during the RIP. The proposed research in each area will be described.

Animated Functional Mimic

In part-task trainers and simulators there are at least two primary ways that the graphic displays such as the animated functional mimic (Fig. 1) can aid in conceptualization and help facilitate the development of appropriate mental models. One way is to provide representations of a complex system at a number of different conceptual levels (Rasmussen, 1986). In the past, understanding the plant from these various perspectives, and the ability to shift between these perspectives as a function of task demands, was developed only through long periods of experience with the system. When combined with graphic displays a simulator or part-task trainer can be used to explicitly provide these perspectives (Hollan, Hutchins, and Weitzman, 1987). A second way that a part-task trainer with graphic displays such as the animated functional mimic can promote a deeper conceptual understanding of complex systems is through a continuous graphical explanation of the process dynamics (Hollan, Hutchins, and Weitzman, 1987). This is especially important for learning about highly complex and interconnected systems such as nuclear power plants and command and control environments. In this type of system a myriad of events are occurring simultaneously and it is virtually impossible to explain these events through language (because of its inherently serial nature). By contrast, high-resolution graphic displays can provide a simultaneous graphic explanation of the

system: the student can see the important systems and subsystems and the flow of information or resources between them. It is predicted that the animated functional mimic will assist the user in understanding the complex system interactions that make the manual control of feedwater such a difficult task, encourage the development of an appropriate mental of the process, and therefore improve performance on the task.

Integral Display

Previous research on integral displays has concentrated on two issues in the integral versus separable display of data: 1) a benefit to decision- making when the variables must be considered together, and 2) a cost when decoding information about the value of individual variables. The first issue is currently being investigated in a second experiment at AFHRL/LRG. The study is designed to see if the integral display shown in Figure 4 can improve performance in the manual control of feedwater task. Five of the six subjects from the first USAF SFRP experiment participated in the second experiment. Recall that although these subjects interacted with both the steam/feed flow and predictor displays, they did not see both displays at the same time. In the second experiment the users will see both the steam/feed flow and predictor information at the same time, but they will see that information in different formats: integral and separable. It is predicted that the integral display of information will improve performance on the task. Additional experiments using a between-subjects, rather than a within-subjects design, are planned to supplement these initial results. To investigate whether or not the integral display incurs the cost that has been previously reported, a second type of experiment is proposed. Consistent with previous research, the users will be checked for their knowledge about the values of the individual variables that were used to construct the integral display.

During the course of a trial the screen will be blanked and the user will be asked to estimate the value for a parameter. It is predicted that the costs related to the decomposition of the integral display will not be incurred.

Enhanced Learning Environment

The use of the enhanced learning environment as an instructional tool raises a host of complex instructional issues. The goal of Phase III is to determine those aspects of both simulator design and instructional strategies that contribute to the most efficient acquisition of skills on the start-up task. What is the most effective use of the tools that the environment provides? Should the learner be required to use each of the the tools and the informational displays that are provided? If so, should there be a particular sequence? One end of the spectrum of instructional strategies can be considered a strictly regimented sequence of training activities. Training at this end of the spectrum is likely to be ineffective, due to the passive role assigned the learner. At the other end of the spectrum is unstructured discovery learning. In this approach a trainee is provided a computerized learning environment and encouraged to explore the domain with very limited or no guidance. At this end of the spectrum training is likely to be ineffective because there is no guarantee that the learner will utilize the tools that are provided in an optimal manner, and no guarantee that the important concepts will be learned. Somewhere in the middle of the spectrum lies what might be considered "guided discovery learning." In this approach the trainee is encouraged to actively explore a domain, but is also provided with sequences of events that are likely to illustrate fundamental concepts that must be learned. The use of the enhanced learning environment and alternative instructional strategies will be the focus of this area of research.

SUMMARY

The R & D program that has been described in this proposal will provide information that can be used to improve the efficiency and effectiveness of programs to train personnel to perform complex, dynamic tasks. The similarities between process control, and command and control environments are such that the findings will transfer directly, with immediate benefits for AFHRL/LRG. From my discussions with AFHRL/LRG personnel and my observations of the systems being developed, it is clear that we share an common interest in a number of issues. The USAF SFRP resulted in the completion of two experiments and the continued development of the part-task trainer. The proposed RIP research will provide information for the effective design of computerized training programs for complex, dynamic tasks including methodological procedures (including transfer-of-training procedures, time-on-task issues, and enhanced learning environments), graphic displays (animated functional mimic, integral displays), and instructional strategies (discovery learning, guided discovery learning).

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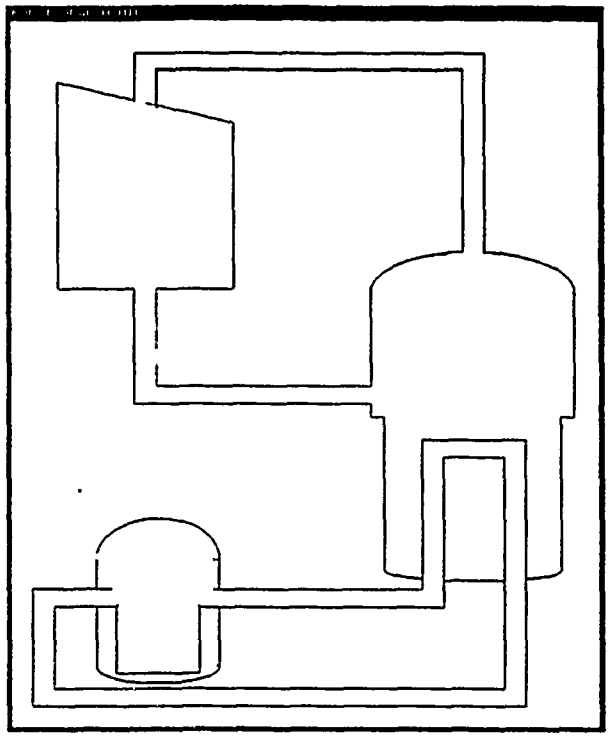


Figure 1. Animated Functional Mimic

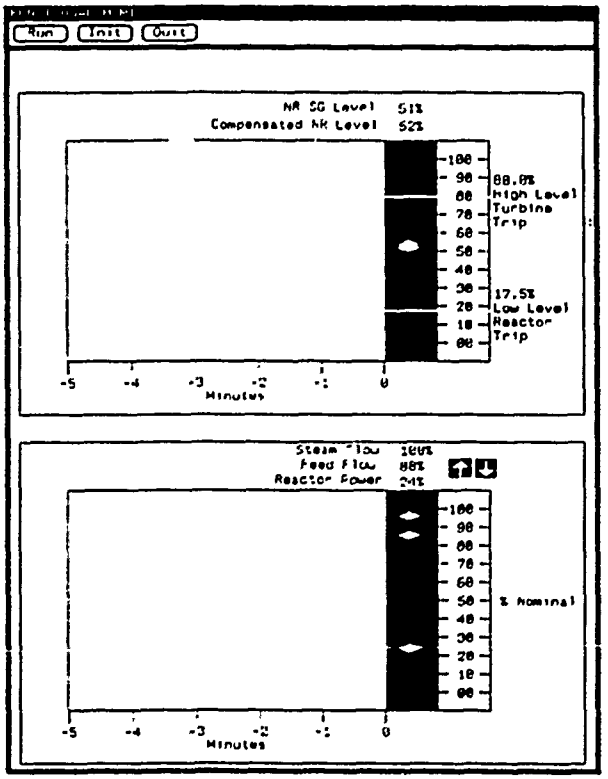


Figure 2. Trend and Flow Meters in Windowing Environment

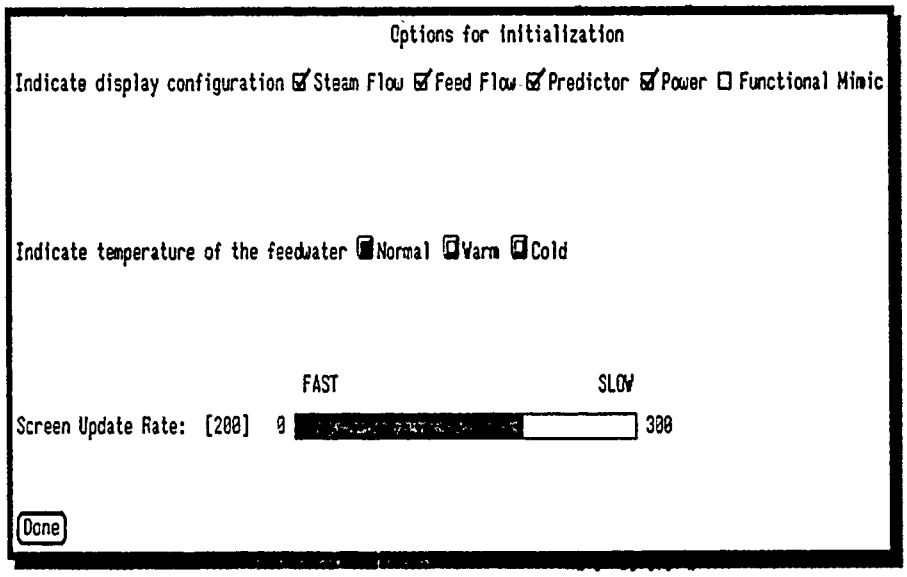


Figure 3. Initialization Options in Enhanced Learning Environment

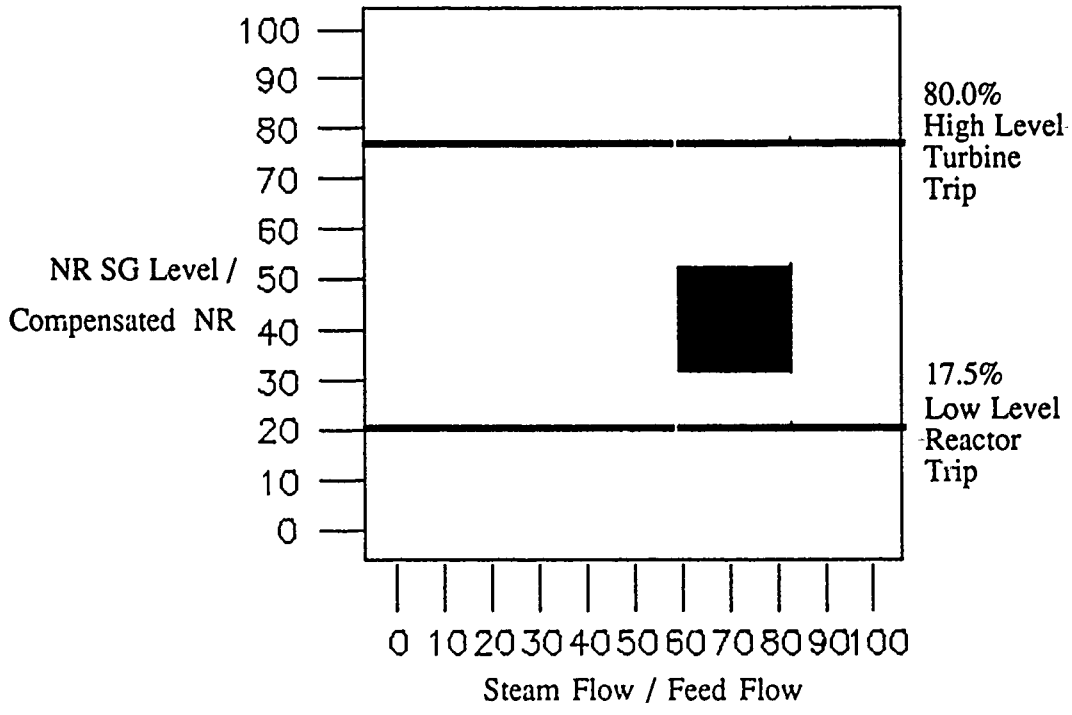


Figure 4. Integral Display

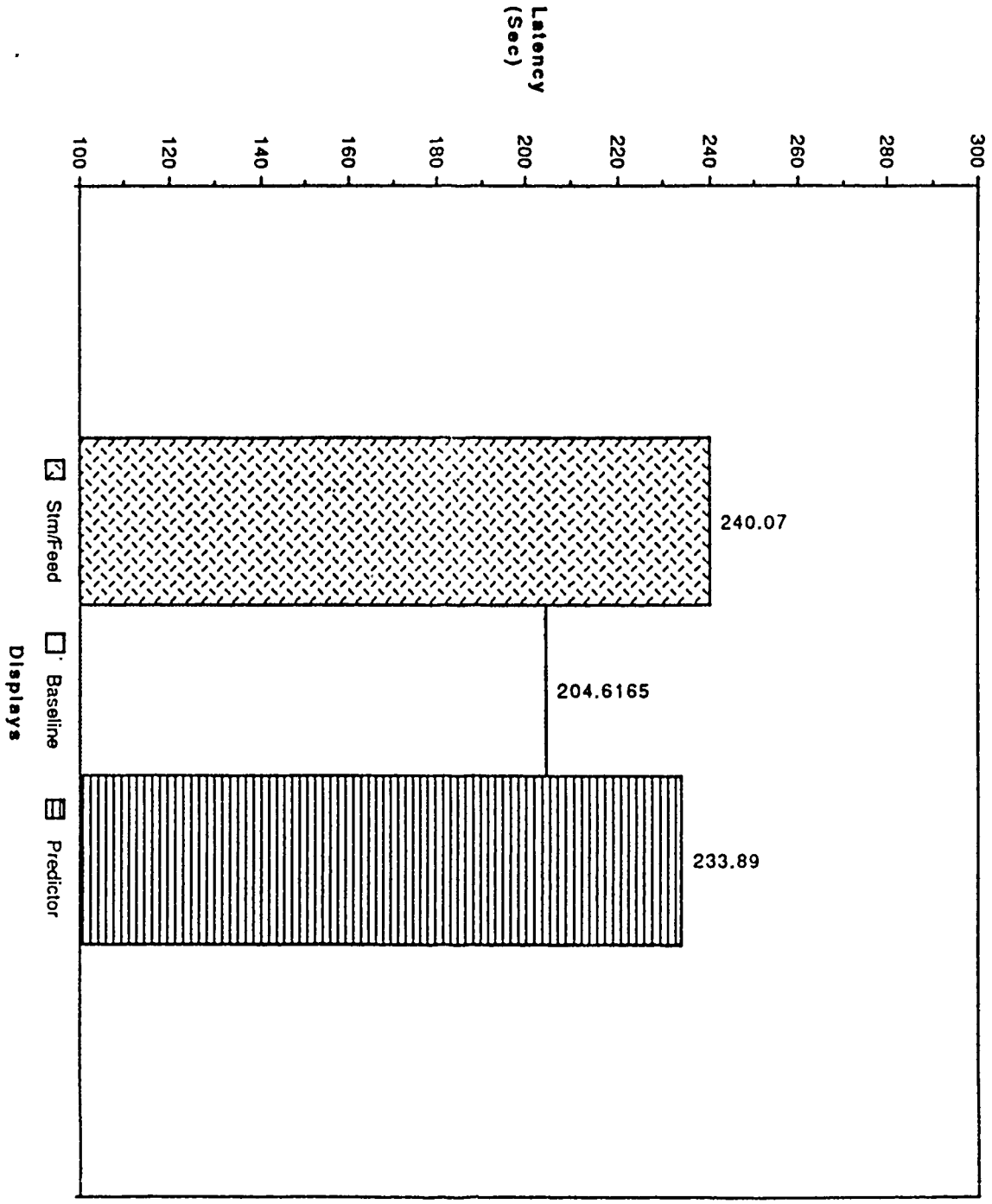


Figure 5. Mean latency scores for display conditions

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FINAL REPORT

Working Memory and Cognitive Structure

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Working Memory and Cognitive Structure

by

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Abstract

The research described in this report was designed in the context of the Learning Abilities Measurement Program conducted at AFHRL, Brooks AFB, Texas. The goal of this research was to investigate the influences of working memory and prior knowledge on the development of conceptual information stored as declarative knowledge in memory, and it is based on the theoretical conceptions of working memory developed by Baddeley and Anderson, and advances in the measurement of this construct made at AFHRL. A modification of Novak & Gowin's concept mapping procedure was developed for computer presentation, and was used as pretest and posttest measures of conceptual understanding in a test battery with five measures of working memory.

Due to unforeseen software incompatibility problems, the data analyses for this research are incomplete. The process of transferring floppy disk information to the mainframe computer at the University of Northern Colorado was more complex and time consuming than expected. This report was delayed as long as possible, but these problems could not be resolved before Oct. 1. A complete final report will be submitted as soon as possible.

Acknowledgements

We would like to thank the Air Force Office of Scientific Research, Universal Energy Systems, and the Air Force Human Resources Laboratory at Brooks AFB, Texas for making this research possible. Special thanks goes to the staff of MOE, especially Dr. William Tirre for continual encouragement to apply for the UES Summer Faculty Research Program, and whose ideas and expertise were invaluable to the development of this work. Valuable comments from and discussions with Drs. Scott Chaiken, Pat Kyllonen (who generously allowed us to share his workspace), Valerie Shute, and Dan Woltz were also very much appreciated. We would also like to thank OAO Corporation staff members Rich Walker for his expert and creative programming and Terri Perdue for her cheerful and extreme dedication to task. This report covers only the beginning of what is expected to be a long and productive relationship with AFHRL/MOE personnel.

I. INTRODUCTION

a. Theoretical Background

The research described in this report is an investigation of the interrelationships between working memory and cognitive structure; that is, on the organization of and relationships between concepts in declarative memory (Shavelson, 1972; West & Pines, 1985). These studies were designed from the perspective of the Four-Source Research Framework (Kyllonen & Christal, 1988) which has been used to structure the Learning Abilities Measurement Program (LAMP) conducted at AFHRL to measure and model basic cognitive abilities.

Two components of this framework, declarative knowledge and processing capacity, were investigated in the present research to assess their effects on the acquisition of conceptual information. Research associated with LAMP has focused more on the second of these components than the first, and has shown that processing capacity or working memory capacity is substantially related to performance on cognitive tasks in a variety of areas, including those related to the development of technical expertise (Kyllonen & Christal, 1988).

The concept of working memory has evolved partially due to the inability of traditional conceptions of short term memory (STM) to predict performance in learning situations with high ecological validity. For example, except for very low ability learners, the digit span has rarely been found to predict individual differences in intellectual ability (Dempster, 1985). Daneman (1982) has suggested that the lack of predictability of the digit span is due, in part, to the

inadequacy of the theory underlying the STM measure, and thus has advocated additional research with the working memory construct.

Working memory has been described as an information processing system that includes typical STM storage functions, and executive or control systems for information organization, reorganization, or synthesis (Baddeley, 1976; Baddeley & Hitch, 1974; Hitch & Baddeley, 1976). The total capacity of working memory (i.e. the amount of attention that can be allocated for use by both storage and processing systems combined) is hypothesized to be quite limited, and Baddeley has proposed that the two systems share this limited capacity in ways that are dependent on current task demands. That is, when the retention of large amounts of information is necessary, a portion of the processing attentional capacity can be allocated to increase storage capacity. Baddeley has measured working memory capacity with a dual task method that requires concurrent storage and processing of information, an approach which has been expanded and refined for the working memory tasks designed for the LAMP research at AFHRL.

The storage component of working memory has been hypothesized to comprise subordinate systems, including a verbal short term store called the articulatory loop, an output buffer, and a visuo-spatial scratchpad (Baddeley & Hitch, 1974). In addition, the processing component has been hypothesized to be related to a variety of information processing mechanisms including rehearsal, chunking, consolidation, long term memory search processes (Baddeley, 1976; Britton & Tesser, 1982; Hitch, 1980), problem solving (Johnson-Laird, 1983), and the integration of newly encoded

information with prior knowledge (Anderson, 1983). Anderson's description of working memory is somewhat different than Baddeley's and is based on the notion that activated declarative knowledge, both that stored in long term memory and that which has been recently encoded, constitutes the contents of working memory.

Research based on both the Baddeley and Anderson positions have shown individual differences in working memory to be associated with performance on a variety of cognitive tasks. Woltz (1988) and Carlson, Sullivan & Schneider (1989) have shown that working memory attentional capacity is related to the initial, i.e. declarative stages of learning. Working memory has also been found to show substantial relationships with reading processes and reading achievement (Baddeley, Logie, Nimmo-Smith & Brereton, 1985; Cochran & Davis, 1987; Hitch & Baddeley, 1976; Mason & Miller 1983; Tirre & Rancourt, 1986). Moreover, evidence also suggests that these effects are domain independent (Turner & Engle, 1989).

b. Working Memory and Reading Processes

One aspect of working memory which has been frequently addressed is concerned with the amount of processing capacity used during the reading of text. Inhoff & Fleming (1989) and Britton and his colleagues (Britton, 1980; Britton, Graesser, Glynn, Hamilton, & Penland, 1983; Britton & Tesser, 1982) have used probe detection methods similar to Baddeley's dual task approach. These methods present readers with short passages of varying difficulty and complexity in self-paced formats, usually with the words individually presented on a computer screen. Response times and accuracy data are collected for both visual and auditory probes to

assess the amount of memory capacity remaining beyond that needed for text processing.

These studies, however, have revealed inconsistent findings. Britton's early work showed that easy text requires more cognitive capacity to process than difficult text, and his later studies showed that syntactic and propositional complexity, presumably a component of difficult text, requires greater cognitive capacity. Inhoff and Fleming identified the inconsistencies in these results and attempted to replicate Britton's earlier work. Their evidence was more consistent with Britton's later findings, and provided support for the position that difficult text does indeed tax working memory capacity to a greater extent than easy text. Inhoff and Fleming conclude that this difference is at least partly a function of the increased amount of propositional information activated from long term memory during the reading of difficult text. This argument must be regarded as tentative, however, since none of these studies included a working memory task separate from the probe measure. In addition, this research has not adequately isolated the effects of prior knowledge and text difficulty. Fincher-Kiefer, Post, Greene & Voss (1988) have shown that low levels of prior knowledge produce greater processing demands than high levels, but only for those task situations that demand the aquisition of conceptual relationships.

A reading task was chosen to be the vehicle for information aquisition for the present research for two reasons. First, much of the working memory literature focuses on reading and so provides theoretical grounding as discussed above. Second, use of technical expository text would allow generalization of these results to training

settings in technical fields such as electronics, and would provide information useful for the development of technical manuals and training materials.

c. Cognitive Structure

The major focus of the current project is the measurement of cognitive structure, the organization of conceptual information stored as declarative knowledge in long term memory. This construct has not been investigated for its relationships with working memory. Although substantial research is available which shows the facilitative effects of knowledge organization on intellectual performance in a wide variety of domains (Eylon & Reif, 1984; West & Pines, 1985), it is unclear to what extent individual differences in working memory capacity and prior knowledge mediate or moderate these effects. If such effects could be demonstrated, performance on cognitive tasks and the prediction of performance on those tasks would be further enhanced.

The assessment of cognitive structure has occurred in a variety of ways, including methods ranging from interviews (West & Pines, 1985) and free-recall protocols (Eylon & Reif, 1984) to association strength ratings requiring multidimensional scaling analyses or cluster analyses (see e.g. Smith, 1986). Novak & Gowin (1984) describe a paper and pencil procedure for measuring cognitive structure called concept mapping which is essentially a technique for representing propositional relationships among concepts in a specific content area. This procedure was chosen for the present research in that it allows both quantitative and qualitative assessment of a learner's knowledge, and because it does not require the assumptions

of associative symmetry necessary for procedures such as multidimensional scaling analysis (Nagy, 1986). Concept mapping has been shown to be related to achievement (Cochran, 1989), and it has an advantage in that it incorporates a general-to-specific hierarchical conceptual organization, which has been shown to be related to recall and problem solving performance (Eylon & Reif, 1984). This task thus serves to measure both the amount as well as the organization of conceptual knowledge.

d. Research Hypotheses

This research was designed to address the following questions:

1. How is working memory (verbal and nonverbal) related to the amount and organization of prior knowledge?

2. How is the use of working memory and prior knowledge related to text difficulty?

3. How do working memory and prior knowledge interact to influence the development of cognitive structure from text?

4. How are working memory, prior knowledge and text difficulty related to changes in cognitive structure as a result of instruction from text?

II. METHODS

a. Subjects and Procedure

The pilot study which has been completed is based on data collected from 126 USAF recruits tested during their 11th day of basic training at Lackland AFB, Texas, 80% of them male. The test battery was programmed in Turbo Pascal (Version 5.5) and designed for presentation during a three and one-half hour session on IBM AT

compatible computers. The battery was presented in two parts, separated by a 5 minute break. The first section of the battery was composed of a series of knowledge measures, including a multiple choice pretest and a cognitive structure pretest (both designed to measure the amount and organization of prior knowledge), a text passage, and a cognitive structure posttest. The second half of the battery contained 5 measures of working memory selected from those developed from the AFHRL Learning Abilities Measurement Project.

b. Materials and Tasks

The knowledge measures in the first half of the battery were based on the content area of electricity, chosen to be representative of learning and cognitive skills in technical fields. One measure of prior knowledge was a 25 item, multiple choice pretest, partially based on the electricity subtest of a study manual for the Armed Services Vocational Aptitude Battery (Department of Defense, 1984).

The other measure of prior knowledge was a version of Novak and Gowin's (1984) concept mapping procedure. The adaptation of this method for the computer used in the present study required subjects to first locate six given concepts in boxes on the screen according to the level of generality of the concepts. The given concepts were battery, circuit, current, electrical potential difference, electrons, and resistance. Subjects were then asked to choose those concept pairs they wished to identify the interrelationships for, and then to select an appropriate relationship from a menu of alternatives designed to assess typical misconceptions (see e.g. Cohen, Eylon, & Ganiel, 1983; Fredette & Clement, 1981). The dependent

variables measured were latencies for all decisions, the number of interrelationships identified, the accuracy of those interrelationships, and the order in which the relationships were specified. The same concept mapping task was used for the posttest measure of cognitive structure.

Two text passages, easy and difficult, were developed from Air Force training materials and were both 756 words in length. They differed in that in the easy text, fewer concepts were presented and the interrelationships among them were made as explicit as possible and repeated throughout the passage. The two texts were checked for similarities in surface structure, e.g. sentence length, types of sentences, and readability, and were found to be comparable. In each text, 92 word probe locations were identified, 34 of them exactly the same in both passages. The texts were presented on the computer using a self-paced rapid serial visual presentation. Two forms of probes were compared. One form, a flash above the presented word (5 x 7 pixels in size), was identical to that used in previous research (Britton & Tesser, 1982; Inhoff & Fleming, 1989). The other probe type was a word color change from white to yellow, and was included to determine the extent to which the flash probe interfered with content comprehension. The subject's task was to initiate the appearance of each word by pressing the keyboard space bar with the left hand and to respond to each probe by pressing the return key with the right hand. The dependent variables were probe response times and reading times for the target words and the two words following the target words.

Five measures of working memory were also included in the present study, three verbal and two non-verbal, all selected from the tests used in conjunction with the Learning Abilities Measurement Program at AFHRL (Kyllonen & Christal, 1988). The first verbal working memory measure was an alphabet order recall task (ABCD ORDER) in which the letters A and B were designated as Set 1 and the letters C and D were designated as Set 2. The order of the sets and the orders of the letters within each set were specified by a series of instructions such as the following:

Set 1 precedes Set 2

A does not follow B

C does not precede D.

The correct sequence is then selected from a list of all eight possible alternatives. Response time and accuracy data were recorded.

The second verbal working memory task (ALPHA WM) was a recoding task in which a sequence of up to three letters is presented, followed by a number ranging from -2 to +2. The subject's task was to determine those letters coming either before the presented letters in the alphabet (if the number was negative) or after (if the number was positive), and to choose the correct alternative from a list.

The third verbal working memory measure was a version of Daneman and Carpenter's (1983) span measure (READING SPAN) and combines a sentence verification task and a word recall task. The two sentences were initially presented and the number of sentences was increased to six across trials. The subject was asked to indicate each sentence to be true or false while maintaining the last word of each sentence in memory. The last word of each sentence was

recalled after each set of sentences was presented. Sentence verification and recall accuracy and response times were recorded.

The two spatial working memory tasks were presented last in the battery and consisted of the Kyllonen/Palmer Working Memory Test and the Tirre Visual Imagery Test. These measures were included in the battery partially to assess the relationships between the spatial and verbal components of working memory, and partially to assess the impact of spatial processing on concept mapping performance.

In the Kyllonen/Palmer Test, a two dimensional grid with three rows and three columns of dots is presented. A simple line drawing is added to the grid, and the subject's task is to retain an accurate representation of the grid and line drawing through a multidimensional rotation, flip, or both. A test stimulus is then presented, and a true/false response is required. For the Tirre Visual Imagery Test, the subject is shown a 2 x 2 matrix with random presentation of the numbers one through four in the four quadrants. Each number is then shown paired with four ambiguously shaped figures. A test matrix with the shapes is then presented and a true/false response is required. Accuracy and latency data were collected for both visual working memory tasks.

III. DATA ANALYSES

Due to unforeseen software incompatibility problems, the data analyses for this research are incomplete. The process of transferring floppy disk information to the mainframe computer at the University of Northern Colorado was more complex and time consuming than expected. Planned data analyses include multiple linear regression to determine those factors that affect conceptual understanding as measured by the concept map posttest, and the interaction of those factors with instructional text difficulty. Analysis of variance will also be employed to investigate group differences and the effects of probe type on working memory capacity utilization during reading. Factor analysis may be used to allow description of the interrelationships among verbal and nonverbal measures of working memory. In addition, detailed qualitative analyses of the nature of the conceptual relationships produced on the concept maps will also be performed. These will include verification of typical misconceptions of electricity found in the literature (e.g. Cohen et al., 1983), as well as characterization of the changes in those conceptual relationships between pretest and posttest maps (especially as a function of the intervening text difficulty level). These analyses will form the basis for revisions and additions to the battery for future data collection.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

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FINAL REPORT

Investigation of Color Appearance within Low Light Levels

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I. INTRODUCTION:

My research interests have been in the area of individual differences in the perception of color and temporal sensitivity. Specifically, my studies on the relationship of iris pigmentation and textureless chromatic displays (Mandra and Anderson,1984) and critical flicker fusion (Mandra and Jordan, 1979) contributed to my assignment to the Human Resources Laboratory/OTD.

The Primary purpose of this study is to investigate color appearance within low light levels (mesopic range), and secondly, to determine the extent to which individual differences can account for the large amount of variability typically found when humans make judgements of hue, saturation and luminosity. The Human Resources Laboratory/OT has been interested in the judgement of color appearance in flight simulator displays. The presentation of computer simulated displays in the dome occurs at low light levels which falls into the range of mesopic luminance; a range which involves a unique interaction of rod and cone functioning. The database which would allow for more accurate predictions of color appearance within the mesopic range is incomplete. The present study provides information on the appearance of color brightness at mesopic levels of luminance. A second issue is concerned with the large variability in human judgements of color appearance. Sources of inconsistent judgment may be due to age, gender and iris pigmentation. Past research demonstrates that individual differences play a role in color appearance, however such variables have not been simultaneously tested in the mesopic range of luminance.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The objectives of the research are to provide a database which describes how bright colors appear as a function of hue, saturation levels and individual differences when viewed under three luminance levels: a low photopic level (near 10 nits) and two mesopic levels (near 1 and 0.1 nits). The experiment employed the method of heterochromatic brightness matching (HCBM) to investigate apparent brightness of colors and is distinguished from brightness equivalence determined by other methods such as flicker photometry or minimally distinct border. Operationally, HCBM for a test color in this experiment is defined as the measured luminance of an adjacent achromatic gray which has been adjusted to match it. If HCBM is represented as B and if L represents the measured luminance of the chromatic patch, then the ratio B/L describes the relation between the HCBM of color and its measured luminance. If measured luminance were a perfect indicator of HCBM, the B/L ratio would be one. It was expected that the B/L ratio would be greater for reds than for blues at a luminance level of ten nits, reflecting the greater contribution of cone elements at that level and their greater sensitivity to red than to blue light. It was also expected that the B/L ratios would change at the lower luminance levels, reflecting the relatively greater contributions of rod elements at 1 and 0.1 nits. Since rods are more sensitive to blue than to red, the B/L ratio is expected to be higher for blues than for reds at 0.1 nits. For the saturation series SA, SB, and SC it was expected that the B/L ratio would be higher for colors with higher chroma values than for less saturated colors. Individual differences such as age has already been related

to differences in color perception due to a yellowing of the lens (Weale, 1963). It was expected that B/L ratios would demonstrate a complex interaction between hue and luminosity levels. Finally, iris pigmentation has been related to hue and afterimages (Wallace, 1979), short-wave sensitivity was found to be related to variation in density of macular pigmentation (Stabell and Stabell, 1980) which is correlated to iris pigmentation and lastly, Sommers and Fry (1974) suggest that brightness matching in the blue region is related to the effect of macular pigment. It was expected that density of iris pigmentation would be related to hue and luminosity levels in a complex interaction. There are no studies that systematically manipulate all these factors within the same experimental design. Thus it is important to conduct this type of study so that each of the variables and their interactions can be evaluated.

My assignment as a participant in the 1988 Summer Faculty Research Program (SFRP) was to design and execute a study which would evaluate the apparent brightness of color for a range of hues, saturation levels, luminances levels using subjects who differ in gender, age, and iris pigmentation. In addition, my assignment was to provide an analysis of the data to determine if the previously described variables affect judgments of apparent brightness, trials to make a match and the confidence one had that the match was correct.

III. Method:

a. Subjects.

Ten observers, five light-pigmented and five dark-pigmented irises, were selected from a pool of available personnel at Williams Air Force base in Arizona. Of the five light-eyes subjects , three were in the 20 to 30 year range (two males and one female) and two were in the 40 to 50 year range (one female and one male). Of the five dark-eyed subjects, three were in the 20 to 30 year range (one male and two females) while there were two dark-eyed subjects in the 40 to 50 year range (one male and one female). Thus, there were an even number of dark- and light-eyed subjects, an even number of males and females, but there were six subjects in the 20 to 30 year group while only four in the 40 to 50 year group. All subjects were judged to be color normal, as determined through a test for color defectives by using the Optical Society of America pseudoisochromatic plates. Finally, subjects who used glasses wore them during all sessions.

b. Apparatus and Calibration.

The stimuli were presented with a SG Iris Graphics System Hitachi monitor (CM2073A). A software program controlled all display characteristics, sequence of presentation and data storage. A mouse and mouse pad was used to produce all responses.

Calibrations were made on the monitor with a Photo Research Pr-703A Fast Spectral Scanner. Both luminance and chromaticity were measured in the chromatic and grey display fields twice daily, in the morning prior to data collection or training and at noon to insure that there were no changes. All observations were taken in a darkened room using either no neutral density filter (NDF) or one (NDF of density approximately 1.0) or

two NDFs to produce the luminance conditions. Luminances for the colors and for the gray series were always measured without the NDFs. Values of luminance for the two dimmer levels were derived computationally from the filter transmissions and the spectral energy distributions of the test fields. A mask was placed around the border of the monitor to limit stray light and in addition, a mask was placed 45 cm in front of the monitor which had a 9 x 9 cm opening to hold the filter. A chin rest was also used.

c. Stimuli for Training and Testing.

All stimuli were presented in a bipartite field (15 degree visual angle) which was framed by thin black lines. The luminosity of the standard stimuli was set at 10 cd/m2. and were presented against a common bright white background having a luminosity of 50 cd/m2. The variable stimuli always consisted of a gray stimulus whose luminosity was randomly selected to be either lighter or darker than the standard. The luminosity measurements of the various grays was determined by measuring a wide range of grays (in graded steps) in the morning and rechecking those values in the afternoon. Since all possible grays could not be measured, interpolation procedures were used to determine the luminosity values of the brightness matches whose grays were not actually measured. Training stimuli consisted of five sets of five pairs. Each of the five pairs was repeated six times in a predetermined random order resulting in a total of 30 trials per set. The first set consisted of five different gray standards paired with the variable grays. The second and third sets consisted of five heterochromatic pairs each; the five standards for these two sets consisted of low saturated (Munsell 5/2) chromatic stimuli paired with a

variable gray. Each of the fourth and fifth sets included five heterochromatic pairs which were more saturated (Munsell 5/4) than the previous sets. The u' and v' coordinates for the test colors are displayed in table 1. The test stimuli consisted of five sets of five standards. They are conveniently presented below:

Munsell Notation For Test Colors

HE	HF	SA	SB	SC
5R 5/6	5YR 5/6	5GY 5/8	10BG 5/6	10GY 5/10
5Y 5/6	5GY 5/6	5GY 5/4	10BG 5/4	10GY 5/6
5G 5/6	5BG 5/6	5P 5/4	10R 5/4	10P 5/6
5B 5/6	5PB 5/6	5P 5/8	10R 5/6	10P 5/10
5P 5/6	5RP 5/6	5P 5/12	10R 5/10	10P 5/16

The HE and HF series comprise hue circles, all at chroma (saturation level) six . The SA, SB, and SC series were saturation series, each arrayed along a complementary hue line (green-yellow to purple or blue-green to red) but differing in chroma.

d. Procedure.

The training and test phases were very similar with the following exceptions: training sessions did not use any filters, feedback was provided by way of a digital code which indicated the relative position of the last judgment. Subjects had been instructed to make judgments as consistent and accurate as possible. Training began with the gray set, then proceeded with low saturated sets and ended with the more saturated sets. The amount of training on each series was very similar for all subjects; training stopped on each set when the subjects standard deviations for their matches was approximately 1.0 or no further decrease was evident. All sessions began with a three minute dark adaptation period. During the experimental sessions, an additional three minutes was given when the two

filter condition (NDF = .1) was used. During the color training and experimental procedures, the observers looked at a bipartite square with a color on the left side and a neutral gray on the right side. Both appeared against a brighter white background with a narrow dark boundary separating the three display areas from each other. Using a mouse and mouse pad, the subjects were instructed to adjust the brightness of the gray area until it appeared to be just as bright as the colored area. To avoid afterimages, the test fields appeared for only two seconds out of every ten seconds; during the eight second blank interval, the white background filled the test fields, although the dark boundaries remained visible and served as a fixation point. During the eight second interval the observers made their match and then were presented a confidence line which had markings from one-to-ten. The extreme values were labeled as Not Confident (one) and Very Confident (ten). The observer could indicate her choice by moving a cursor along the line which was controlled by a mouse. The observers were allowed to look at a pair of test fields as many times as they wished before signaling that they had achieved a satisfactory match. Each of the five colored stimuli were repeated six times before the session was ended. The observers typically completed one session per day but on occasion were able to complete two sessions. The second session would repeat the same color set but used a different filter condition. The computer recorded the number of times a test color was viewed, as well as the digital code for the gray selected as a brightness match and the confidence judgements.

IV. RESULTS:

The scores used for analysis include Brightness-to-Luminance ratios (B/L), the number of trials to achieve a match (TL), and a confidence rating of the certainty that the match is correct (CR). Each of the three dependent measures were averaged over six matches made for each of 25 stimuli. To facilitate analysis, three separate sets of stimuli were formed and each set was analyzed separately. The first set included 10 of the 25 stimuli presented during two separate sessions. All hues in this set were equal in luminosity, saturation and were evenly spaced around the Munsell color wheel : 5R 5/6, 5YR 5/6, 5Y 5/6, 5GY 5/6, 5G 5/6, 5BG 5/6, 5B 5/6, 5PB 5/6, 5P 5/6, 5RP 5/6. The formation of the two remaining sets was based on similarity of wavelength composition while varying saturation levels. Thus the second set included 9 hues which were red and purple colors differing in saturation: 10R 5/4, 10R 5/6, 10R 5/10, 5P 5/4, 5P 5/8, 5P 5/12, 10P 5/6, 10P 5/10, 10P 5/16. The last six hues formed the final set and included middle wavelength stimuli differing in saturation: 5GY 5/4 5GY 5/8, 10GY 5/6, 10GY 5/10, 10BG 5/4, 10BG 5/6.

Set 1 Analyses.

B/L Ratios. The B/L data were submitted to a 2 (Iris Pigmentation) X 2 (Age) X 3 (Filter) X 10 (Hue) analysis of variance with repeated measures on the latter two variables. The results yielded a significant interaction between the Hue X Filter condition, $F(18,108) = 2.896$, $p < .001$ and a significant four way interaction: Iris Pigmentation X Age X Filter X Hue , $F(18,108) = 1.784$, $p < .05$. No other effects were significant (p's > .05). The means, standard deviations, and coefficient of variability for the Hue X Filter interaction are found in Table 1 for all three sets of stimuli. In

addition the Munsell notation and corresponding 1976 CIE u' v' coordinates are listed along with the Munsell notation. In order to facilitate comparisons between color set A and color stimuli of the other two sets all 25 hues have been plotted as a function of filter condition in figure 1. Furthermore, it is possible to make comparisons between the different sets by observing the connecting line drawn between the 14 data points having the same saturation level (six) as noted in the legend. A comparison of the means for the five principle hues (5R,5Y,5G,5B,and 5P) used in the current analysis (set A) demonstrate little change in mean B/L Ratios for the no-filter and one-filter conditions but an increase for the two-filter condition. In contrast, the five intermediate hues (5BG,5GY,5YR,5RP,and 5PB) showed a steady increase in the mean B/L Ratios as the display became dimmer. Thus observers required more white light to make a brightness match for the intermediate hues relative to the principle hues for both filter conditions. It is interesting to note that only the hue 5R does not show any appreciable change in B/L ratio (approximately 1.0) for the three filter conditions. Also, only the 5Y hue required less white light to make a brightness match for all three filter conditions (the B/L ratios were less than one). Lastly, there seems to be a transition point at or around the 5GY hue for all saturation levels (Munsell 6,8,and 10). These findings are further complicated by the significant four way interaction between Eye-Color, Age, Filter Condition, and Hue as noted above. The overall pattern of results suggest that for observers with a more heavily pigmented iris (dark-eyed) who were also in the older age group (40-50 yrs.), the mean B/L ratios were lower than their younger dark-eyed counterpart for all hues across all three filter conditions. The mean B/L ratios taken across all

hues for the dark-eyed observers within the Age X Filter conditions are: NDF=0, 20yrs vs 40yrs, mean B/L ratios were .944 and .760 respectively; NDF=1, 20yrs vs 40yrs, mean B/L ratios were 1.177 and .722 respectively; and finally for NDF=2, 20 yrs vs 40yrs the mean B/L ratios were 1.079 and .911 respectively. This pattern was not evident in the same Light-eyed groupings. The B/L ratios were more equivocal for the no-filter and one-filter condition (e.g., approximately 1.0) but for the two-filter condition, all the observers in the light-eyed, 40+ group had slightly higher mean B/L ratios for all hues. The mean B/L ratios for the two age groups were: .981 and 1.091, 20yrs. and 40yrs respectively. In general, the light-eyed subjects had slightly higher mean B/L ratios than their dark-eye counterparts for all filter conditions (e.g., for the NDF=0, NDF=1, and NDF=2 conditions, the mean B/L ratios are 1.01 vs .852, and 1.024 vs .950, and 1.036 vs .995 respectively). Thus the dark-eyed observers required less white light to match the ten hues in Set A for all filter conditions. It is of interest to make comparisons of the variability associated with the B/L ratio judgments for the two significant interactions. Both the standard deviation (measure of absolute dispersion) and coefficient of variability (measure of relative dispersion) were calculated for the Filter X Hue conditions. Both indices of variability show a decrease in range as the stimuli became dimmer. The range of SD's and CV's for the NDF=0, NDF=1, and NDF=2 were .31 and .25, .16 and .18, .11 and .13 respectively. The SD's are less than one-third of those reported by Booker (1981) but the CV's are somewhat similar to those reported by Booker (CV grand mean = .32) for the NDF=0 and NDF=1 conditions (both means = .27) vs the NDF=2 condition (mean = .15). The SD's associated with the mean B/L ratios for the four way

interaction (table 2) show a pattern which is related to the eye-color X age conditions. To begin with, almost every SD associated with the dark-eyed observers is higher than the SD's associated with the light-eyed observers. There are only four exceptions which are due entirely to the 40+ light-eyed observers in different hue conditions; of these exceptions, two are negligible. When comparing the SD's of the Dark- and Light-eyed observers within the age X filter condition for all hues, the following pattern emerges: for the 20+ and 40+ year dark-eyed observers, there is an increase in the SD's for all hues in the NDF=1 condition relative to the other filter conditions which are approximately equal. The drop in the SD's in NDF=2 condition is much greater for the 40+ year group. This pattern is much less evident in the light-eyed 20+ year group while the 40+ year group shows higher SD's for the NDF=0 condition relative to both other filter conditions.

Trials and Confidence Ratings. The mean number of trials required to match the chromatic and achromatic stimuli was analyzed using a design identical to the B/L ratio data. The results demonstrated significant effects for Hue, $F(9,54) = 2.18$, $p<.05$; Hue X Eye-Color, $F(9,54) = 2.857$, $P<.01$; Hue X Age, $F(9,54) = 2.345$, $p<.05$; Hue X Eye-Color X Age, $F(9,54) = 3.354$, $p<.001$; Hue X Filter, $F(18,108) = 2.661$, $p<.001$; and finally Hue X Filter X Age, $F(18,108) = 2.127$, $p<.01$. When both measures are contrasted with each other, it appears that B/L ratio matches requiring a greater number of trials were also judged as less confident by observers. In other words, although more difficult matches required more repetitions observers were still less confident about their choices. It is important to point out that these confidence judgments were not reliably different. The finding of an

Eye-Color X Age interaction, $F(1,6) = 6.056$, $p<.05$ for confidence judgments was due entirely to the light-eyed, 40+ years observers who were less confident (mean = 3.30) than the light-eyed, 20+ (mean = 6.05) or either of the dark-eyed age groups (means 5.61 and 5.91).

Analyses of Sets 2 and 3.

B/L Ratios. The B/L ratios for Set 2 were submitted to a 2(Eye-Color) X 2(AGE) X 3(Filter) X 9(Hue) analysis of variance whereas Set 3 involved a 2(Eye-Color) X 2(Age) X 3(Filter) X 6(Hue) ANOVA. Set 2 includes six purple hues varing chroma and saturation level as well as three red hues varing in saturation level. Set 3 includes four GY hues varing in saturation and two BG hues varing in saturation. The only significant effects found for Set 2 were the Hue X Filter interaction, $F(16,96), = 2.564$, $p<.01$. Similarly, the Hue X Filter interaction was significant for Set 3, $F(10,60) = 2.596$, $p<.05$ (Figure 4). Two general patterns emerge from a comparison of the NDF=0 and NDF=1 filter conditions. The first is that more saturated the hue is, the greater the B/L ratio with the exception of 5P and 5GY. The second general finding is that for all filter conditions, the effects of satuaration decrease as the stimulus display becomes dimmer. In the dimmest condition, NDF=2, the effects of saturation reversed for the 10R hue condition. These data are in partial agreement with Booker (1981) and Sanders and Wyszecki (1963) who found that, the B/L ratios increase as saturation increases. Both studies note some exceptions to the general rule in both the higly saturated blue region and the yellow region. In the present study, the B/L ratios for the 10R hues(/4, /6, and /10) decreased as saturation increased for the NDF=2 condition. The latter effect is in agreement with a model devised by Kokoschka (1972 cited in Wyszecki &

Stiles,1982) which predicts that under mesopic luminous conditions, red cones are increasingly inhibited.

Trials and Confidence Ratings. The ANOVAS performed on both Trials and Confidence Ratings were identical and included Eye-Color, Age, Filter and Hue variables as described above. The pattern of results was very similar for the trials data of Sets 2 and 3: significant effects were found for Hue, Hue X Filter, and Hue X Filter X Age. For Set 2, the effects were $F(8,48) = 4.414$, $p<.001$; $F(8,48) = 1.791$, $p<.05$; and $F(16,96) = 2.136$, $p<.01$ respectively. In addition, a Hue X Age interaction was found for Set 2, $F(8,48) = 2.441$, $p<.05$. For Set 3, the effects were $F(5,30) = 4.05$, $p<.01$; $F(10,60) = 4.292$, $p<.001$; and $F(10,60) = 2.213$, $p<.05$ respectively. The Hue X Filter interaction affords an opportunity which would allow a comparison with the B/L ratio analysis and will be discussed. For both Sets, the number of trials required to make a match for all hues in the NDF=0 filter condition were more than for the NDF=2 filter condition. The NDF=1 filter condition was tended to be intermediate to the other two conditions. Thus, as the stimulus display became dimmer, fewer trials were required to make matches. Analysis of the confidence ratings for Set 2 revealed a significant interaction for the Eye-Color X Age condition, $F(1,6) = 8.315$, $p<.05$. The effect is primarily due to the Light-eyed, 40+ observers rating their confidence lower (mean = 3.12) than either the other Light-eyed, 20+ group or the two Dark-eyed groups (means = 5.89,5.74 and 6.16 respectively). The ANOVA for Set 3 yielded significant effects for Eye-Color, $F(1,6) = 7.518$, $p<.05$; for Age, $F(1,6) = 6.532$, $p<.05$; and for the Eye-color X Age interaction, $F(1,6) = 12.035$, $p<.05$. As before, the Light-eyed observers had a lower confidence rating that the other groups (mean = 3.3 vs 6.45,6.43 and 5.81).

V. RECOMMENDATIONS:

The present study provides important data concerning the appearance of color within mesopic luminance levels as well as the importance of individual differences in making heterochromatic brightness matches. If the data of the present study can be generalized, then the B/L ratios presented in table 1 may be used to adjust the color display in the dome for some average trainee. However, because of the finding that Eye-Color and Age interact with Hue and Luminance conditions, a more appropriate adjustment of the color in dome displays would involve data from table 2. Since, it is not likely that a trainee would be in the 40+ year range, only the 20+ year range should be given serious consideration. According to the data of the present study, the density of iris pigmentation (Eye-color), which is correlated with macular pigmentation, contributes to the brightness of some colors. Thus, the B/L ratios could be used to alter the luminosity of a chromatic stimulus depending upon the eye-color of the trainee. Additional hues need to be studied along with a range of background colors since real or simulated displays involve multiple colors in a single display. How color interactions affect brightness of hues needs further investigation.

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Table 1. Mean Brightness-to-Luminance (B/L) Ratios, Standard Deviations and Coefficient of Variation for Hue and Filter Conditions.

Color System Munsell Notation	1976 CIE u'	v'	Filter = 0 Mean	SD	CV	Filter = 1 Mean	SD	CV	Filter = 2 Mean	SD	CV
5R 5/6	.2687	.4801	0.97	0.22	.23	1.01	0.31	.30	0.98	0.16	.16
5Y 5/6	.2306	.5349	0.91	0.24	.26	0.91	0.31	.34	0.97	0.19	.20
5G 5/6	.1517	.4897	1.01	0.25	.25	1.01	0.32	.32	1.06	0.16	.15
5B 5/6	.1528	.4193	1.01	0.27	.27	1.00	0.38	.38	1.06	0.10	.10
5P 5/6	.2173	.4147	1.01	0.32	.32	1.02	0.38	.37	1.03	0.18	.17
5YR 5/6	.2644	.5126	0.87	0.18	.20	1.00	0.24	.24	0.96	0.19	.19
5GY 5/6	.1877	.5321	0.89	0.19	.22	0.98	0.24	.24	0.94	0.13	.14
5BG 5/6	.1463	.4561	0.94	0.21	.22	1.09	0.22	.20	1.07	0.13	.12
5PB 5/6	.1796	.4045	0.90	0.24	.27	1.07	0.22	.21	1.09	0.13	.12
5RP 5/6	.2519	.4493	0.91	0.20	.22	1.02	0.30	.29	1.03	0.17	.16
5GY 5/8	.1828	.5490	0.94	0.24	.26	1.00	0.27	.27	0.94	0.10	.10
5GY 5/4	.1929	.5107	0.90	0.17	.19	0.98	0.23	.24	0.95	0.10	.11
5P 5/4	.2117	.4306	0.94	0.20	.21	1.02	0.32	.31	1.03	0.14	.14
5P 5/8	.2229	.3991	0.95	0.24	.25	1.02	0.32	.31	1.07	0.13	.12
5P 5/12	.2333	.3698	0.94	0.23	.25	1.07	0.33	.31	1.10	0.16	.15
10BG 5/6	.1466	.4359	0.92	0.27	.29	1.07	0.26	.24	1.05	0.13	.12
10BG 5/4	.1635	.4452	0.90	0.22	.25	1.05	0.23	.22	1.04	0.11	.10
10R 5/4	.2462	.4853	0.91	0.22	.25	1.05	0.29	.27	0.95	0.13	.13
10R 5/6	.2713	.4968	0.92	0.23	.25	1.04	0.30	.29	0.91	0.14	.16
10R 5/10	.3229	.5158	0.95	0.28	.29	1.11	0.38	.34	0.89	0.21	.23
10GY 5/10	.1396	.5431	1.08	0.42	.39	1.09	0.24	.22	1.04	0.18	.18
10GY 5/6	.1649	.5134	1.00	0.32	.32	1.06	0.22	.21	1.03	0.17	.17
10P 5/6	.2355	.4298	0.99	0.33	.33	1.05	0.22	.21	1.04	0.15	.15
10P 5/10	.2578	.4083	1.02	0.38	.37	1.09	0.22	.20	1.05	0.14	.14
10P 5/16	.2900	.3764	1.10	0.48	.44	1.13	0.28	.24	1.06	0.20	.19

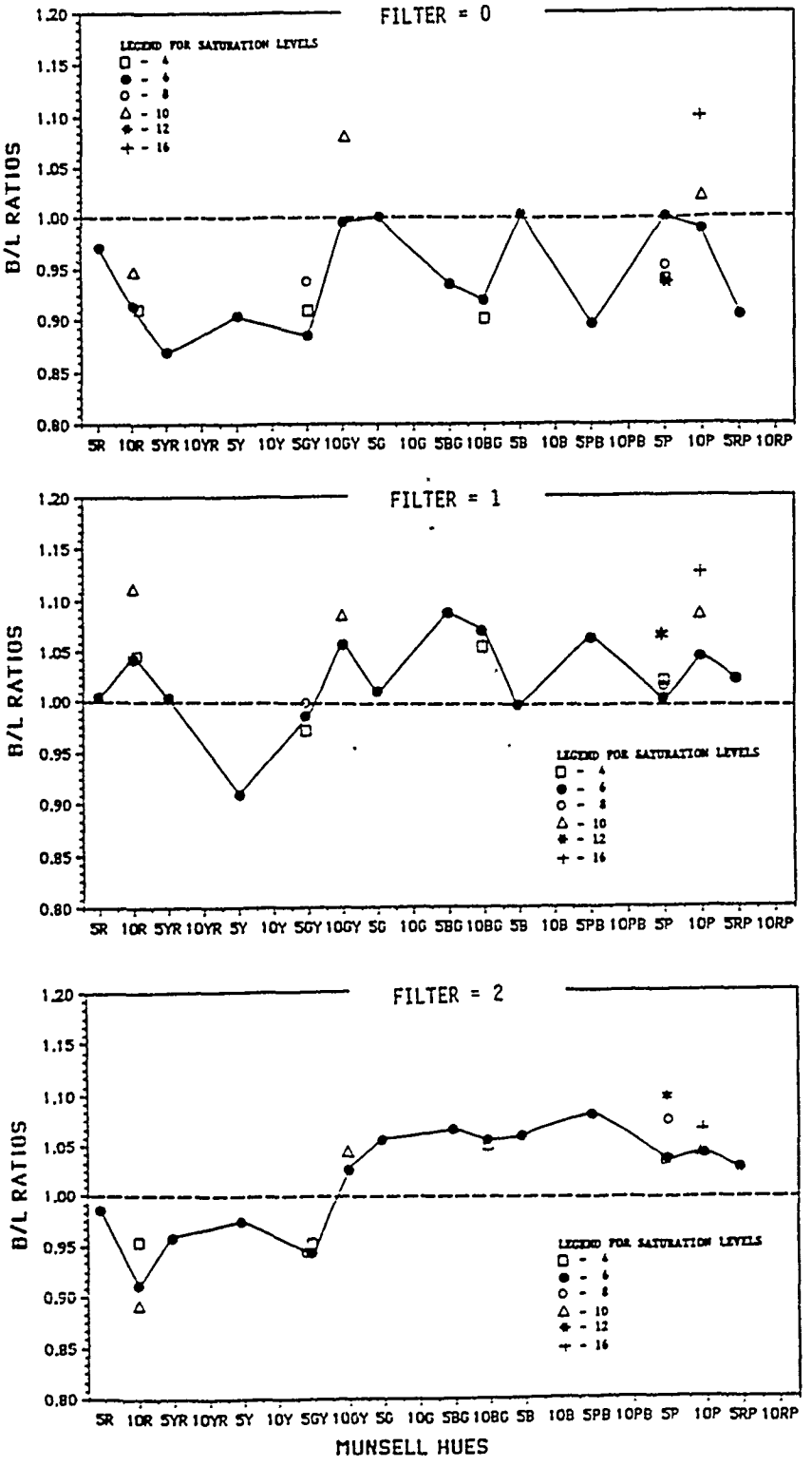
Note: Each data point represents an average of 6 matches across 10 subjects. Spatial separation of the five color series shows the stimuli that were matched within a session.

Table 2. Mean Brightness-to-Luminance Ratios, Standard Deviations for the Eye X Age X Filter X Hue Interaction

Filter		NDF=0				NDF=1				NDF=2			
Eye Color		Dark		Light		Dark		Light		Dark		Light	
Age		20+	40+	20+	40+	20+	40+	20+	40+	20+	40+	20+	40+
Munsell Hue													
5R 5/6	M	0.95	0.75	1.09	1.03	1.14	0.66	1.02	1.13	1.05	0.88	0.94	1.05
	SD	.25	.35	.03	.21	.33	.52	.08	.13	.26	.09	.11	.07
5YR5/6	M	0.84	0.83	0.96	0.84	1.12	0.82	1.06	0.92	0.97	0.82	0.94	1.11
	SD	.20	.32	.13	.17	.36	.22	.16	.07	.28	.09	.08	.22
5Y 5/6	M	0.87	0.65	1.04	1.01	1.05	0.57	0.95	0.98	1.03	0.85	0.89	1.14
	SD	.27	.31	.05	.22	.30	.56	.09	.17	.29	.02	.10	.17
5GY5/6	M	0.88	0.76	0.96	0.93	1.12	0.78	1.04	0.91	1.03	0.89	0.89	0.94
	SD	.18	.41	.15	.04	.34	.24	.18	.07	.23	.07	.04	.001
5G 5/6	M	0.98	0.79	1.09	1.17	1.22	0.69	1.01	1.02	1.13	0.95	1.01	1.13
	SD	.27	.45	.03	.20	.38	.55	.10	.01	.28	.08	.04	.10
5BG5/6	M	0.95	0.85	0.95	0.99	1.22	0.89	1.08	1.08	1.12	0.97	1.03	1.13
	SD	.28	.39	.13	.15	.30	.30	.11	.03	.17	.16	.08	.12
5B 5/6	M	1.03	0.74	1.05	1.18	1.26	0.59	1.00	1.02	1.10	0.98	1.03	1.11
	SD	.33	.45	.08	.19	.42	.61	.13	.04	.16	.05	.05	.06
5PB5/6	M	0.97	0.77	0.93	0.87	1.20	0.87	1.08	1.04	1.14	0.96	1.07	1.13
	SD	.31	.34	.12	.37	.27	.33	.17	.05	.21	.15	.05	.05
5P 5/6	M	1.04	0.66	1.07	1.22	1.27	0.62	1.05	1.01	1.15	0.86	1.02	1.06
	SD	.36	.49	.04	.28	.41	.63	.10	.05	.25	.21	.07	.08
5RP 5/6	M	0.93	0.81	0.96	0.91	1.17	0.74	1.10	0.96	1.07	0.95	1.01	1.11
	SD	.23	.42	.09	.16	.38	.45	.16	.05	.25	.23	.06	.21

Note: Each data point represents an average of 6 matches across 10 subjects.

Fig. 1 MEAN B/L RATIOS FOR HUES AND SATURATION LEVELS



1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/
GRADUATE STUDENT RESEARCH PROGRAM

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FINAL REPORT

FISHEYE REPRESENTATION OF INFORMATION: IMIS USER INTERFACE

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FISHEYE REPRESENTATION OF INFORMATION: IMIS USER INTERFACE

by

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ABSTRACT

A computer interface capable of presenting information in a usable format improves the quality of human-computer interaction. A user often experiences difficulties in accessing and interpreting information because his knowledge of both the underlying structure of the information and the relationships between a currently observed information element and other elements in the structure is limited. Another problem typically occurs during routine human-computer interaction: the amount of information to be presented exceeds the amount of space provided by the display medium. One technique recently developed as a means of enhancing the quality of human-computer interaction is known as the fisheye iens viewing strategy. This technique presents information concerning a point of interest (focus point) in great detail; less relevant information is presented as more of an abstraction. In this manner, the global aspects of the entire information data structure are not eliminated from the user's view.

This report demonstrates how fisheye views are used as a mechanism for filtering details associated with maintenance data. Results to be presented extend the original fisheye concept. The first extension allows the fisheye technique to be applied to information described by any general network, not only information represented by tree graphs. In a tree network, arcs connect nodes that are at most one level apart such that the link between any two nodes defines a parent-child relationship. By allowing information to have a general network structure, additional "family" relationships are available (e.g., sibling or grandparent links). The second extension illustrates that fisheye views resulting from multiple focus point selections are possible. In these views, detail associated with each focus point is presented, and the global context associated with each point is maintained.

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I. INTRODUCTION:

The Air Force Human Resources Laboratory (Combat Logistics Branch of the Logistics and Human Factors Division) is currently developing an integrated computer-based information system to aid in tasks associated with aircraft maintenance. This system is known as the Integrated Maintenance Information System (IMIS). The purpose of IMIS is to provide a comprehensive information system, consolidating existing information systems and data bases used in aircraft maintenance. IMIS will provide a maintenance technician with a direct link to various maintenance information systems and data bases: supply data, historical data bases, and automated technical orders. IMIS is to provide diagnostic/troubleshooting recommendations, test procedures, appropriate graphics (e.g., locator diagrams, schematics), and obtain fault data from built-in tests. Eventually IMIS will provide specialized data for aircraft battle damage assessment tasks, enable technicians to order parts from supply, and feature an automated training capability. It is expected that IMIS will also enable the technician to receive work orders efficiently and report maintenance actions.

It is suggested that IMIS will provide the following benefits for Air Force maintenance technicians: (1) a reduction in the amount of training necessary for technicians to learn aircraft maintenance tasks, (2) ready and easy access to a comprehensive set of data, including management data and job aids, and (3) an improvement in the diagnostic capabilities of maintenance technicians. Thus, researchers and developers of the IMIS concept have proposed that IMIS will enhance technician productivity.

Discussions with Combat Logistics Branch personnel have indicated that human-computer interface issues associated with IMIS are of significant importance and should be addressed. One issue is the importance of effective information presentation: appropriate representations of maintenance information that enable ready interpretation by maintenance personnel and allow these personnel to operate the system easily and effectively. Thus, the development of any tools that can be incorporated into the IMIS interface to facilitate user-IMIS interface interaction is considered to be appropriate.

One IMIS feature is a portable, battery-powered computer intended for flight line maintenance tasks. (A research prototype of this portable computer is known as PCMAS.) Data entry and system inputs are performed through a keyboard consisting of a set of function keys, a numeric keypad, and a set of arrow keys for scrolling. Due to the portability characteristic, all relevant information must be presented on a visual display for which the amount of information displayed per viewing screen is likely to be restricted. This limitation becomes especially significant when multiple page ("foldout") diagrams or circuit schematics must be presented. Note that if a larger graphic is sufficiently reduced to fit a single display screen, its finer details are likely to be obscured. Typically this problem is solved by either displaying a hierarchy of functional units, or by displaying a fully detailed window with scrolling and/or zoom capabilities. The problem with the scrolling/zoom approach is that, while a technician can obtain detailed information about a specific subunit or subsystem, his perspective on how this subunit or subsystem fits into the entire system is lost (Gunning, 1987). In another example, a problem arises when trying to select the right amount of detail for presenting maintenance procedures to an experienced technician. Well developed formats for displaying detailed step-by-step procedures for the novice exist; however, the problem is in deciding how to abbreviate this information for the experienced technician. He remembers most of the major steps but has forgotten some specific details. Hierarchical levels of abstracted data obscure the facts he needs. Fully detailed procedures contain an excess of unwanted information.

Another IMIS feature is the Content Data Model (CDM). The CDM is AFHRL's neutral (declarative) maintenance data base. This data model defines the content of maintenance information elements and specifies relationships between these elements. CDM maintenance data is hierarchically structured. A top level element might, for example, describe a certain VEHICLE; successive lower levels might describe VEHICLE SYSTEMS, SUBSYSTEMS, and finally SUBASSEMBLIES. Users responsible for interacting with this type of structure and obtaining information from the data base are required to navigate through various levels of information. A navigational aid, intended to maintain a user's global orientation as he progresses through hierarchical data levels, would help in the accurate interpretation of IMIS information.

Consider a PCMAS scenario in which a technician is required to navigate through a number of screen displays to obtain solutions to diagnostic or troubleshooting problems. Note that navigation is required due to the inherent hierarchical structure of maintenance data. One risk associated with this type of prolonged navigational strategy is that the system user becomes disoriented and is lost within the interface. Typical symptoms of this type of problem within a PCMAS session might be that the technician is unable to determine how/why a given diagnostic or troubleshooting procedure will lead him to a problem solution or to understand the reasoning strategy underlying a system recommendation.

From the discussion in the preceding paragraphs, it appears that a navigational tool capable of enhancing users' abilities to interpret and locate information conveyed through the IMIS interface would be of special benefit. Incorporation of such a tool would aid in ensuring accurate interpretation and efficient searching of the following IMIS information: diagnostic test procedures, repair/maintenance procedures, and graphics.

II. OBJECTIVES OF THE RESEARCH EFFORT:

A computer interface capable of presenting information in a usable format improves the quality of human-computer interaction. A user often experiences difficulties in accessing and interpreting information because his knowledge of both the underlying structure of the information and the relationships between a currently observed information element and other elements in the structure is limited. Another problem typically occurs during routine human-computer interaction: the amount of information to be presented exceeds the amount of space provided by the display medium. Traditional solutions for solving both the navigational and information quantity problems described above are hierarchical menus, windowing, and scrolling/zooming features.

One technique recently developed as a means of enhancing the quality of human-computer interaction is known as the fisheye lens viewing strategy (Furnas, 1986). This presentation approach shows information related to a point of interest (focus point) in great detail and

more remote information in less detail. In this manner the global aspects of the entire information data structure are not eliminated from the user's view. The underlying motivation for incorporating a fisheye representation is to provide interface users with the proper balance between local detail and global context.

The research objective for the 1989 summer research period was to examine the fisheye strategy as a means of appropriately representing IMIS maintenance information. This formatting/presentation approach should enable IMIS users to access desired information easily, quickly, and accurately. Specifically, the summer research period was dedicated to implementing the fisheye viewing strategy on a subset of IMIS maintenance data. (A simple circuit schematic and the mechanical parts diagrams associated with the 20 mm Gun System were selected as candidate data items.) An interface for the fisheye facility was designed, and a prototype incorporating the selected data subset and the fisheye interface was developed. The prototype system will serve as an initial guideline for future fisheye implementation research.

III. THE FISHEYE LENS VIEWING STRATEGY:

In a poster entitled "New Yorker's View of the United States," now a classical example of the fisheye presentation strategy, information is provided at two levels. The New Yorker has detailed facts concerning features within his immediate domain (e.g. "Where is the closest post office?") and can still observe the relationships between features of his immediate domain and other distant but important features (e.g. "If I am traveling from New York to Phoenix and I must make an intermediate connection, does it make more sense to connect in Dallas or Seattle?").

The primary requirement for implementation of the fisheye presentation strategy is for information to be structured as a network. Each network node (information element) is assigned a metric known as its degree of interest (DOI). The DOI indicates the presentation value or priority of the information contained at the node, that is, whether the information is important enough to present on the display medium. Applying the fisheye concept to the presentation of interface information requires implementation of a DOI function. This

function assigns a DOI to each node, where the DOI value is the degree of interest a user has in viewing a particular information element for a given scenario. Furnas' (1986) implementation of the fisheye strategy was applied to graphs having tree structures, that is, acyclic, hierarchies of nodes originating with a top level root node and branching to nodes at lower hierarchical levels.

Each node's degree of interest is expressed as the difference of two values: importance and distance. For a network consisting of the set of nodes $\{x_1, x_2, \ldots, x_n, y\}$, where y is the focus point, the DOI function is given below:

$$DOI(x_i) = API(x_i) - D(x_i,y), \quad (1)$$

where $API(x_i)$ is the global importance rating of node x_i and $D(x_i,y)$ is the path distance between node x_i and focus point y. Importance rating $API(x_i)$ is actually the distance of node x_i from the root node. Note that the degree of interest assigned to a given node increases with its importance rating and decreases with its distance from the current information focus point. In addition arithmetically larger numbers imply a greater presentation value for information contained at a particular node. For example, suppose nodes x_i and x_j have degrees of interest $DOI(x_i) = 4$ and $DOI(x_j) = 10$, respectively. Given the focus point y, the information contained at node x_j has a higher presentation priority than information associated with node x_i. These priority values imply that if an interface user is currently viewing information specified at node y, node x_j provides information that is more useful to successful human-computer interaction than the information provided at node x_i.

Furnas' (1986) implementation also allows for the setting of DOI thresholds. By selecting a threshold level, t, and displaying the information content contained at nodes with $DOI \geq t$, fisheye views with varying degrees of information content can be displayed. By lowering the threshold level, a greater amount of information is displayed. Nodes having the highest DOI are placed in a zero-order fisheye view. The zero-order view is the least detailed of all views and contains the minimum amount of information necessary to show detail and

global context. As the order of a view is increased, more detail is provided. Furnas (1986) applied a fisheye approach to the presentation of C programming code (on-line text), as a navigation tool for a hierarchically structured data base, and with an interactive fisheye calendar that allowed a user to obtain hourly information for a given day, as well as weekly and monthly data.

Recently, a fisheye viewing strategy was applied to the presentation of topographic information (Hollands, 1988; Hollands, Matthews, McCann, and Carey, 1989). In a topographic network, nodes represent locations within a geographic environment. A fictitious subway map was developed to implement fisheye views. Two tasks for using the map were selected: (1) determining the shortest route from one station to a given flashing destination station and (2) selecting the shortest route through a set of specified stations.

IV. MAINTENANCE DATA AND FISHEYE VIEWS:

This report demonstrates how fisheye views are used as a mechanism for filtering details associated with aircraft maintenance data. The results to be presented extend the original fisheye concept. The first extension allows the fisheye technique to be applied to information represented by any general network, not only information represented by tree graphs. Note that in a tree network, arcs connect nodes that are at most one level apart such that the link between any two nodes represents a parent-child relationship. By allowing information to have a general network structure, additional types of information element relationships are available. The second extension illustrates that fisheye views resulting from multiple focus point selections are possible. In these views, detail associated with each focus point is presented, and the global context associated with each point is maintained.

As stated in the previous section, the primary requirement for implementing the fisheye presentation strategy is for information to be represented as a network or graph. Furnas (1986) applied his presentation strategy to information represented by a strict hierarchy, in which arcs connect nodes that are separated by a distance of at most one hierarchical level. A link between any two information elements demonstrates a parent-child (also system-

subsystem) relationship. By allowing data base information to have a structure represented by a more general network, additional "family" or system relationships are available, for example, sibling (system-system) or grandparent (supersystem-subsystem) links. It is interesting to note that system relationships, or links between functional units of a system, are not the only types of relationships available. Physical relationships typically shown to maintenance technicians through graphics (mechanical parts diagrams or circuit schematics) can also be introduced. For example, a network can indicate that Part A "snaps_into" Socket B or Output Pin 1 on Gate G1 "is_connected_to" Input Pin 2 on Gate G2.

A very simple example of the correspondence between a graphic and its underlying network structure is given in Figures 1 and 2. Figure 1 illustrates a schematic consisting of three major functional units: I/O units and the internal operational units of the circuit. Each unit in turn has its own components and subcomponents.

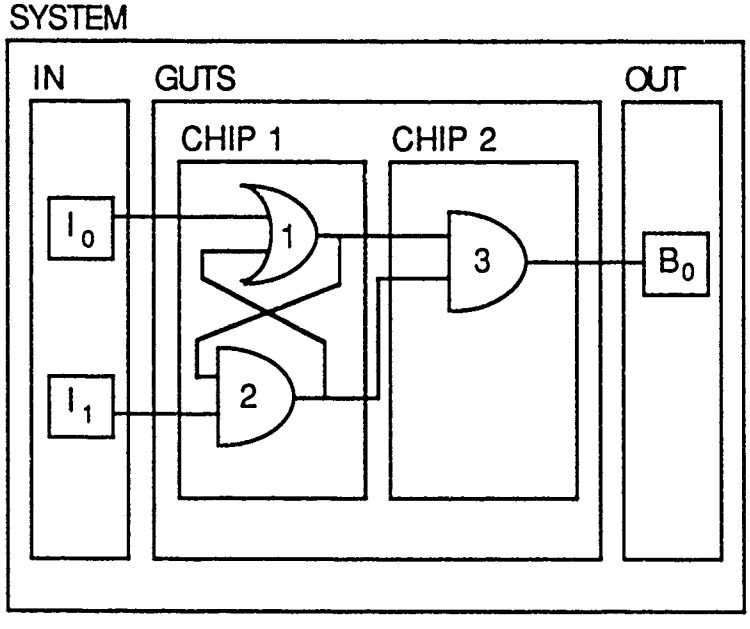


Figure 1. Simple Circuit Schematic.

Figure 2 is a network representation of this information. Note that in Figure 2, system hierarchy (functional) relationships are captured (e.g., the I/O unit named IN has components I_0 and I_1; Gate 1 (G1) has two inputs, i_{11} and i_{12}, in addition to an output o_1),

as well as physical relationships (e.g., Chip C1 is connected to Chip C2; input port I_0 is connected to Gate 1 (G1) input i_{11}).

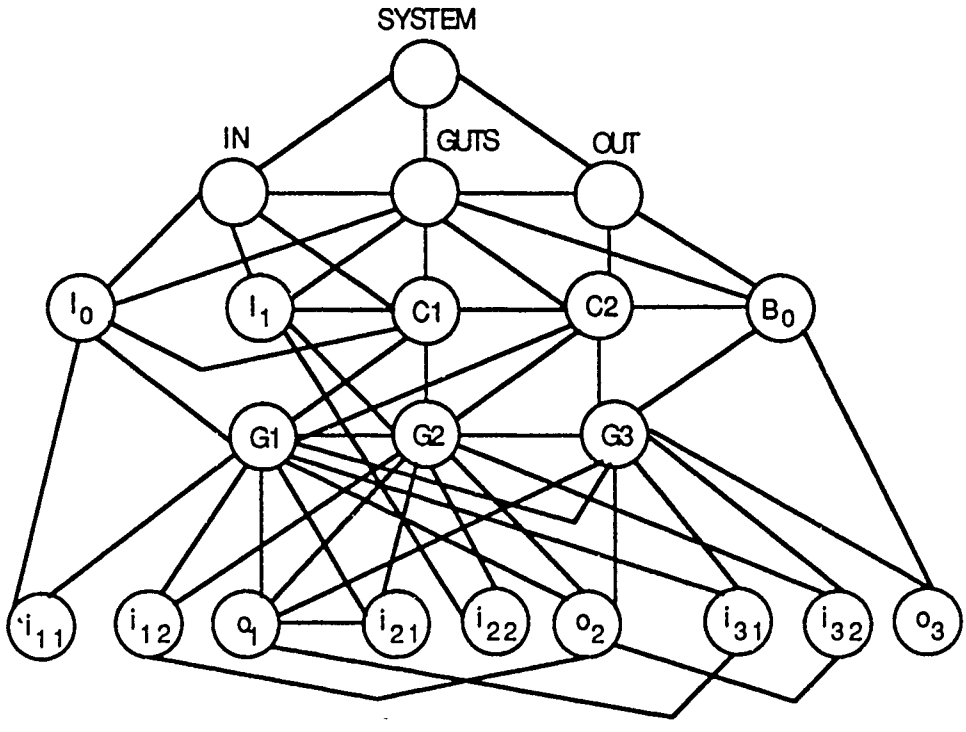


Figure 2. Network Representation of Circuit Schematic.

Furnas (1986) describes scenarios in which multiple focus points are of potential interest. In such instances, it is perhaps desirable for detail associated with two or more focus points to be important, while global context associated with each point is maintained. This type of interaction scenario is appropriate from a maintenance perspective. For example, a technician may be performing an electronics troubleshooting task, suspecting that two chips, C1 and C2, are likely to be responsible for a particular fault. Chips C1 and C2 would be the focus points. Fisheye views based on these focus points would provide detailed information about the physical connections and hierarchical relationships associated with each chip. Additionally, these views would provide global context information, that is, the physical and hierarchical relationships existing between C1 and C2 and the electronics system of which they are components.

To incorporate multiple focus points, a modification to the original DOI function given by Equation (1) is made. Degrees of interest for each network node x_i are calculated according to the following function:

$$DOI(x_i) = API(x_i) - \sum_j D(x_i, y_j), \tag{2}$$

where, for a node x_i, $DOI(x_i)$ and $API(x_i)$ are the degree of interest and the importance rating, respectively, assigned to node x_i; $D(x_i,y_j)$ is the minimum path distance between x_i and focus point y_j.

The importance rating, as defined by Furnas (1986), is actually the minimum path distance between node x_i and the root node, where distance from the root node is assigned a negative value. Equation (2) can be rewritten as follows:

$$DOI(x_i) = -D(x_i, root) - \sum_j D(x_i, y_j). \tag{3}$$

Thus, Furnas' original function given by Equation (1) in effect incorporates multiple focus points, if one views the root node as a focus point.

V. PROTOTYPE SYSTEM:

To demonstrate how the fisheye concept might be applied to aircraft maintenance data, a prototype system was developed. The primary motivation for implementing the fisheye presentation strategy was to provide a mechanism for filtering maintenance data. Conceptually, a fisheye environment would enable a technician to view additional detail only after a specific request. Example fisheye views were developed in HyperCard®. The information content presented in each nth-order view was determined from DOI values calculated from Equation (3). It should be noted that the HyperCard® environment will not serve as the final presentation medium; however, it was a convenient software tool for initially demonstrating the information filtering concept.

Several types of maintenance data were selected for fisheye presentations. These data were various types of graphics: circuit schematics and mechanical parts diagrams. Both types of graphics typically appear in repair manuals and technical orders used by Air Force technicians. Figures 3 and 4 are examples of actual mechanical parts diagrams as they might appear in technical orders. Figure 3 is an exploded view of a mechanical assembly called a clearing solenoid; Figure 4 illustrates how the clearing solenoid assembly is linked to a larger system of mechanical assemblies.

Suppose during a routine maintenance task, a technician is required to disassemble the clearing solenoid assembly. If screws 1 and 9 (Figure 3) are selected as focus points, Figures 5 and 6 illustrate how the fisheye algorithm was used to eliminate levels of detail from the original graphics. The MORE and LESS buttons allow access to higher and lower order views, respectively. Note that the physical and hierarchical relationships associated with these mechanical assemblies are defined through a network, where network nodes specify system components, and network arcs specify the types of relationships existing between these components. Hierarchical relationships define the functional structure underlying the mechanical system, for example, SUBASSEMBLY, PART, and LINKING DEVICE. Three examples of the hierarchical (functional) relationships established from Figures 3 and 4 are given below:

(1) clearing solenoid assembly IS_SUBASSEMBLY_OF rear housing assembly

(2) solenoid mounting plate IS_PART_OF clearing solenoid assembly

(3) screw 9 IS_LINKING_DEVICE_OF solenoid mounting plate.

Physical relationships establish physical orientations of elements in the mechanical system: ATTACHMENT, ADJACENCY, and INSERTION. The following physical orientations of various system components are described as follows:

(1) rigid connecting link IS_ATTACHED_TO solenoid plunger

(2) solenoid shell IS_ADJACENT_TO solenoid mounting plate

(3) solenoid plunger INSERTS_INTO solenoid shell.

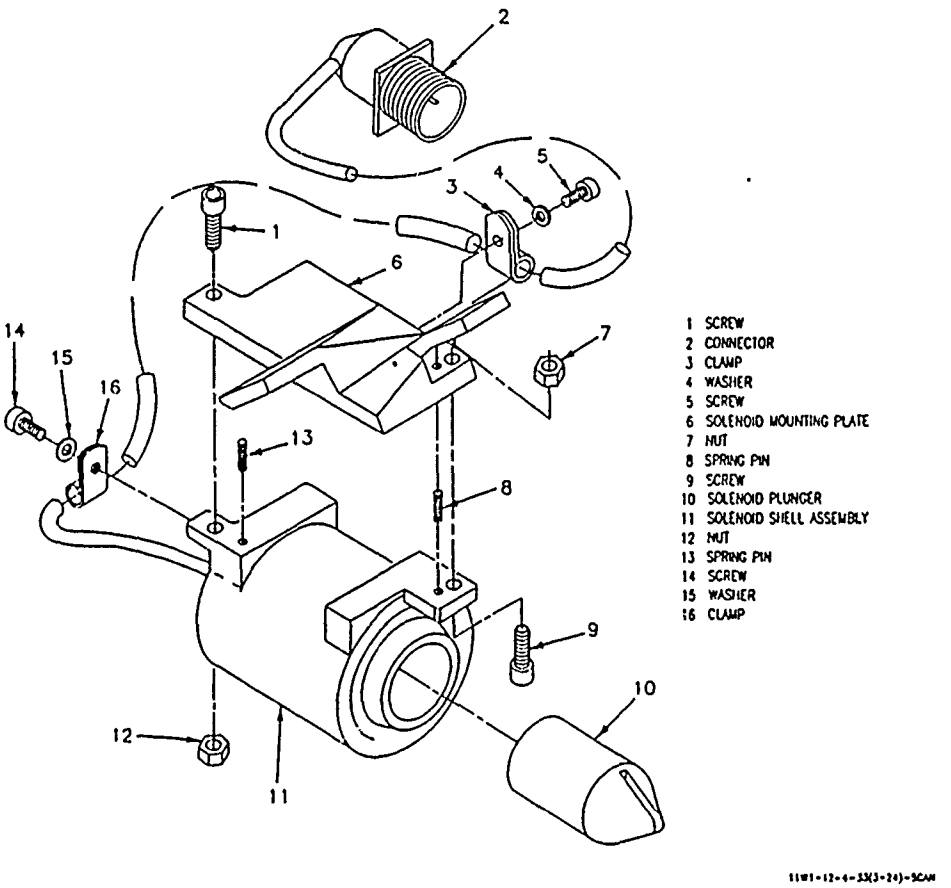


Figure 3. Clearing Solenoid Assembly.

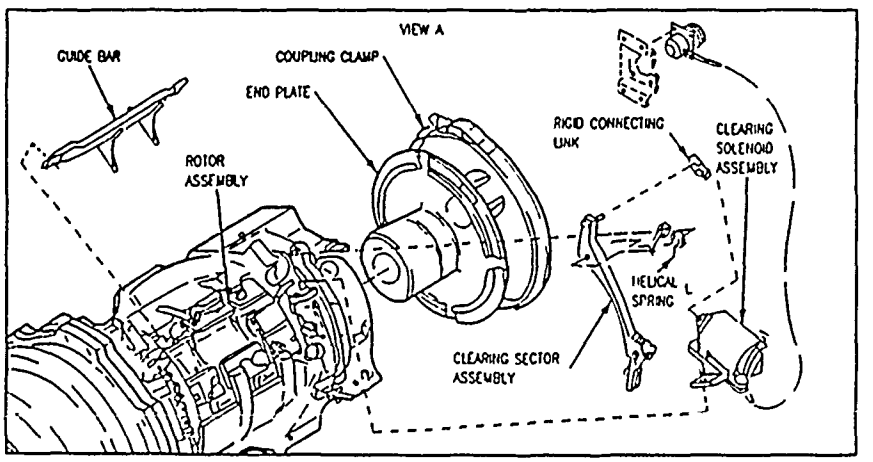


Figure 4. System S1 of Mechanical Assemblies.

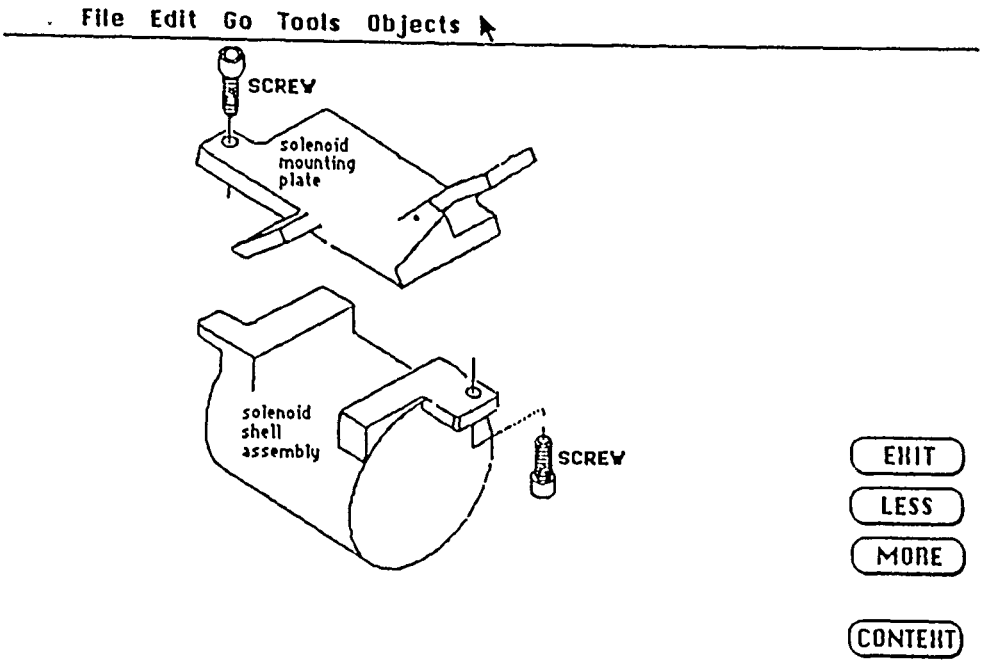


Figure 5. Clearing Solenoid Assembly: Zero-Order Fisheye View.

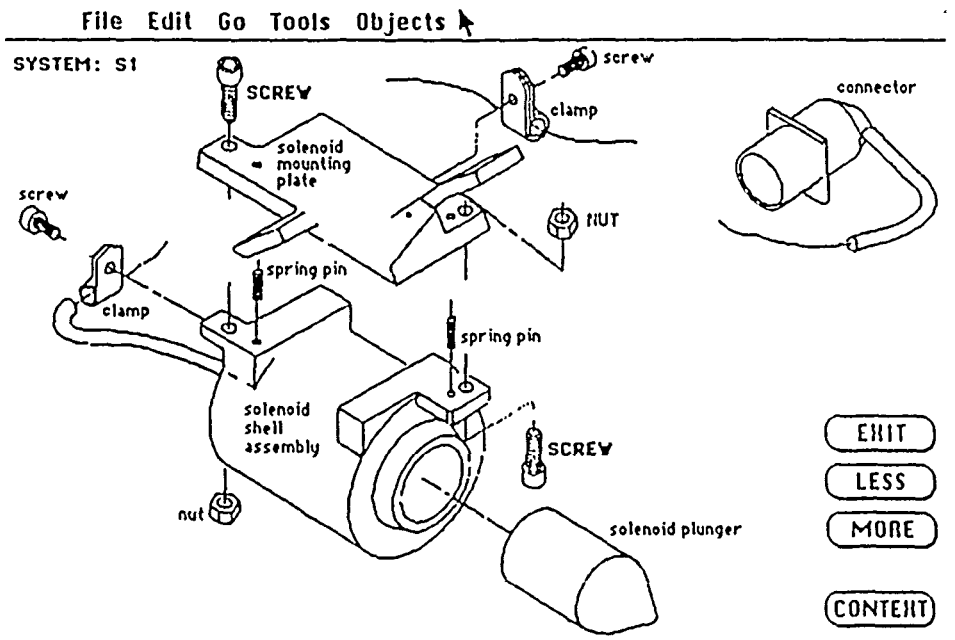


Figure 6. Clearing Solenoid Assembly: Third-Order Fisheye View.

The fisheye views of Figures 5 and 6 present both physical orientation and functional relationships. The CONTEXT feature provides global context information relevant to the clearing solenoid assembly and allows a user to view the clearing solenoid assembly as a function of a larger system (Figures 7 and 8).

In the graphics-based fisheye views of Figures 5-8, the actual functional relationships, as originally established, are not explicitly stated. However, functional information describing the organization of system components can be presented through text-based fisheye views, as illustrated in Figures 9 and 10. In these views, two focus points, the solenoid shell and connector, have been selected; they appear in boldface font to distinguish them from other system components. Figure 9 provides a second-order fisheye view of the system S1: (1) the solenoid shell serves as the FRAME for the clearing solenoid; (2) the connector (in conjunction with the solenoid mounting plate and solenoid plunger) is a PART associated with the clearing solenoid; and (3) the clearing solenoid is a SUBASSEMBLY of the rear housing ASSEMBLY. Figure 10, a fifth-order view, provides a more detailed perspective of system S1's organizational structure: a new functional relationship (LINKING DEVICE) is introduced, and additional FRAME, PART, SUBASSEMBLY, and ASSEMBLY relationships are defined.

VI. RECOMMENDATIONS:

The results of this research represent an initial examination of the fisheye presentation strategy and its potential application to the presentation of aircraft maintenance data. The intent of the research was to demonstrate how this particular presentation approach might be used as an information filter for an aircraft maintenance data base. Graphics-type data were considered. It should be noted from the examples of fisheye views provided in this report that further research on the design of a user interface is required. As presented in this report, the existing interface allows users to (1) access higher or lower order fisheye views (more or less information, respectively), (2) obtain additional global context, or (3) exit the fisheye environment.

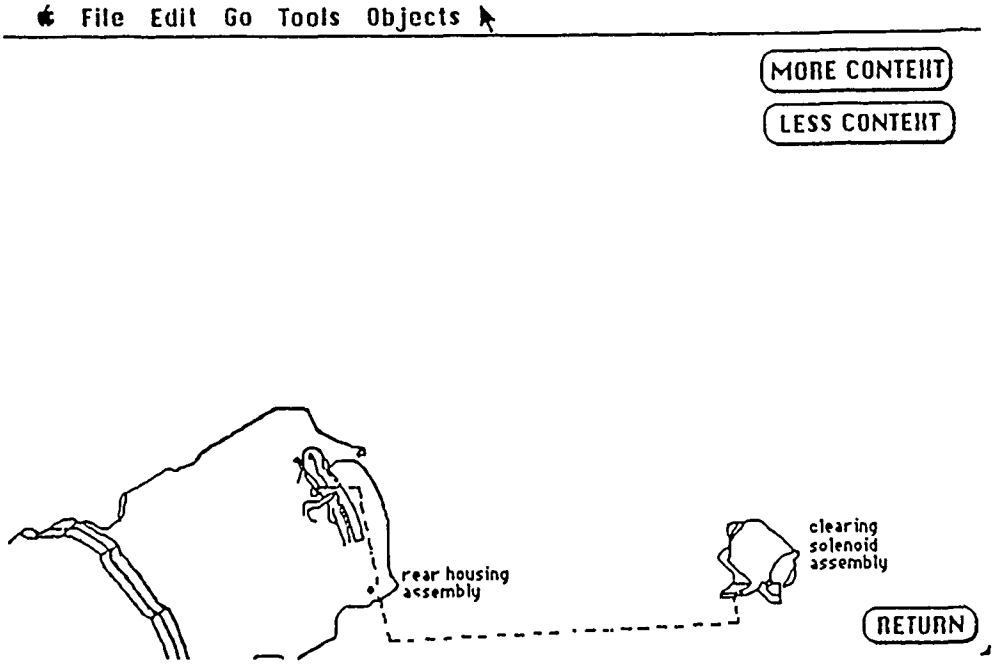


Figure 7. Global Context: First-Order Fisheye View.

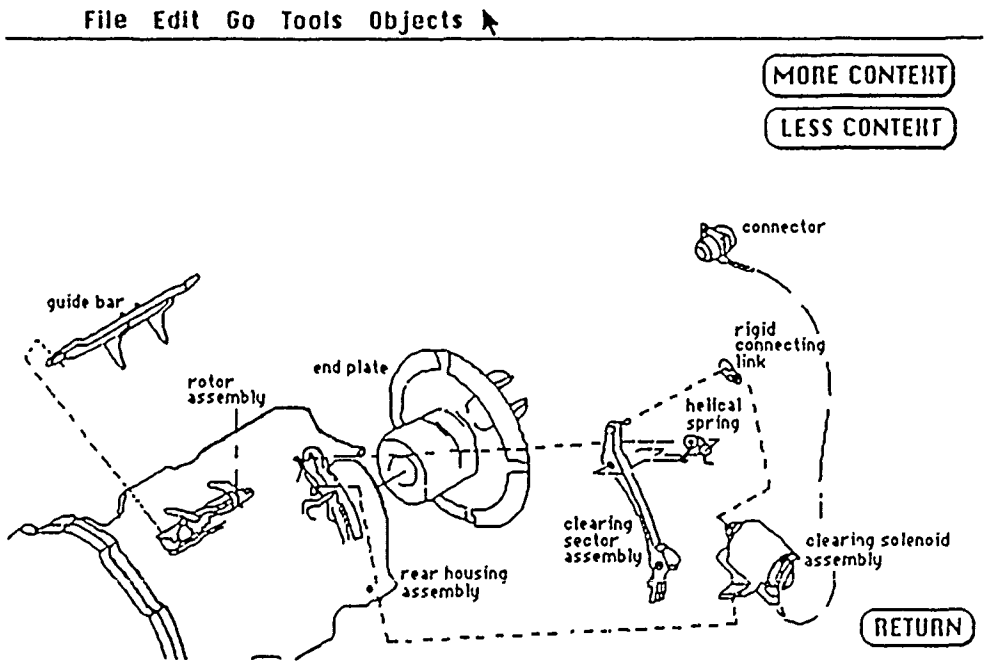


Figure 8. Global Context: Fourth-Order Fisheye View.

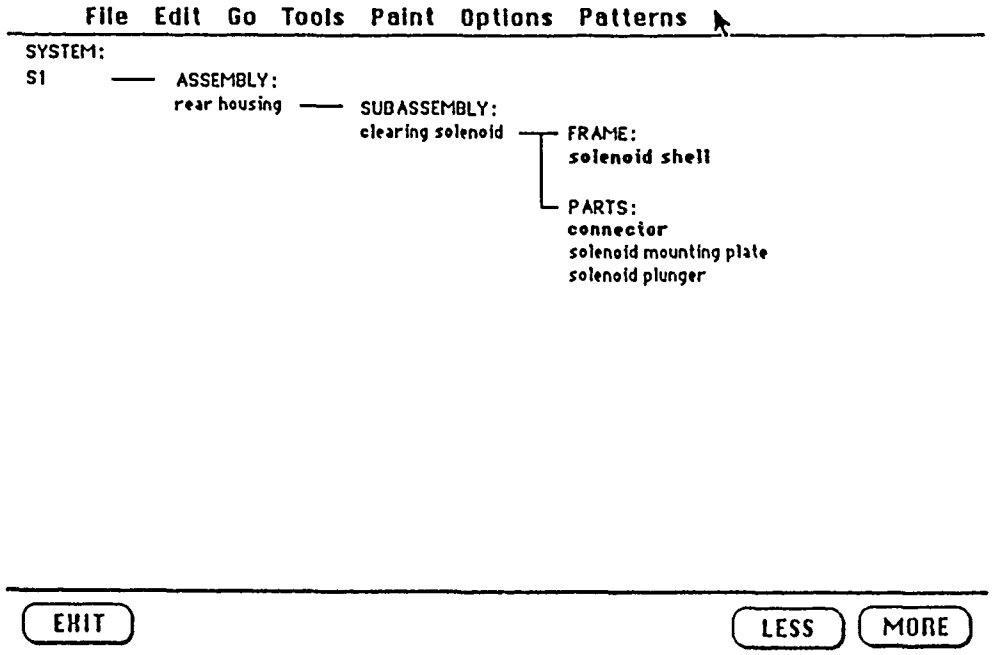


Figure 9. Organizational Structure of System S1: Second-Order Fisheye View.

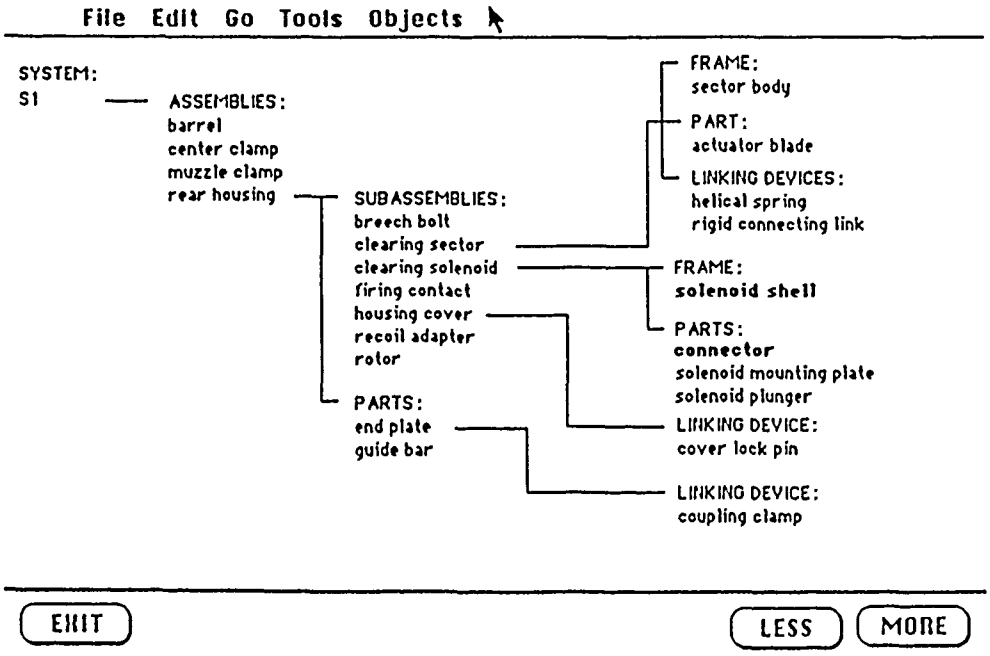


Figure 10. Organizational Structure of System S1: Fifth-Order Fisheye View.

It is suggested that several interface design issues require further examination. One issue is the selection of focus points. At this point, an emphasis has been placed on using the fisheye algorithm to determine what information should be presented for each nth-order view for a given set of focus points, the assumption being that focus point selection had occurred earlier. Thus, future research efforts might investigate how information should be selected, as well as what information should be presented. Design of an interface feature facilitating the process of focus point selection is suggested. Additionally, this feature should enable users to select new focus points from within any nth order fisheye view.

Another topic that merits further examination is the selection of "focus relationships," as well as focus points. In terms of the network defining a given system, focus relationships represent the network's arcs. Thus, in addition to providing a mechanism for the selection of information elements (or nodes), the fisheye interface would enable users to specify the links between information elements and in turn view relevant fisheye presentations. A user, for example, might wish to view varying levels of complexity associated with a CONNECTED_TO relationship. One problem resulting from focus relationship selection is the potential for the network to become disconnected. For any relationship R_i, we cannot guarantee that the R_i arc will be incident to all network nodes.

As with any research pertaining to human-computer interaction, the motivation behind the fisheye presentation strategy is to enhance the quality of interaction and thus improve human performance. Both Furnas (1986) and Hollands (1987) document somewhat of an improvement with a fisheye presentation approach. At this point it appears that more extensive experimentation is perhaps required to obtain an understanding of the full benefits researchers and designers might expect from this particular information presentation technique.

This research has demonstrated the results of using the fisheye presentation strategy as a mechanism for filtering maintenance information. Two extensions to the original concept have been presented. Information represented by a general network structure can be represented through fisheye views. In addition the original DOI function has been modified to incorporate the selection of multiple focus points.

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FINAL REPORT

The Validation of the Occupational Learning Difficulty (OLD) Index as a Predictor of Retrainee Performance

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Date:	21 July 89
Contract No:	F49620-88-C-0053

The Validation of Occupational Learning Difficulty(OLD) Index as a Predictor of Retrainee Performance

by

Miles E. Simpson

ABSTRACT

This study began a validation of the Occupational Learning Difficulty Index was to be validated through predicting of retrainees' time to upgrade and time to promotion of retrainees. retrainees' former Air Force Specialty's Occupational Learning Difficulty Index was to predict their advancement rates. The data was from the study of Air Force enlisted personnel who retrained between 1972 and 1978. A demographic analysis of the AFSs during this period revealed many major changes in the opportunity structure. Some AFSs lost most of their personnel; a few AFSs were relatively stable and a handful experienced expansion. Also, some AFSs were top heavy; others were bottom heavy. Validation of the OLD's Index or measures of individual characteristics must take the AFS's opportunity into consideration.

Acknowledgments

I thank the Air Force Systems Command, the Air Force Office of Scientific Research, and the Air Force Human Resources Laboratory for the opportunity to do this research. Universal Energy Systems provided solid administrative support.

I can't begin to mention all the HRL personnel who helped me this summer. But I thank them all. Special thanks goes to Mr. Wayne Archer and Dr. Bruce Gould of MOD for their guidance and cutting away the administrative underbrush. I thank Dr. Jacobina Skinner of MOA for graciously providing me the retrainee study data set and for the time and patience to explain its intricacies. Special thanks also go to Ms. Laurel Betz, Ms. Doris Black, and Ms. Janice Buchhorn for going out of their way to provide critical technical support. Others, who provided assistance on this project, include Ms. Nancy Maybi, and Airman Robert Zook, MOD. Administrative support was provided by Lt. Col. Larry Short, MOD, and Dr. Bill Alley, MO. I will also extend thanks to some of many persons who I have talked including Bill Phalen of MOD; Jim Earl, Dr. Lonnie Valentine, and Dr. Malcolm Ree of MOA, Dr. Jim Weeks of MOE and Dr. Bob Pakorny of MOM. Lastly, Dr. Michelle Lynskey who shared her office and micro-computer with me for ten weeks and Lieutenant Todd Dart who let me use his mini-computer to access the UNISYS computer. I want to give a special thank you to Nancy Maybi who put together the AFS tables and set them up on a mini computer.

I. INTRODUCTION:

Manpower, Personnel, and Training requirements change with the introduction of new technologies and missions. Trade-offs and potential shortfalls of critical personnel need to be anticipated and worked through before a new project is undertaken. Once a new position and its tasks are identified, reliable estimates of the training and ability levels must be made available to planners. AFHRL developed the Occupational Learning Difficulty (OLD) measure to meet such a goal. Two studies provided indirect evidence for the validity of OLD (Mumford, Weeks, Harding & Fleishman,1987; Davis,1988) but none of these efforts measure learning difficulty directly or the individual level outcomes of such training. In this study we will examine retrainees' movement from Air Force Specialties (AFSs) with different OLD ratings and Armed Services Vocational Aptitude Battery (ASVAB) requirements. Our criterion variables will be time to promotion and training school scores. As retraining comprises the main source of personnel for a new or modified weapon system and the only source of personnel from the upper grades, it is critical that we assess the difficulties associated with movement between positions with different OLDs.

Occupational Learning Difficulty: Since 1973 the Air Force has worked on perfecting a means of assessing the difficulty of tasks and occupations. This effort addresses the critical need for a benchmark to estimate the abilities and training needed of a given specialty. Personnel administrators must rationalize manpower allocation such that the talents of the manpower pool are maximized

with regard to the structure of Air Force Specialties (AFS). Early efforts failed. They constructed occupational difficulty ratings from the assessments of incumbents which confounded difficulty with other job characteristics such as time pressure, interpersonal relationship, and leadership problems (Madden, 1962). Recent efforts stress the difficulty of learning a new task. This parallels the suggestion of educational researchers who speculated that the more difficult a task the longer it would take to learn (Cronbach & Snow, 1977; Gettinger & White, 1979; Krumboltz, 1965). To date this assumption has not been tested directly though a comparison of tasks with learning times. Instead, researchers have relied on perceived learning times of Subject Area Experts or others who are close to the task. Persons in difficult positions tended to view their tasks as requiring more training and taking longer to learn than less difficult jobs (Lecznar, 1971; Mead & Christal, 1970).

In order to operationalize learning difficulty (OLD), Air Force Human Relations Laboratory personnel (Burtch, Lipscomb, & Wissman, 1982; Weeks, 1981; Weeks & Wissman, 1980) developed a benchmark scale of many easily identified tasks through experts' ratings of the task learning difficulty. The procedure involves three stages: detailed task-level stage, a position-level stage and an occupational-level stage. In the first stage, between 50 and 100 senior-level technicians and a smaller number of occupational experts provide independent ratings of each tasks' learning difficulty. Supervisors ratings were taken of the relative difficulty of specialties' tasks. The supervisors' ratings were not comparable across occupations. These scores were then converted to weights for each task. During the second stage, incumbents of the

AFS in question were studied. Their time spent on each task was recorded. Then the task times were weighted by each tasks' learning difficulty weights and the products summed. Generally, if the AFS had less than 300 incumbents, the staff surveyed all the AFS's incumbents. Any incumbent with less than six weeks in the specialty was excluded. The return rate for the mailed out surveys ran between 75 and 95 percent (Weeks, 1984, p. 10)

In the third stage, the staff derived an occupational-level index through averaging the position-level weighted-summed measures of occupational learning difficulty(OLD). The procedure proves to be flexible and allows management to create separate indices of learning difficulty for jobs within a specialty such as first term, second term, or all groups.

Task-anchored benchmark ratings were constructed with which tasks' learning difficulty could be compared across AFSs but within a given attitude area (Burtch, Lipscomb, & Wissman, 1982). These benchmark ratings allowed the movement from a research base to a cost effective operations based collection procedure. Ongoing collection of OLD data were now possible through the OMCs occupational surveys of Subject Matter Experts (SME)s. Up to date OLD's ratings can be collected on new or updated older Air Force specialties (Garcia, Ruck, and Weeks, 1985).

To date, 200 enlisted specialties have OLD indexes. The construction of these indexes involved the evaluation of over 100,000 tasks and r[illegible] of their learning difficulty. In turn, AFHRL surveyed over 170,000 incumbent positions. The reliability and validity estimates of the task level judgments were examined and the results proved satisfactory (Burtch, Lipscomb, and Wissman, 1982).

Aptitude minimums (SAI) failed to correspond well with OLD. While there is a correlation between OLD and SAI, Weeks (1984) shows that although some specialities, e.g.,. Jet Engine Mechanic, have high OLDs and SAIs, other specialities, e.g., Tactical Aircraft Maintenance have low OLDs and high SAIs, other specialties with the same OLD have very different SAIs, e.g., Air Passenger and Materiel Facilities, and some specialities differ widely on OLD while having the same SAI, e.g., Avionics Aerospace Ground Equipment and Missile Systems Maintenance. Weeks (1984) concludes that these misalignments result in a suboptimal use of talent. High aptitude individuals learn faster than low aptitude individuals in the same subject matter area. The Air Force, therefore, cannot afford to waste high aptitude individuals on low difficulty positions. the results of the OLDs have been used subsequently to realign the SAIs. But, then does OLD measure performance beyond school and get at performance on the job?

Retrainees:

Every year approximately three percent of the manpower pool retrains. Retraining costs the Air Force a great deal with additional losses from the decommissioning of old skills and knowledge. But, retraining bestows benefits as well. Personnel who have roven their capacity to handle military discipline an to be mature airmen and airwomen should have a greater probability of succeeding in their new AFS than new recruits. Of course, retraining personnel from AFSs that are being phased out, or which are undergoing force reduction, retains seasoned personnel rather than

losing their experience. Personnel who are dissatisfied with their career in one AFS may find greater reward through retraining into a new AFS and thereby may be more productive airmen.

Experience and success of retraining may be non-linear. The more experience in the service up to 16 years, the better retrainees did on their course grades. After sixteen years experience, the older the retrainee the poorer they did. But among retrainees, those coming from AFSs within the same SAI area as the AFS into which, they are moving, do better in courses than those retrainees moving into an AFS with a different SAI. Airmen who retrained from an AFS with a Mechanical SAI to an AFS with a Mechanical AFSs, had higher school scores than retrainees moving into Mechanical AFSs from different AFSs with different SAIs. Retrainees with Electronic SAIs for their former AFSs did as well as retrainees from AFS with the same SAI as their new field. Also, selective retrainees do less well than volunteers.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The predictive power of the occupational learning difficulty index has been only validated against school variables. Real learning time should be the ideal criteria. But, that is not available. When we inspected the LAMPS project at Lackland last March, this computer based research program was doing a "Pascal Course." Although no outline of the material was available, it appeared to be ideal given that all activities of the student and their time at each task was recorded by the computer. But a complete description was made available one week after I arrived in May. Preliminary data was made

available two weeks latter. It was obvious that the course was more or less an elementary computer programming course and after an SME (high school computer science teacher and summer graduate student) reviewed the Pascal course, I knew that the material was unsuitable even for a pilot study. We reluctantly abandoned this approach. Next, we negotiated to get data but the process was going to take two to four additional weeks. Although promising this line was dropped as well. Finally we settled on the Retrainee Data and gained the cooperation of Dr. Jacobina Skinner of MOA. Seven weeks into the ten week period the computer center made the data available.

In lieu of school time, the OLD index of a retraining airman's old AFS should influence his length of his training time into a new AFS. This learning time should extend beyond the school. Hence, retrainees from high OLDs former AFS should learn quicker on the job than airman from low OLDs former AFSs. OLD will be employed as a measure of ease of movement (EOM). Holding ability constant, when Airmen with the same length of experience in two different AFSs with on having a higher OLD are retrained into the same AFS, the Airmen with the former AFS with the higher OLD should have an advantage that will result in a faster rate of promotion and upgrade on average.

The following are the general hypotheses:

1. Holding ability and years of experience constant, the occupational learning difficulty (OLD) index of an former AFS should be associated positively with success in the new AFS.

2. Holding ability and years of experience constant, the occupational learning difficulty index of the new AFS should be negatively related to success in the new AFS.

The plan was to operationalize these hypotheses with data taken from the retrainee study which included a sample of some 29,000 Airmen that retrained from the years 1972 and 1978. Also interview data from some 8000 of these retrainees and their supervisors in 1980. Success in the new AFS was operationalized by time to upgrade of skills for those who have had been promoted and the percent who were promoted. In addition, peer, self, and supervisor's rating of 8000 of the retrainees was also available.

After reviewing the literature on validation of predictors of career success, it was apparent that little attention had been paid to the complications created by the opportunity structure and rapid change in such structures. Yet, it is critical in considering mobility between and within AFSs. A third objective was therefore necessary: Control for differences in opportunity for advancement posed by different AFSs. The new hypothesis is:

3. Those AFS with a steep opportunity structure-few positions at skill levels seven and nine relative to levels five and six-will have lower promotion and skill upgrade rates than AFS with a top heavy opportunity structure with a greater proportion of the positions at the seven and nine skill level.

Before validation of the OLD index employing upgrading and promotion rates of retrainees can proceed, the opportunity structure must be shown either not to be a factor through demonstrating hypothesis three to be false or by controlling for the effects of the AFS's opportunity structure.

III. FINDINGS.

The period from which the retrainee study was conducted ran from 1972 to 1978. This was a dramatic period of Air Force history. To examine the effects of the skill-level structure of AFSs, I employed the Uniform Airman Record to compile a distribution of the enlisted force with breakouts of all AFS by skill level for each year of the retraining study. I then selected approximately 100 AFS into which more than 100 airmen retrained during the period. These AFS were then displayed with the number and percent of the airmen at each skill level for each of the seven years. During this period the structure of the AFSs changed dramatically. The ending of the Viet Nam conflict brought about a drop in the enlisted force of 19.8% and 14.1% of this drop came between 1975 and 1976. But, the change varied dramatically. While some virtually disappeared and retraining into these AFSs was virtually nil, other AFS were expanding. While most AFS were experiencing declines in personnel at skill level seven and often at level five almost all AFS, some only declined modestly or were expanding, and many AFSs experienced an increase in the number of airmen at skill level nine.

AFSs' Differential Mobility Potential Since we will be examining promotion and upgrading rates across AFSs, it is critical that we explore the possibility that mobility potential is not the same across the AFSs. The following table presents several AFSs skill level break down for the years of the study. This is from the UAR for the entire Air Force. It is apparent that technical positions such as 431 303 etc. have far more sevens than skill level fives. On the other hand, the 811 with both the security specialist 811X0 and law enforcement specialty 811x2 have far more skill level 5 than 7. For the 8110 in 1972, 16.6% were at skill level 3, 68.3% at level 5 ,and 15.1% at level 7. Although the totals remained the same over the six year period, in 1978 the concentration at the 5 level increased to 73.1% and level 7 decreased slightly to 14.9%. Opportunity for promotion to skill level 7 is at best scant in this profession. The odds are about one in five that a level 5 in 811X0 will rise from level 5 to 7 in this AFS. Possibly mobility out of the AFS produces alternative routes of mobility but that seems unlikely to be a very large number given the profiles of the security people. This AFS is important for our analysis since it is the most populous in the Air Force.

Airman in the populous clerical AFS 702X0 had only slightly better odds of upgrading to level seven. This AFS declined some 18.8% which just about matches the decline in Air Force enlisted personnel. In 1972, 42.0% of the 702X0 clerks were at the 5 level and 38.4% at the 7 level. Again six years latter in 1978 level 5 expanded to 45.8% and level 7 declined to 34.3%.

Some of the technical AFS such as 305X4 started out with one ratio in 1972, a favorable 58.3% at level 7 and 25.2% at level 5 and ended up in 1978 with a slightly unfavorable 40.1% in 5 and 36.4 in level 7. Yet, the AFS declined only 2.7% during this period so the total reduction in force does not account for the drop. Other AFSs have a similar shift with or without a drop in size: 291X0, 303X1, 303x2, 303X3, 464X0, 511X0, and 511X3. In fact while 291X0 show a dramatic drop in personnel 29.4%, 303x3 increased 6.7%. The demobilization had very different effects on different AFSs.

To complicate matters further, while showing changes, some AFSs were top heavy at the beginning and at the end of the period. In 1972, 511x1 had 71% at the 7 level and 25% at 5 level while in 1978 47.2% were at the 7 level and 41.1% were at level 5. And 431x1 showed the reverse trend despite a drop of 38% in force. In 1972, 431x1 level 5's were 43.1% and level 7 at 39.8%. in 1978 the percents stood at 36.2% for 5 and 38.8% for level 7. We must take care not to infer that the opportunity structure has improved much given the heavy attrition.

The age structure of the force is critical for the validation of OLD. Opportunity for advancement in skill level was not uniform across the AFSs. Technical AFS in electronics, mechanics, and training were top heavy with the greatest concentration at skill level 7. As these AFSs had higher SAI cutoffs and OLD, care will be taken to include the structure of the AFS as variable in the final validation.

I communicated these results to Prof. David Woehr, UES Summer Fellow, who is working on the same data set validating the ASVAB. This has altered his approach to the validation of the ASVAB through

promotion and skill upgrades. Individuals with high ASVAB scores tend to be selected for AFSs that are top heavy and have high promotion rates while airmen with low ASVAB are in the main relegated to bottom heavy AFS with slow promotion rates. The effects of ability as measured by ASVAB and the difficulty of a retrainee's former AFS as measured by OLD must validated within current AFS and only after the within AFS slopes are determined can we look across AFSs.

At present the validation is incomplete. Given the extremely late start and the difficulties with the UNISYS system, the project is incomplete. I received a copy of my data set Friday and it will put up on our university's system today. The completion of project will be a matter of adding the OLD index to the retrainee files. Latter, I will employ a new procedure to deal with the random effects through a restricted maximum likelihood Bayesian approach to regression, EM analysis, with the GENCO package.

IV. RECOMMENDATIONS:

As the project is incomplete I must draw on the literature review and analysis of the demographic structure of the AFS from 1972 to 1978.

A. The OLD index combined with the SAI of in and out AFSs should be explored as he basis for a quick and inexpensive EOM index. The tasks rated for the OLD could be compared for similarity with existing techniques and a similarity index could be a supplement to the OLD and possibly measure an additional dimension of Ease of Movement.

B. Validation of any predictor of success must be done within AFSs and not across AFSs. The analysis of AFS from 1972 to 1978 provides ample evidence for each AFS being a different environment for promotion and upgrading.

C. The demographic study of the AFS should continue from 1978 to until the present. Force deployment and personnel resources utilization could be enhanced through the study of streams of retrainees and personnel who leave the service. Additional information on those who move and at what juncture and climate, could deepen our understanding of the retraining potential of Air Force Personnel. A complete discussion of this topic is beyond the scope of this report and is precluded by page limitations but study of movement within the force must incorporate a complete understanding the structure of AFSs and the dynamic changes that AFSs are undergoing at any given time.

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FINAL REPORT

Assessment of Intelligent Tutoring Systems

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Assessment of Intelligent Tutoring Systems

by

William L. Smith

ABSTRACT

Intelligent tutoring system (ITSs) have a relatively short history, about one decade. It is not surprising, therefore, that the primary focus of research on ITSs has been on functionality and on short-term effectiveness in the tutoring context. Yet to be conducted is assessment research focussing on how ITSs affect "on the job" performance or on how using a sequence of ITSs affects performance on each subsequent ITSs. A continuum of research from pre-experimental and laboratory studies to job-effect studies is necessary in order to interpret the results of research at any point in the continuum. Such research was initiated using CONFER, a text-based ITS designed to help students better understand a text they have read. Because CONFER can be used in a university freshman composition course setting, both small-scale sampling (case studies and intensive analyses of rich data) and large-scale sampling (for generalization) can be done. The data collected includes think-aloud-protocols (done while using CONFER and while writing an essay) and interviews to provide rich data and comparative text-analyses of the transcript of the conference with CONFER and the written essay to provide evidence of direct effects.

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I. INTRODUCTION:

Although the bases for intelligent tutoring systems (ITSs) trace back at least three decades, ITSs per se have a relatively short history, about one decade. It is not surprising, therefore, that the primary focus of research on ITSs has been on designing the systems. Assessment research is rare and has focussed almost exclusively on what Steuck and Fleming (1989) have called "pre-experimental" and "laboratory" research; that is, research on functionality and on short-term effectiveness in the tutoring context. Thus far no research exists on "job effect", that is, how using an ITS affects performance on the job. The Air Force education and training research community, therefore, must pursue research in the evaluation of intelligent training systems. Consequently, even though there is considerable evidence that ITSs should affect job performance, the knowledge that they do, and the knowledge about how they do it, is yet to come.

There are many types of ITSs, but one is of relevance to my research: the text-based ITS which attempts to help students in what is called the "incubation" process. During this process, students develop knowledge about a domain, making connections among concepts within the domain and connections with their own prior knowledge. The best example of this type of this type of ITS is CONFER, which provides a Socratic tutorial on a text students have read.

The research on CONFER (Parlett, 1987) thus far has indicated that it has face validity. Professors who have taught the text have used CONFER and have concluded that it resembles a teacher conferencing with a student, that it enacts a pedagogy which is appropriate, and that it should be able to help student incubate. CONFER provides a unique opportunity to conduct research which is generalizable to ITSs. A large population, college freshmen, can be studied while they are in a composition course, thus allowing "job performance" research. Furthermore, the research requires triangulation of multiple data sets, thus providing needed knowledge about the most profitable types of data, the data which provides the most information at the least cost in time/effort-to-collect and time/effort-to-analyze. And, the research allows further investigations into the use of "fuzzy" data in determining short-term and long-term effects.

My research interests have been in the area of assessment of teaching effectiveness, especially in a tutoring situation, and of assessing student ability based on samples of those students' writing. Both of these areas are called "fuzzy" assessment areas because they involve direct assessment (e.g., determining writing ability by reading an essay written by a student rather than by having the student answer multiple choice questions, questions which have "right" answers but which can only indirectly assess writing ability).

My research on human tutors in a university writing center, where any student receives individualized help with any type of writing problem, has indicated that expert tutors have internalized a hierarchy of problems (i.e., a "nesting" of the interrelationships among problems and of which problems, when "cured", affect the "cure" of other problems) and that they determine the focus of the tutorial by diagnosing not only the students' problems by also the level in the abstraction ladder at which to interdict. This dual diagnosis allows the expert tutor to work at the students' highest possible level and thus effect the greatest cascade of cure.

My research on composition placement testing of college freshmen, essentially the same population as Air Force recruits, has resulted in the reformulation of the ways one tests for reliability among human raters of student essays and in the creation of new methods for determining the validity of raters' judgments. The reliability research has shown that while two raters may disagree and thus appear unreliable, they are, as a set, very reliable. Another set of raters will produce the same ratings.

II. OBJECTIVES OF THE RESEARCH EFFORT:

There were two primary objectives in my research effort in the Summer Faculty Research Program. The first was to review the research on assessment of ITSs, particularly the procedures used, the types of data collected, and the generalizability of the results. The second was to analyze the need for systematic assessment and

to determine how best to design ways to gather the types of information needed to make decisions about immediate and long-term effectiveness of ITSs and cost-effectiveness of ITSs. In particular, my focus would be be on ways to use more sophisticated methods for eliciting and analyzing what is called "fuzzy data," i.e., data which requires human judgment as opposed to such methods as multiple choice.

a. It is important for any piece of assessment research to be embedded with a continuum of assessment research, moving, as Steuck and Fleming (1989) indicate, from pre-experimental to field tests and, at each point, examining issues of functionality, effectiveness, and cost. The continuum of such assessment research is long and broad, and much of what is to be learned about ITSs, about how students learn from ITSs, and about how to assess ITSs is yet to come. As Baker (1989) states, "The design of seriously planned embedded assessment systems that includes the full range of input, process, and outcome data, such as individual differences, process, trainee performance, and transfer data could be undertaken in a *long-term study* ." [italics mine] The absence of such "embedded assessment" is not unexpected in relatively new fields such as intelligent tutoring systems. The early focus would be on the design of the ITSs and on immediate effects. However, only through embedded research, on a continuum such as the one Steuck and Fleming propose, can any research on a point in the continuum be

evaluated. Until systematic embedded research is conducted, the results from all research provide only partial pictures and no clear guidance about the real effects of ITSs.

At a conference in September, 1986, Littman & Soloway stated that "No one we know, including ourselves, has yet carried out [internal and external] evaluations in an elegant, comprehensive way." (cf Littman & Soloway, 1988) More recent literature does not show that the state of evaluation has radically improved. Overall, only about twenty ITSs have been evaluated, yet none of these have been evaluated extensively. Instead, the evaluations are piecemeal, most focussing on pre-experiment and laboratory testing (cf Steuck and Fleming, 1989). Other evaluations seem to be underway, but it will be some time before they are published.

Recognizing this lack of systematicity, Kyllonen and Shute (1989) have proposed a taxonomy for studying learning, a taxonomy consisting of four dimensions. The first dimension is the learning environment, moire specifically, the learning strategy invoked by the ITS. This dimension ranges from rote learning to induction by observation. The second dimension is the knowledge type which results from learning. This dimension is a continuum from proposition to mental model, but it is not a strictly linear continuum, for it allow such moves a proposition to schema to mental model as well as proposition to rule to skill to mental model. The third dimension is the domain. For this dimension, Kyllonen and Shute propose a two dimensional "domain space," one axis being the

degree to which the domain is quantitative/technical, the other axis being the speed of processing, from fast processing resulting in quick decisions to slow processing resulting in quality decisions. The fourth axis is the students' learning style. Here Kyllonen and Shute focus on those characteristics which can be manipulated or controlled by the instructional designer, e.g., holistic vs serial processing, theory-driven vs data driven, and deep processing vs superficial processing.

There is one example of an attempt to examine several different evaluations of ITSs. Baker and her colleagues at UCLA (Baker 1989) are, apparently, attempting meta-analyses of ITSs, but those results have not been published. Such meta-analyses are made problematic by the unsystematic manner in which assessment research has been conducted. These studies have not tested the same variables, not have they examined ITSs under the same conditions. Even more problematic is the quality of the outcome data produced in the assessments. According to Baker (1989), "Relatively few studies of intelligent systems use outcome data of any sort...." In particular, extant assessments of ITSs have not examined students' learning beyond declarative knowledge and have not been designed to collect fine-grained data.

Even though some intelligent tutoring systems use no written language (e.g., INFLITE), most use it to varying degrees. For example, ORBITAL MECHANICS uses written language only in its help and information windows whereas CHALLENGER requires users to read

what amounts to a textbook. The ability to interact with written language is, therefore, a prerequisite for using ITSs (Hull, 1989). ITS users must understand what they read and comprehend it at functional levels. That is, they must appropriate the text, making the necessary connections among ideas presented in the text (intra-textual connections) and making connections to their prior knowledge and to other texts (inter-textual connections). Since prior knowledge can be seen as a type of text, making connections to prior knowledge is also making inter-textual connections. Inter-textuality is extremely important, for creating such relationships is necessary for long term memory, for generalization, and for problem solving given new information or situations.

Venezky, Kaestle, and Sum (1987), in their review of the National Assessment of Educational Progress data, note that the respondents in that study were able to perform well when asked identification questions when the language of the question matched the language of the text the respondents had read. However, those respondents could not perform well when required to make inferences from the text. They were ineffective problem solvers because they had not made the necessary intra- and inter-textual connections.

In ITSs, help and information windows are common used, even in primarily graphic ITSs. These windows commonly contain explanatory text, and, thus, reading ability becomes a factor. Whereas others have conjectured that the help feature may be problematic, Hull (1989) studied the use of help on MINA and found

that Ss used it "infrequently and poorly." When they did use the help feature, "they misread or misinterpreted much information" (Hull, 1989, p. 148). In other words, the Ss did not, or were not able to, make inter-textual connections. Similar research on other ITSs has not been conducted, but there is no reason to believe that the results would be different.

b. What is most obviously missing in current ITS assessment is 1) a method which will allow designers and evaluators to determine whether and how ITSs have an effect beyond immediate increases in declarative knowledge, beyond what Kyllonen and Shute call rote and didactic learning, and 2) knowledge about the effects of students' being put through a sequence of ITSs within the same domain or in more than one domain, a topic which has not yet been addressed in the theory literature.

Although the first problem could be addressed by using any extant ITS, it would be preferable to address both problems at once, for that would provide much more insightful evidence about learning in an ITS environment. One ITS, CONFER (cf Parlett, 1987) in particular provides a unique opportunity to address both issues. CONFER is a text-based ITS designed to help students in what is called the "incubation" process. To do this, CONFER, through a Socratic tutorial about a text they have read, helps students to increase their knowledge about a domain and to make connections between that domain and their related prior knowledge.

What makes CONFER appropriate is that the text which is the focus of the tutorial can be replaced with another text of the same genre or domain, thus allowing research on a single ITS and on sequenced ITSs. Furthermore, the expert within the ITS can be held constant to changed, thus allowing additional research on the expert module. Finally, because CONFER was designed to be used with the context of a college composition course, the use of writing as a means of evaluation is not unexpected or disruptive as it might be with other ITSs, most of which are in computing, math, or science and technology domains, domains where one would expect "objective" tests.

As Baker and others have noted, there is a need for more fine-grained data, such as the data produced in think-aloud-protocols (TAPs). Although TAPs can be used with any ITS, using them with CONFER offers a distinct advantage. The students "converse" with CONFER using real language, their own language. Consequently, the transcripts of their "conversations" with CONFER are a data source which can be used to triangulate with the data produced in the TAPs and their resulting essays. The analysis of transcripts to determine changes in the students' knowledge, and increases in their connections with prior knowledge can be compared with results from more direct assessment of knowledge change. The comparison of transcripts with the resulting essay allows one to trace the effect of the ITS. One could also use TAPs while the students are writing to gather additional information about the effects of

CONFER. This may be of considerable importance, for the comparison of transcripts and resulting essays would show only overt effects. The data from TAPs during writing would show covert effects, those which could be seen only if one were privy to what is called "pre-texts," that is, the texts one writes in one's head before one commits to written text.

III. RECOMMENDATIONS:

a. The research design outlined above should produce important information about the direct and indirect effects of text-based ITSs, and, by inference, about all ITSs. However, perhaps the more important product will be the methods which can be used to assess the degree to which students, using any ITS, have moved beyond declarative knowledge to what Kyllonen and Shute (1989) label mental models. The problem in assessing such knowledge has been both the difficulty in eliciting such data and the fuzzy nature of both the data and the means for judging the data. For example, reading researchers (e.g., Tierney and Pearson, 1983; Tierney and Cunningham, 1984) have, in the past decade, claimed that the best method for testing comprehension is to have students write a response in which they connect the reading material to their own knowledge. Yet the judging of written responses is either efficient and fuzzy (e.g., holistic rating by expert judges) or tedious and more

precise (cf, Spivey, 1984). Consequently, methods such as writing have not been used to assess either the immediate effects of ITSs or the students' on-the-job performance because they seem to lack reliability and are difficult to use.

b. The design for adequately assessing CONFER must be multi-phased and multi-faceted with recursive looping. During the first phase, the focus is on the immediate effects of CONFER with its current text (Walker Percy's "The Loss of the Creature"). If the results of this direct assessment indicate that CONFER does increase inter-textuality, then the next phase will be a replication but with a different text of the same genre. This phase is necessary in order to determine whether the positive results were text-specific or generalize within the genre. During the first phase, it will be necessary to create and test an authoring program for CONFER so that various experts can be used with various texts and with various genres of text. Without this authoring program, it will be impossible to determine whether the effects were due to the expert used in the Percy version of CONFER.

Because the various data bases collected during phases one and two are tedious to collect and analyze, requiring many man-hours, one facet of the research will focus on comparing these data bases to determine which provide the most and best information for the least effort. Such research has implications for all research on what are called "fuzzy" areas of assessment; that is, areas where one attempts to collect data requiring human judgment of quality.

During the third phase, the authoring program will be used to create several versions of CONFER, each with different genres of text. The research will have two foci: 1) to determine whether there is a genre specific effect, and 2) to create comparable base-lines for each genre.

The fourth phase will focus on the issue of sequential use of ITSs. Students will use two or more versions of CONFER, some within the same genre, some across genre, to determine whether there is an incremental or decremental effect. That is, whether performance on a subsequent ITS is affected by having used the previous ITS. This effect has been noted in research on human teachers and human tutors.

During the fifth phase, the procedural knowledge gained in the first four stages (e.g., methods which yield the most profitable data for the least effort) would be applied to the assessment of students' learning from other ITSs. This phase is necessary both to provide base-line data to be used in future studies (e.g., studies comparing ITSs with other types of instruction) and to determine whether certain types of assessment procedures (e.g., writing an essay) are too disruptive to be useful.

c. If such long-term research is sufficiently funded to allow it to be fully realized, the pay-off will far exceed the investment, in knowledge concerning ITSs per se (e.g., refined student models and improved interfaces) and in knowledge concerning the best, most

robust yet least expensive and least disruptive measures to use to determine immediate, delayed, and transfer effects of ITSs. "Inexpensive" measures such as objective tests of declarative knowledge are considered to be less robust than more "expensive" measures such as tests of prior knowledge and think-aloud protocols for determining delayed and transfer effects, but sufficient comparisons of the two data types may well indicate that the less expensive measures are sufficient for predictions of performance, especially of the coarse-grained measures the Air Force is forced to use to assess on-the-job performance.

The research on CONFER itself addresses two issues of considerable importance to the Air Force and the nation: literacy and critical thinking. CONFER is designed to address both of these issues. Research on CONFER will lead to more and better versions of CONFER, versions which will be appropriate to the education and training of the full spectrum of Air Force recruits, from airmen to the future officers at the Academy, and for the continuous retraining and education necessary in a dynamic, highly technological world.

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FINAL REPORT

THE ROLE OF THE INSTRUCTOR IN COMPUTER BASED TRAINING (CBT)

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THE ROLE OF THE INSTRUCTOR IN COMPUTER BASED TRAINING (CBT)

by

Stanley D. Stephenson

ABSTRACT

The question of what an instructor should do in CBT has not been answered. Although research on the role of the instructor in traditional instruction (TI) has produced a relatively high degree of consensus on what an effective TI instructor does, CBT research has not examined the instructor's influence on achievement. However, because of the differences between TI and CBT, the two learning environments may not be equivalent. The results of comparing the CBT environment with known, effective TI instructor behaviors suggests that in CBT effective TI instructor variables related to presenting the course material are allocated to the computer-based software, while effective TI instructor variables related to classroom management remain with the instructor. Compared to a TI instructor, a CBT instructor has fewer variables to use to influence academic achievement. A research program to study the CBT instructor's role with regard to academic achievement is recommended.

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I. INTRODUCTION:

A large body of research on computerized instruction has accumulated, and the results have typically been positive. CBT (which is used here as a generic label for all computer-based education) generally, but not always produces increases in learning and retention while concurrently requiring less time than traditional instruction (TI) (e. g., Fletcher & Rockway, 1986; Goodwin et al., 1986; Kulik & Kulik, 1986, 1987; McCombs, et al.; 1984; O'Neil, 1986).

However, the role of the instructor has not been a major issue in CBT. Other than to assume that CBT instructors should be computer literate (e. g., Troyer, 1988), the role of the CBT instructor with regard to academic achievement has received little research emphasis. For instance, Bear (1984) concluded that ". . . future research will find CAI (Computer Assisted Instruction) to be effective in those classrooms that are characterized by the same elements of instruction that previous research has shown to be associated with effective teachers" (p. 12).

Unfortunately, the future research mentioned by Bear has not been conducted, and the issue of the role of the instructor in CBT is still an open question. Moreover, feedback from CBT instructors, training managers, and reviews of CBT courses suggests that the CBT environment is different from the TI environment and that the TI instruc-

tor's role may not be directly transferable to CBT.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Since the primary purpose of any instructor is to promote academics, a primary question in CBT is what can an instructor do to maximize achievement? Achievement-related research in TI has produced a relatively high degree of consensus on what an effective instructor does versus what a not-so-effective instructor does. Are these results being considered in CBT? Do these results transfer to CBT? If the behaviors identified as being effective in TI do not transfer to CBT, what instructor behaviors do influence achievement in CBT? Can the CBI instructor influence achievement? At the present time, there are no answers to these questions. As a result, during CBT design and implementation the role of the instructor is not addressed, perhaps to the detriment of the CBT system.

Therefore, the role of the instructor in CBT seems to be a problem area. The purpose of this paper is to review the role of the CBT instructor with regard to academic achievement and to identify instructor behaviors which may influence achievement.

III. ENVIRONMENTAL DIFFERENCES BETWEEN TI AND CBT:

At a macro level, CBT and TI share a general description. Both involve bringing students and course material together for the purpose of the students learning the material.

Trying to compare CBI and TI at a micro level poses more of a problem in that there is not an accepted definition of what is a CBT environment. CBT can range from using material which contains an example of a computer printout to using a completely automated training system, one in which all the instruction is presented on a terminal. As the basis for comparing TI and CBT environments, this paper will use a completely automated, formal (versus OJT) CBT system, a system which is acknowledged to exist in only a few instances.

Traditional Instruction

The typical TI environment contains an instructor who is knowledgeable in the subject material and who presents the course material in one or more modes. The most typical mode is the traditional platform lecture. Other TI modes are question and answer, discussion, seatwork, and audio-visual aids (e. g., films, sound-on-slide, television, etc.).

The instructor not only presents the material but also controls what is presented, the method of presentation, the rate of presentation, and the atmosphere of the learning environment (e. g., course management, classroom discipline, student attention, student attitude, etc.). In addition to being a source of expertise, the instructor is the course administrator and the one who establishes the

learning atmosphere in the classroom.

Because of the large number of student-instructor contacts that occur in TI, the student also comes to view the instructor as a source of warmth, advice, and social interaction. Due to the social nature of the TI classroom, the instructor becomes a provider of a wide and varied amount of collateral learning; e. g., attitudes about adult life in general as well as attitudes about school.

Computer Based Training

The completely automated CBT system differs markedly from a TI system. Course material is presented on a terminal; therefore, method of and what is presented are fixed. Rate of presentation is under the control of either the software or the student, provided the software has an interactive capability.

Who or what controls classroom management, discipline, and the atmosphere of the course is not clearly defined in CBT. In a completely automated system, much of the course management can be built into the software. Discipline is obviously still the responsibility of the instructor. However, the atmosphere of the class is a combination of the behavior of the instructor and how the student interacts with the terminal.

A CBT system is not designed for student-instructor inter-

action. Consequently, the opportunity for naturally occurring, collateral learning may not exist. For example, the opportunity for the instructor to spontaneousiy transmit an attitude may never occur.

In summary, from the instructor's perspective, CBT and TI are different learning environments. It is not clear that they are equivalent. Instructor behaviors in one may or may not be appropriate in the other.

IV. REVIEW OF THE TI INSTRUCTOR LITERATURE:

A recent series of meta-analyses (e. g., Brophy, 1986) summarized TI instructor behaviors which seem to influence student achievement. Although there were minor differences of opinion among researchers about specific instructor behaviors, overall there was agreement about those behaviors which are positively and which are negatively linked to academic gain. These results are summarized in Tables 1 and 2.

Table 1

TI Instructor Behaviors Positively Related to Achievement

Course content knowledge demonstrated

Business-like atmosphere created

Organized atmosphere created

Focus placed on academics

Praise (for task performance) given to students

Mild reproach given to students

Table 1 (Cont.)

Neutral feedback given to students

High percent of time spent on task

Student behavior controlled (academic)

Student behavior controlled (non-academic/physical)

Students warned versus threatened over misconduct

Questions asked

Feedback provided

Student behavior monitored

Attitude created that students are accountable for their performance/achievement

Frequent but short interactions held

Presentations are:

- clear
- structured
- organized

Table 2

TI Instructor Behaviors Negatively Related to Achievement

Strong criticism given to students

Negative climate created

Non-response questions asked

Time spent on non-academics

Affective nature of course emphasized

Instructor fails to interact with students

Table 2 (Cont.)

Instructor employs silent reading, independent study, or written assignment

Besides the specific behaviors listed above, the literature suggests that a critical aspect of a successful (i. e., high academic achievement) classroom is the atmosphere established by the instructor early in the course. Table 3 summarizes the critical aspects of the successful classroom atmosphere.

Table 3

Atmosphere Created by the Effective TI Instructor

This is my classroom

I am in control

You are here to learn

I am here for you to learn

I am going to teach you

I can teach you

You can learn

If you do not learn, it is my fault

You must abide by the rules if you are to learn the material

You will be held accountable for your performance

We must spend a high percent of our time on the task

It should be noted that Table 3 contains both behavioral

and attitudinal dimensions.

V. REVIEW OF THE CBT INSTRUCTOR LITERATURE:

Although several studies have touched upon the issue (e. g., Bear, 1984), no studies have been found which specifically investigated the effects of CBT instructor behavior on academic achievement. A common attitude is the one expressed by Bork (1984) in a discussion of the LOGO program: "While very competent Logo teachers do do something, it is never entirely clear exactly what kind of help, assistance, or guidance they provide" (p. 54).

There have been attempts to determine what the CBT instructor's behaviors should be, as based on observed behavior of in-place CBT instructors. For example, Summers, Pelletier, and Spangenberg (1977) presented the results of a job analysis of 82 computer-based instruction instructors. These instructors reported that their most time-consuming tasks were not learning-related but consisted of such behaviors as observing student progress on terminals, maintaining discipline, and interpreting computer printouts and displays.

McCombs, Dobrovolny, and Judd (1979) reported the results of the implementation of an Air Force computer managed instruction (CMI) course. In addition to information about the course software, CMI instructors were given training in

problem solving skills, diagnostic strategies, remediation procedures, and listening/probing skills. This instructor training represented what was thought to be new to the role of the instructor in CMI versus the instructor's previous role in TI.

McCombs and Lockhart (1984) compared actual time spent data by CBT instructors with a theoretical time-spent model. They postulated that Counseling would be the primary job dimension but found that Counseling ranked fifth in actual time spent. They also found that CBT instructors spent nearly 20 percent of their time in job dimensions not included in the theoretical model and that they spent more time on administration and clerical tasks than on tutorial and learning facilitator activities. There was no direct attempt to link the job dimensions in the McCombs and Lockhart model to achievement.

In summary, little work has been done on the effects of CBT instructor behavior on academic achievement (Gillingham & Guthrie, 1987). The work that has been done has focused on either what in-place CBT instructors are doing or on theoretical job dimensions of what CBT instructors should be doing.

VI. RELATIONSHIP BETWEEN TI INSTRUCTOR EFFECTS AND CBT:

Given both that there are instructor behaviors which affect academic achievement in TI and also that the TI and the CBT

environments can differ, a useful analysis would be to speculate whether effective TI instructor behaviors are controlled by the software or by the instructor in CBT. The results of this analysis are presented in Table 4.

Table 4

Effective TI Instructor Variables

Compared to CBT Environment

Positive Behaviors

BEHAVIOR	WHERE CONTROLLED IN CBT: SOFT/INST
Course content knowledge demonstrated	both
Knowledge about CBT (?)	?
Business-like atmosphere created	inst
Organized atmosphere created	both
Focus placed on academics	inst
Praise (for task performance) given to students	soft
Mild reproach given to students	soft
Neutral feedback given to students	soft
High percent of time spent on task	both
Student behavior controlled	inst
Students warned vs. threatened over misconduct	inst
Learning tasks controlled	soft
Thinking tasks controlled	soft

Table 4 (Cont.)

Questions asked	soft
Feedback provided	soft
Student behavior monitored	inst
Attitude created that students are accountable for their performance/achievement	both
Frequent but short interactions held	inst
Presentations are:	
clear	soft
structured	soft
organized	soft

Negative Behaviors

BEHAVIOR	WHERE CONTROLLED IN CBT: SOFT/INST
Strong criticism given to students	soft
Negative climate created	inst
Non-response questions asked	soft
Time spent on non-academics	inst
Affective nature of course emphasized	inst
Instructor does not interact with students	inst
Instructor employs silent reading, independent study, or written assignments	both

Compared with Tables 1 and 2, Table 4 contains another

variable: Knowledge about CBT. In TI the instructor is primarily oriented towards the subject material. However, in CBT the instructor may have to be expert in the computer-based instructional system as well as (or in place of?) the course material.

VII. CONTROL OF EFFECTIVE TI INSTRUCTOR BEHAVIORS IN CBT: Section Six suggests that effective TI instructor behaviors are dichotomized in CBT. Effective TI instructor variables related to presenting the course material are allocated to the computer-based software, while effective TI classroom management variables are allocated to the CBT instructor. Therefore, it appears that in CBT (versus TI) the instructor's role may have shifted from one of presenter and course administrator to one of just course administrator.

If this conceptualization is accurate, then the number of instructor-controlled behaviors which can affect student achievement is reduced in CBT. Instead of being able to manipulate both course material presentation and classroom management variables, the CBT instructor primarily has just classroom management variables at his or her disposal. However, it is acknowledged that as a CBT course moves away from complete automation (which is the assumption for this paper), the role of the CBT instructor may be more similar to that of the TI instructor.

Table 5 is an attempt to merge the information presented in

Tables 1, 2, 3, and 4. Table 5 summarizes the behaviors and attitudes presented earlier into eight effective instructor dimensions and assigns their control in CBT to the instructor, the software, or both the instructor and the software.

Table 5

Effective TI Instructor Dimensions

Dimensions Controlled by the Instructor in CBT

1. Instructor attitude/orientation/role definition
   - internal locus of control
   - can do attitude
   - planning/time management oriented
   - must spend time-on-task
   - personally responsible for student's performance
   - task oriented
2. Classroom atmosphere/ethos
   - learning environment
   - pro-performance versus pro-compliance
   - task oriented
   - warmth
   - student held accountable for work
3. Classroom management
   - few target errors
   - few timing errors
   - pro-active

Table 5 (Cont.)

organized (plan ahead)

brief transitions

Dimensions Controlled by Both the Instructor and the Software in CBT

4. Knowledge of material

5. Active teaching

available for/offer assistance

monitor class work

interact with students (task and non-task)

praise (for task performance)

Dimensions Controlled by the Software in CBT

6. Content covered/time on task

7. Consistent success/Academic Learning Time

70 - 80 % correct response

8. Presentation skills/behaviors

clarity

variety

information provided

feedback provided

number of questions asked

type of questions asked

VIII. RECOMMENDATIONS:

The recommendation of this research effort is that CBT instructor behaviors which maximize achievement should be determined. The basis for this recommendation is that if

there are instructor behaviors which maximize academic achievement in TI, then the logical assumption must be that there are also instructor behaviors which maximize achievement in CBT. Specifically, the following actions are recommended:

1. Continue to review the relevant literature. It is difficult to believe that there are not more studies which have been conducted on instructor effects in CBT. Going beyond published sources (e. g., surveying industry training personnel) should be done.

2. Conduct a research program to study the relevant CBT instructor variables identified in this report. This line of research will answer the initiai question of what a CBT instructor should do to maximize academic achievement. Once this question is answered, research can proceed to broader questions; e. g., the impact of CBT on instructor selection, training, and evaluation; and the loss of collateral learning opportunities in CBT.

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FINAL REPORT

Evaluation of Air-Intercept Performance: Observer Reliability Issues

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Evaluation of Air-Intercept Performance: Observer Reliability Issues

by

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and

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ABSTRACT

Four F-16 Instructor Pilots viewed videotaped Radar-Electro-Optical (REO) and Head-Up (HUD) displays of 16 air intercepts performed by student pilots. A 16-item rating form was employed to evaluate each intercept. A 5-point rating scale was used. Ratings were made of three types of intercepts: head-on, beam, and front quarter. Analyses were made of Instructor Pilots' ratings when the three intercept types were combined and when the intercept types were separated. Interrater agreement was greatest on global evaluations of performance; there was less agreement among the scores of raters on specific air-intercept maneuvers, particularly radar utilization. Improved observer reliability may be engendered through the use of rater-training sessions to familiarize them with the behaviors to be assessed and the evaluation criteria to be employed.

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I. Introduction

The study of individual differences in behavior has attracted the attention of many cognitive psychologists. Indeed, it has been suggested that the evaluation of conditions which produce systematic differences or similarities in human performance is crucial to developing useful theories of behavior (Underwood, 1975). The study of individual differences in cognitive ability is also of considerable importance to the development of methods of personnel selection and training.

The research interests of the principal investigator address both theoretical and applied issues in individual difference psychology. Our expertise was well suited for our assignment to the Human Resources Laboratory (HRL) at Williams AFB. Considerable ongoing research in the HRL evaluates individual differences among fighter pilots as a function of specific training techniques. Innovative instructional methods are developed to facilitate the acquisition and maintenance of fighter pilot flying skills. One ongoing project is assessing the effectiveness of part-task simulators as training devices. The Air-Intercept Trainer (AIT) has been designed to enhance F-16 pilots' performance.

Another source of variability in performance assessment lies in the psychometric properties of the instrument developed to measure behavior. Siegel (1986) has identified several sources of experimental error which influence observers' ratings. Interrater reliability improves when test items are described unambiguously and when the meaning of individual items is agreed upon by the observers selected to rate behavior. Further, observers who receive training in rating procedures prior to data collection tend to show greater agreement among scores than observers who do not receive pretraining.

A study was conducted in which various air-intercept maneuvers performed by student pilots were rated by four Instructor Pilots who participated in the AIT study conducted by Edwards (1989). The objective of the research was to determine the degree of interrater reliability among the Instructor Pilots.

III.

a. Four civilian Instructor Pilots participated in the study. All were under contract by the U. S. Air Force to provide flight instruction to F-16 pilots receiving training at Luke AFB, Arizona. The four Instructor Pilots participated in an earlier study that evaluated the effectiveness of a part-task AIT experience on the development of air-intercept skills.

II. Objectives of the Research Effort:

A study recently conducted by Edwards (1989) evaluated the influence of AIT part-task training experience on the development of air-intercept skills. The performance of pilots who received supplemental AIT training was compared to the performance of pilots who received standard flight training. Each pilot's air-intercept performance was rated during whole-task simulator conditions by an Instructor Pilot. Pilots given AIT training were found to perform a number of air-intercept skills more efficently than non-AIT trained pilots.

An important methodological issue related to the AIT study and virtually all simulator-based training methods concerns the reliability of performance measurement. The issue of reliability of measurement is particularly important when data are obtained from the direct observation of several raters. Caro (1988) provides an extensive overview of methodological problems that are encountered in flight training. He suggests that instructor selection and education plays a key role in effective flight training. Considerable individual differences may exist among instructors that may affect reliable assessment of pilot performance.

Raters were instructed to judge 18 videotaped air intercepts. Each intercept video consisted of the simultaneous presentation of a Radar-Electro-Optical (REO) and a Head-Up (HUD) display. The REO and HUD displays were shown on separate 25-in monitors placed adjacent to each other. These displays conveyed information that reflected the performance of pilots under simulator flight conditions.

The videotape was developed in the following manner. Films were taken of REO and HUD displays on the Instructor Pilots' consoles during student pilots' full simulation training sessions. The students were F-16 pilots in a TX class at Luke AFB. Fifty-seven intercepts were taped over a two-day period. Individual air intercepts were reviewed by HRL personnel and categorized on the basis of three types of intercepts: head-on, beam, and front quarter. Within each category, the air-intercept skills shown by each pilot were rated and graded according to four levels of ability; i.e., good to poor. A single air intercept was randomly selected from each of the three intercept types and from each level of skill, yielding a total of 12 intercepts. Four additional intercepts were selected at random from the

remaining film segments. The 16 intercepts selected consisted of six beam, six front quarter, and four head-on encounters. Each intercept was edited to provide sufficient information to allow the Instructor Pilots to rate the student pilot's air-intercept skills. The average length of each intercept was 2 min 42 sec. The intercepts were sequenced in a random fashion, yielding a 48 min 31 sec videotape. REO and HUD displays provided only a visual representation of each pilot's performance; auditory information was not included. In addition to the 16 intercepts selected for review, two intercepts were placed at the beginning of the videotape. These intercepts were used for instructional purposes and to provide practice for the Instructor Pilots, they were not included in data analysis.

Instructor Pilots were briefed prior to viewing the videotape by a flight officer assigned to the HRL. They were told that their ratings would be used to aid in the analysis of the previously conducted AIT study. It was explicitly stated that the rating style of individual Instructor Pilots was not being assessed. They were asked to provide independent ratings of the videotaped air intercepts using the same scoring criteria employed in the AIT study.

Each Instructor Pilot was given a booklet containing 18 rating forms, one for each intercept to be evaluated. An example of the rating form is seen in Table 1. The ratings made by Instructor Pilots were designed to address three issues relevant to measuring pilot performance: (1) Specific abilities - items 1-11 were constructed to evaluate specific air-intercept maneuvers; (2) General abilities - items 12-15 were designed to provide ratings of global piloting abilities; (3) Overall ability - item 16 was designed to elicit from raters a single score that best reflected the student's overall air-intercept ability.

The rating form was similar to one developed by Edwards (1989) and used in the previous AIT study. The form was modified to correspond to the information available to the Instructor Pilots from the videotape displays. The Instructor Pilots were requested to score each of 16 items on the rating form using a five-point scale ranging from 0 (lack of ability) to 4 (high degree of ability). If the intercept did not provide sufficient information for the Instructor Pilot to rate a particular item, the rater was asked to score the item as unknown ("U"). In addition, raters were allowed to score the performance of any pilot as potentially dangerous ("D"). Raters were free to score pilot performance during the

Table 1

Air-intercept Performance Rating Form

Intercept # _____

RADAR USE		RATING SCALE
A. Search	U D 0 1 2 3 4	0 = Lack of Ability
B. Range (Scope)	U D 0 1 2 3 4	1 = Limited Ability
C. Azimuth	U D 0 1 2 3 4	2 = Essentially Correct
D. Symbol Placement	U D 0 1 2 3 4	3 = Correct and Skillful
COLLISION		4 = High Degree of Ability
A. Attain CATA (if applic)	U D 0 1 2 3 4	
B. Maintain CATA to range	U D 0 1 2 3 4	
OFFSET		U = Unknown
A Turns in proper direction	U D 0 1 2 3 4	D = Dangerous
B. Adequate offset	U D 0 1 2 3 4	
C. Maintain offset to parameter	U D 0 1 2 3 4	
CONVERSION		
A. Rollout parameter	U D 0 1 2 3 4	
B. Weapons employment	U D 0 1 2 3 4	
COMPOSITE RATING		
A. Radar utilization	U D 0 1 2 3 4	
B. Aircraft control	U D 0 1 2 3 4	
C. Intercept geometry	U D 0 1 2 3 4	
D. Situational awareness	U D 0 1 2 3 4	
E. Overall intercept	U D 0 1 2 3 4	

intercept or to complete their evaluation immediately following each intercept. The videotape was paused manually at the end of each intercept by an experimenter to provide the Instructor Pilots sufficient time to complete their scoring. Two sessions were required to obtain the ratings from the four Instructor Pilots. Three raters attended one of the sessions and the remaining rater viewed the videotape independently.

b. Separate analyses were conducted for each of the 16 items scored by the Instructor Pilots. An Analysis of Variance procedure described by Winer (1971, p 289) was employed to derive a correlation score that represented the degree of agreement among the four raters. With this model, individual raters are viewed as a treatment factor and the 16 intercepts are viewed as a repeated-measures factor. The correlation among the ratings of the Instructor Pilots on each item are seen in Table 2.

The computation of the interrater agreement correlation is based on the following formula:

$$r = \frac{K\hat{\theta}}{1 + K\hat{\theta}}$$

where: K = the number of judges and

$$\hat{\theta} = \frac{\text{MS b.judges} - \text{MS w.judges}}{K\ \text{MS w.judges}}$$

Table 2

Reliability estimates for each of the items rated

	Reliability	# of intercepts rated by all raters
RADAR USE		
1. Search	-.59	15
2. Range (Scope)	.11	15
3. Azimuth	.52	15
4. Symbol Placement	.66	10
COLLISION		
5. Attain CATA	.90	6
6. Maintain CATA to range	.84	6
OFFSET		
7. Turns in proper direction	.87	13
8. Adequate offset	.88	13
9. Maintain offset to parameter	.74	13
CONVERSION		
10. Rollout parameters	.74	15
11. Weapons employment	.61	14
COMPOSITE RATING		
*12. Radar utilization	.29	16
*13. Aircraft control	.59	16
*14. Intercept geometry	.78	16
*15. Situational awareness	.87	16
16. Overall intercept	.90	16

*Theses items were rated by 3 raters only

The calculation of the correlation coefficent (r) using this statistical technique requires that the ratings of each repeated-measure item (i.e., intercepts) be made by all Instructor Pilots. It was necessary to omit from analysis scores of individual intercepts not rated by all Instructor Pilots. The number of intercepts used to derive the interrater agreement coefficient for each item is seen in Table 2.

An evaluation of those items developed to measure specific pilot abilities suggest that Instructor Pilots tend to agree in their assessment of student pilots' skills in performing collision, offset, and conversion maneuvers; they tend not to agree in their rating of radar-utilization skill. Composite ratings of student pilots' general abilities tend to confirm these findings. The Instructor Pilots' composite ratings of student pilots' radar utilization was low compared to their ratings of aircraft control, intercept geometry, and situational awareness. There was strong agreement among the Instructor Pilots' ratings of student pilots' overall air-intercept abilities.

A second analysis was conducted to evaluate the agreement among Instructor Pilots' ratings of student pilots' abilities to execute specific types of air intercepts. The correlation coefficients for three types of intercepts are seen in Table 3. The statistic derived for each item was based on the number of intercepts scored by all raters. In some cases, a correlation score could not be computed due to the low number of intercepts rated. The derived correlation coefficients were quite high in the majority of items rated, despite the limited number of intercepts scored by the Instructor Pilots. Inspection of these data suggest that the Instructor Pilots' ratings of student pilots' radar-utilization skills tends to have the lowest level of rater agreement, particularly in the case of beam intercepts.

IV. Recommendations:

While the agreement among the ratings of student pilots' abilities by Instructor Pilots was quite close on many of the items rated, there are two areas that should be investigated.

a. One Instructor Pilot did not provide composite ratings of student pilots' abilities. He stated that the items could not be scored. This observation suggests

TABLE 3

Reliability estimates for each intercept type

	Head On	Beam	Front Quarter
RADAR USE			
1. Search	.64 (4)**	0 (5)	0 (6)
2. Range (Scope)	.59 (4)	0 (5)	.27 (6)
3. Azimuth	.65 (4)	-.92 (5)	.73 (6)
4. Symbol Placement	NA (1)	.64 (4)	.68 (5)
COLLISION			
5. Attain CATA	NA (0)	.90 (6)	NA (0)
6. Maintain CATA to range	NA (0)	.84 (6)	NA (0)
OFFSET			
7. Turns in proper direction	.92 (4)	.96 (3)	.60 (6)
8. Adequate offset	.82 (4)	.97 (3)	.76 (6)
9. Maintain offset to parameter	.76 (4)	.90 (3)	.40 (6)
CONVERSION			
10. Rollout parameters	.63 (3)	.73 (6)	.09 (6)
11. Weapons employment	.85 (3)	.55 (5)	.01 (6)
COMPOSITE RATING			
*12. Radar utilization	.64 (4)	-.95 (6)	.38 (6)
*13. Aircraft control	.90 (4)	-.01 (6)	.02 (6)
*14. Intercept geometry	.89 (4)	.80 (6)	.14 (6)
*15. Situational awareness	.97 (4)	.84 (6)	.51 (6)
16. Overall intercept	.98 (4)	.86 (6)	.80 (6)

*Theses items were rated by 3 raters only

**Number of intercepts rated

that the Instructor Pilots' task was ambiguously stated. It is recommended that a pretraining and discussion session be conducted for all raters prior to data collection.

b. Low interrater agreement was observered on ratings of student pilots' radar utilization. It has been suggested by HRL flight training personnel that some items selected for rating these skills may not address critical aspects of air-intercept maneuvers. It is recommended that each of the items of the rating scale be reviewed to determine the degree to which the item measures actual air-intercept behaviors.

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FINAL REPORT

Career Progression in Air Force Enlisted Personnel: An Examination of Two Alternate Criterion Measures

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Career Progression in Air Force Enlisted Personnel: An Examination of Two Alternate Criterion Measures

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ABSTRACT

Two career progression measures for USAF enlisted personnel were examined as possible alternate criterion measures to training school final grades in ASVAB validation studies. One measure, Skill Level Acquisition Rate (SLAR), was found to have too little variance at lower levels and failed to show a substantial relationship with aptitude measures to serve as a viable criterion measure. The other measure, Grade Level Advancement Rate (GLAR), however was demonstrated to be a potentially valuable alternative criterion measure.

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I. INTRODUCTION:

Typical test validation studies examine the relationship between some measure hypothesized to be predictive of job performance and some criterion measure representing actual performance. The strength of this relationship indicates the validity, and is one component of the potential utility, of the test battery for the selection and classification of job applicants.

The MOA branch of HRL has been concerned with the validation of the Armed Services Vocation Aptitude Battery (ASVAB) for several years. The ASVAB is the primary source of information for the selection and classification of enlisted personnel for all branches of the service. Since 1976, various forms of the ASVAB have been used to select enlistees into the military and assign individuals to specific training programs. The ASVAB consists of several subtests which are combined to produce five separate composites. One composite, the Armed Forces Qualification Test (AFQT), is used to screen out applicants unqualified for enlistment. The remaining composites assess enlistees' aptitudes in four basic areas: mechanical, administrative, general, and electronics (MAGE). These aptitude composites serve two purposes: (a) to supplement AFQT scores in determining the qualifications of applicants for military service, and (b) to determine eligibility for assignment to specific military jobs.

Past validation work with the AFQT and MAGE composites has primarily utilized performance in specific training programs as criterion measures. Training performance measures are usually based on a dichotomous pass-fail variable or end-of-course grades. These criteria are in turn assumed to be indicative of later job performance. A more ideal situation involves the validation of enlistment tests against measures of actual job performance. The preference for performance in training over job performance as criteria has come about primarily due to the accessibility of training data, the limitations of job proficiency measures as criteria (Wagner, Dirmeyer, Means, & Davidson, 1982), and the poor reliability demonstrated by supervisory ratings of performance (Vineberg & Joyner, 1983; Wagner, et al., 1982).

Cory (1982) attempted to circumvent these problems by using alternate measures of performance. These consisted of measures of job outcomes related to performance, such as job level attained and speed of advancement. Substantial relationships were found between these alternate measures and AFQT scores obtained between 1967 and 1976. No studies, however, have assessed the usefulness of alternative criterion measures for more recent AFQT or for the other ASVAB composites.

Of critical importance is the degree to which career progression measures of performance overlap (or do not overlap) with training performance measures. To the extent that job

demands (as reflected by the career progression measures) might differ from training demands, information regarding the direct link between AFQT/ASVAB scores and actual job performance would be extremely useful.

Our research interests have primarily been in the area of performance measurement, with a particular focus on the judgment processes involved in performance ratings, as well as in test development and validation. Performance measurement and particularly judgmental measures of performance are an extremely important aspect of test development and validation. In order to validate a test, a performance standard or criterion must be established. Because objective measures of job performance are rarely available for most jobs, such criterion measures are often subjective (i.e. judgmental) measures. Our work in these areas is complimentary to much of the research done at HRL/MOA and contributed to my assignment to this branch.

II. Objectives of the Research Effort

The present study examines two measures of career progression for USAF enlisted personnel. These measures, Grade Level Advancement Rate (GLAR) and Skill Level Acquisition Rate (SLAR), reflect rate of career advancement for enlisted personnel and as such can be viewed as an indication of job performance. The primary question of interest, for the present study, is what factors influence the rate of career progression and how much

variance can be accounted for by measures of pre-training aptitude. More specifically, we evaluate the feasibility of the career progression measures for use as alternative criterion measures in ASVAB validation studies. Here the question is do ASVAB scores predict the rate of career progression for Air Force enlisted personnel. It is typically postulated that valid entry level aptitude testing increases all forms of organizational productivity by supplying the organization with workers who will become productive members of the work force in a shorter period of time. Consequently, ASVAB scores should show a reliable relationship with the proposed criterion measures [Skill Level Acquisition Rate (SLAR) and Grade Level Advancement Rate (GLAR)].

In addition, answers to this question will provide insight into the relative utility of surrogate criteria relative to the standard training performance criteria.

III. Method

Data Set. The data used in this study were provided by Air Force Human Resources Laboratory (AFHRL). The data file was originally compiled as part of a study on aptitude and career progression differences between AFSC retrainees (enlisted personnel who changed AFSC and consequently went through retraining) and non-retrainees. Data was obtained from records of the administration of ASVAB Forms 5, 6 & 7 and December personnel record updates for 1977 - 1979. The data file

contained approximately 326,000 records (the actual number of cases with complete records, however, was much smaller).

Variables of Interest

Time in Grade (TIG) - This variable is a measure of the rate of promotion for enlisted personnel and reflects Grade Level Advancement Rate (GLAR). It is operationalized as the number of months in grade prior to the most recent promotion.

Time at Skill Level - This variable is a measure of the rate of skill level advancement for enlisted personnel (SLAR). It is operationalized as the number of months in the skill level prior to the most recent level advancement.

Date of Enlistment (DOE) & Time in Service (TIS) - These variables reflect the year of enlistment and the amount of time (months) in the service. These variables are almost perfectly correlated (as would be expected) and are used synonomously throughout the analyses.

Air Force Specialty Codes (AFSC) - The AFSC codes reflect the job specialty area for enlisted personnel in the Air Force. This variable was included to see how much variance in career progression could be accounted for by job specialty area. In the present study, AFSC's containing more than 150 people were selected for inclusion in the analyses. This resulted in 23 AfSC

codes being used in the analyses. These codes were then dummy coded for use in the regression analyses.

Final School Grades (FSG) - This variable is a measure of training school performance. Final school grades, however, were only available for the sample of enlisted personnel who had received retraining (N = 11,177). Consequently, all analyses involving training performance scores used this smaller sample.

AFQT and ASVAB Composite Scores - These variables represent the pre-training aptitude measures. They are operationalized in terms of percentile scores on the five ASVAB composites.

Table 1 presents descriptive information for all variables.

Analyses. The analyses for the present study were conducted in two phases. First, hierarchical multiple regressions were run to determine the total amount of variance accounted for by DOE, TIS, AFSC, and AFQT score and the ASVAB composite scores in both the Grade Level Advancement Rate and Skill Level Acquisition Rate variables. In addition, these analyses provide an indication of the amount of unique variance accounted for by the addition of each subsequent independent variable.

Second, separate regression analyses were run for each of the criterion variables (GLAR and SLAR) on each of the predictor variables (DOE/TIS, AFSC, AFQT/ASVAB composites). These analyses

were designed to examine the amount of variance accounted for by each of the predictor variables independently.

In addition, the results of the two steps indicated above were used to calculate both partial and semi-partial correlations for each predictor variable. The partial correlation for each predictor addresses the question: "How much of the variance in the criterion that is not estimated by the other predictors in the equation is estimated by this variable?" The semi-partial correlations provide an indication of the amount of increase in total criterion variance accounted for that occurs when this particular predictor variable is added to the equation. These indices provide a measure of the common variance or degree of overlap between predictor and criterion variables.

Finally, since the regression analyses were conducted across grade level and AFSC categories, the validity of the aptitude measures with the career progression measures (reported as the proportion of criterion variance accounted for) was estimated as the proportion of variance accounted for by the aptitude measures to the total criterion variance minus the variance uniquely accounted for by DOE/TIS and AFSC code.

IV. Results

Grade Level Advancement Rate. Table 2 presents a summary of the initial hierarchical regression analyses and the subsequent

individual regressions for each dependent variable. These results suggest:

a. Approximately 44% of the total variance in time in grade before promotion is accounted for with all of the independent variables in the equation.

b. The largest single influence on the number of months spent in grade prior to promotion is Date of Enlistment (Time in Service). This variable accounts for approximately 39% of the total variance. It is important to note that this finding was not unexpected. The current analyses were collapsed across all grade levels. Consequently, the variance in number of months in grade that is due to differences in the grade level to which individuals are being promoted is accounted for at this step. In other words, since promotions are far more rapid at lower grade levels than at higher levels, it is not surprising that a large proportion of the total variance is accounted for by the amount of time in service (an indirect indicator of an individual's probable grade level).

It is also important to note that the variance accounted for by DOE/TIS is additional variance added to time in grade by collapsing across grade levels. Therefore, results pertaining to the remaining independent variables should be considered in terms of the proportion of variance accounted for of the total variance minus the variance uniquely accounted for by DOE/TIS.

c. Other than DOE/TIS, the largest proportion of variance is accounted for by AFSC. While AFSC accounts for only 15.9% of the total variance, it accounts for 21.6% of the variance remaining after the DOE/TIS unique variance is removed. This indicates that a substantial proportion of the variance in the number of months in grade is due to AFSC. In other words, promotion rates are quicker for some job codes than for others.

Similar to DOE/TIS, the variance accounted for by AFSC is variance added to time in grade by collapsing across AFSC codes. Therefore, the results pertaining to the AFQT and ASVAB composite should be considered with the variance uniquely accounted for by AFSC removed from the total time in grade variance.

d. Finally, AFQT and the remaining ASVAB composites account for approximately 6.5% of the total variance. However, if the variance uniquely accounted for by DOE/TIS and AFSC is removed, ASVAB scores account for 10.2% of the remaining variance. Thus, AFQT and the four ASVAB composites appear to account for a substantial proportion of the variance in the number of months before promotion.

For purposes of comparison, we also calculated the amount of variance accounted for in training school final grades (the standard criterion measure used in ASVAB validation studies) by the AFQT/ASVAB composites scores, using the current data set. Results indicate a multiple correlation coefficient of .361.

Thus, approximately 13% percent of the variance in final school grades is accounted for by ASVAB composites scores. It is important to note that this validity coefficient is somewhat lower than those typically found in ASVAB validation studies (Wilbourn, Valentine & Ree, 1984). There are a number of possible reasons for this finding. In the current data set final school grades were only available for enlisted personnel who had changed AFSC's and consequently gone through retraining. In addition, the current analyses were conducted at a very broad level, collapsing across AFSC's and aggregating the ASVAB composite measures. Finally, the validity coefficients reported here have not been corrected for restriction of range due to selection based on the ASVAB composite indices. These factors tend to result in lower validity coefficients, however, they affect both the final school grade and time in grade analyses. Thus, it appears that time in grade yields validity coefficients comparable to those using the final school grade criterion.

Skill Level Acquisition Rate. Analyses for Skill Level Acquisition rate were computed within rather than across skill levels. Time to upgrade for levels 1 to 3 and 3 to 5 contained too little variance to run the regression analyses. Analyses were conducted, however, for upgrade levels 5 to 7 and 7 to 9, and are presented in Table 3. These results suggest:

a. Only 14% of the total variance in time to upgrade measure at the 5 to 7 level and 12% at the 7 to 9 level were accounted for

with all of the predictor variables (DOE/TIS, AFSC, and ASVAB composites) in the equation.

b. DOE/TIS accounted for the largest proportion of the total variance at both the 5 to 7 (13%) and the 7 to 9 (10%) upgrade levels.

c. Both AFSC codes and ASVAB composite variables accounted for less than 2% of the total time to upgrade variance for both the 5 to 7 and 7 to 9 upgrade levels. Thus, it appears that the ASVAB scores only account for a trivial proportion (if any) of the variance in time to skill level upgrade.

V. Recommendations

We examined two career progression measures for USAF enlisted personnel as possible alternate criterion measures to training school final grades in ASVAB validation studies. One measure, Skill Level Acquisition Rate (SLAR), was found to have too little variance at lower levels and failed to show a substantial relationship with aptitude measures to serve as a viable criterion measure. The other measure, Grade Level Advancement Rate (GLAR), however, was demonstrated to be a potentially valuable alternative criterion measure. Based on our results we offer the following recommendations:

a. GLAR as measured by time in grade prior to promotion be considered as an alternate criterion measure in ASVAB validation

studies.

b. A follow-up study be conducted that elaborates on the findings of this study. Results of the present study indicate a substantial effect of grade level and AFSC classification on time in grade. A follow-up study should examine the aptitude/GLAR relationship within grade levels and AFSC classifications. This breakdown would yield more specific validity and regression estimates. These, in turn, would prove more useful in terms of prediction of advancement rates and consequently prediction of manpower utility increases.

A follow-up study should also generate and use an updated data set. Several characteristics of the data set used in the present study present limitations to the generalizability of the results. The data set used was initially generated as part of a study comparing AFSC retrainees with non-retrainees and consequently results of the present study are based in large part on data from the retrainee sample. This sample has been shown to yield lower validity coefficients in previous studies.

In addition, the data used in this study were from ASVAB versions 5, 6 and 7. These versions of the ASVAB are substantially different from more recent ASVAB versions. The present data set also only contained data on ASVAB composites as opposed to subtest data used to generate the composites. More specific subtest data would allow more valuable results.

Finally, the data set used contains personnel information ending in 1979. Policy and manpower composition have most probably changed greatly since this time and consequently the aptitude/GLAR relationship would be greatly affected.

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Table 1: Mean, Standard Deviation and N for Time in Grade before Promotion, Time to Skill Level Upgrade and the Predictor Variables

Variable	Mean	Standard Deviation	Cases
TIG	10.11	4.157	2590
TIS	19.24	9.015	2593
FSG	87.28	6.822	11177
AFQT	61.28	22.034	7069
Admin.	60.52	19.686	7421
Elect.	62.70	20.681	7422
General	62.82	18.619	7424
Mech.	59.31	21.273	7424
Time at Skill Level			
1 to 3	1.58	.595	3184
3 to 5	2.21	.747	49025
5 to 7	7.65	4.821	29331
7 to 9	9.02	4.663	3086

Table 2: Multiple Regression Results for the Time in Grade Criterion Measure

	R	R^2	R^2	df
Time in Grade =				
Step 1: DOE/TIS	.623	.391	.391	(2, 2462)
Step 2: AFSC	.651	.424	.033	(23, 2441)
Step 3: AFQT & ASVAB COMPS.	.666	.443	.019	(28, 2436)

	R	R^2	df
Time in Grade = AFSC	.398	.159	(21, 2568)
Time in Grade = AFQT & ASVAB COMPS.	.251	.063	(5, 2459)
Time in Grade = AFSC & AFQT & ASVAB COMPS.	.462	.214	(26, 2430)

Table 3: Multiple Regression Results for the Time at Skill Level Criterion Measure

Level 5 to 7 Upgrade

	R	R^2	R^2	df
Time at Skill Level =				
Step 1: DOE/TIS	.359	.129	.129	(2, 27696)
Step 2: AFSC	.363	.132	.003	(23, 27675)
Step 3: AFQT & ASVAB COMPS.	.372	.139	.007	(28, 27670)

	R	R^2	df
Time at Skill Level = AFSC	.133	.018	(21, 29309)
Time at Skill Level = AFQT & ASVAB COMPS.	.085	.007	(5, 27693)

Level 7 to 9 Upgrade

	R	R^2	R^2	df
Time at Skill Level =				
Step 1: DOE/TIS	.318	.101	.101	(2, 2521)
Step 2: AFSC	.321	.103	.002	(23, 2500)
Step 3: AFQT & ASVAB COMPS.	.348	.121	.018	(28, 2495)

	R	R^2	df
Time at Skill Level = AFSC	.140	.019	(21, 3064)
Time at Skill Level = AFQT & ASVAB COMPS.	.127	.016	(5, 2518)

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FINAL REPORT

Development of the "City of Quality (Coq)" Group Decision Support System

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Development of the "City of Quality (Coq)" Group Decision Support System

by

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ABSTRACT

A general theory for Group Decision Support Systems (GDSS) is here investigated. Mathematical models for multi-person multi-attribute decision making (MPMADM) are developed, and justification for use of a modification of the Taguchi cost function is presented for general use by non-profit organizational MPMADM problems. Total quality and unified life cycle engineering are just two examples of areas where this group information system may be applied.

The House of Quality paradigm is then examined as a framework for the "knowledge acquisition" of the parameters needed for the modified Taguchi loss function, and Hypertext is is shown to be an effective platform with which to produce a fully automated version of an integrated, linked series of houses of quality into a City of Quality (Coq) that serves as the basis of our GDSS.

New informatics for the production of hypertext systems were developed, including self-modifying hypertext documents. As a corollary to the development process, techniques for management and structured development of hypertext projects of were created.

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I. Introduction:

In March 1988, memorandum 30452 was sent from the office of the Secretary of Defense stating that "It is critical at this time that the Department of Defense ... focus on quality as the vehicle for achieving higher levels of performance." A number of Air Force efforts are currently being directed toward this Total Quality Management (TQM). One important facet of these efforts is their need for group technology: many aspects of systems being developed for the Air Force must be considered by many different decision makers.

In particular, the Air Force wants to include reliability, maintainability and other "ilities" in the design phase of projects, rather than addressing these issues after production.

In order to do this, group decision support tools are needed to allow decision makers to assess trade-offs and to communicate their expertise among themselves.

Both a mathematical theory of multi-person multi-objective decision making, and working software are needed for this effort. For some time, the Japanese have had success using a mathematical model by Taguchi for computing optimal system specifications. More recently, a promising technique for capturing the parameters needed by the Taguchi model was developed by Hauser and Clausing,. An integration of the two approaches into a software package that automates the mechanical details would be of great practical benefit to the Air Force.

My current research interests are currently in the area of using Hypertext to support group decisions. I recently presented a paper on the development of inexpensive group decision support systems that could be widely distributed, and which would therefore be suitable for use by lower and middle-level decision makers. I had previously developed a decision support systems to enhance combat effectiveness of the F-16 by improved reliability and maintainability, and it was this system which contributed to my assignment to the Logistics Systems branch of the Human Resources Laboratory.

II. Objectives of the Research Effort:

My objective for the summer's research was the study of group decision support aids for concurrent engineering.

I initially performed an extensive review of relevant literature, both journal articles and relevant reports,with a goal of developing a system to support multi-person, multi-attribute decision making (MPMADM) in support of Quality Function Deployment (QFD) and concurrent engineering (CE). The review of MPMADM included a a taxonomy of such systems, and the mathematical theory underlying them. I next reviewed articles and reports on concurrent engineering and quality, including English descriptions of the Taguchi model, and reviewed techniques for developing Hypertext prototypes.

To actually implement the system, I began by outlining the mathematical theory of MPMADM. The specific version chosen for the implementation was the team approach. The specific formulation of the MPMADM problem is based strongly on the Taguchi model, as described in (Elmaghraby et al., 1986). The "House of Quality" paradigm as described in (Hauser and Clausing, 1988) was then chosen as the framework to acquire the parameters required by the Taguchi model.

In order to implement the "House of Quality," I investigated Hypertext as a potential software vehicle. Using Hypertext, I designed a linked ensemble of "Houses of Quality," leading to a tool best described as a "City of Quality (COQ)" a tool that allows the decision makers to communicate their concerns among themselves, that provides a record of decisions made and rationales behind those decisions, that encourages quantification of goals and objectives, and that encourages hierarchical decision making.

I next implemented a very preliminary Hypertext prototype of the system as a "Proof of Concept," and as a guide to the sorts of systems which might be most useful to Air Force decision makers. This prototype is a

model "City of Quality" (Coq) system which consists of a linked series of "Houses of Quality."

III. Multi-person Multi-attribute Decision Making (MPMADM)

A study of decision making indicated that the basic formulation of the MPMADM problem can be written

$$\min_{\mathbf{x}} L(\mathbf{x},\theta) \quad (1)$$
$$\text{S.T. } \mathbf{x} \in \Omega$$

as in (Luce and Raiffa, 1957).

The vector $\mathbf{x}$ is the decision vector, to be set by the several decision makers acting in conjunction; the vector θ is a random vector, called the state of nature; the set Ω is the technology set of feasible decisions; $L(\mathbf{x},\theta)$ is the (possibly non-linear) loss function, which, for this problem, is a matrix of losses, with $L_{ij}(\mathbf{x},\theta)$ the loss of attribute j incurred by decision maker i.

Because of the following:

> Proposition 1. If L is a countable ordered set, then there is an order-preserving mapping
>
> $$\Phi: L \rightarrow Q \quad (2)$$
>
> where Q is the rational numbers.

the problem of multi-attribute decision making can be reduced to finding a suitable Φ. The simplest Φ is, of course, the linear functional:

$$\pi^T l = \Phi(l) \quad \forall l \in L \quad (3)$$

with the vector π chosen to induce the correct ordering on L. Unfortunately, if $|L| > n$, (3) is overdetermined, and, has no solution, except in a least-squares sense.

Group decision making is complicated by Arrow's theorem, which is explained in (Luce and Raiffa, 1957). A first simplification which partially circumvents Arrow's theorem is to reduce from the group decision making problem to the distributed decision making problem, in which each decision maker has complete control over some of the decision variables. Arrow's theorem does not apply, since each decision

maker is allowed to select a preference for the value of some decision variables independent of the choices of the other decision makers. A further simplification is made to team decision making as in (Marschak and Radner, 1972). Each player i in this game has access to an information function $\zeta_i(\theta$, and may signal, or communicate $\zeta_i(\theta)$ to team members $j \neq i$ at some cost χ_{ij}, which is included in the loss function. One method of improving the expected value of the loss function is to reduce the cost of communication, which is the goal of Coq.

The problem of dealing with uncertainty may be dealt with by taking expected values, which is a variation on taking a real-valued function Φ for the multi-attribute problem.

Specializing to the team decision problem, it is necessary to develop appropriate $\mathbf{L}$ and Φ for QFD. For the sake of notation consistent with the literature, introduce the production process. Decision makers are given a decision vector $\mathbf{s}$, which may also be interpreted as input to the production process. The process then produces quantifiable outputs

$$\mathbf{x} = \mathbf{p}(\mathbf{s},\varepsilon) \tag{4}$$

where ε is a random vector, $\mathbf{x} \in \Re^n$ is a random vector, $\mathbf{s} \in \Re^m$ is determined by the decision makers in a way that minimizes the loss $\mathbf{L}(\mathbf{x},\theta)$ associated with $\mathbf{x}$ and the random state of the world, θ. In general, unfortunately, producers do not know $\mathbf{p}$ except for some set $S \subseteq \Re^n$ (often a small, finite set.)

The general form for $\mathbf{L}$ is given by:

$$\min_{\mathbf{s}} \; C_p(\mathbf{s}) + C_c(\mathbf{x}) \tag{5}$$

where $C_p(\mathbf{s})$ is the producer's cost of selecting the decision variables $\mathbf{s}$ and $C_c(\mathbf{x})$ is the consumers' cost associated with production of output $\mathbf{x}$. Taguchi's first contribution is the notion that a producer should minimize total cost to the organization (or nation) rather than maximizing its own profit. In his original formulation, however, Taguchi omitted the producer's cost. Equation (5) has been called the extended Taguchi cost function in (Tse and Cralley, 1988).

In addition to the general form (5), Taguchi also specified the specific form to be used for $C_c(\mathbf{x})$: the consumer is presumed to have a target τ for $\mathbf{x}$. Then

$$C_c(\mathbf{x}) = \sum_i w_i (x_i - \tau_i)^2 \tag{6}$$

where $\mathbf{w}$ is a vector of weights to be applied to the deviations. The scalar loss function is then

$$L(\mathbf{s}) = C_p(\mathbf{s}) + E(C_c(\mathbf{x})) \tag{7}$$

$$= C_p(\mathbf{s}) + \mathbf{w}^T\sigma^2 + \mathbf{w}^T(\bar{\mathbf{x}} - \tau)) \tag{8}$$

where $\bar{\mathbf{x}}$ is the mean of $\mathbf{x}$ and σ^2 is the variance of $\mathbf{x}$.

We assume that $C_p(\mathbf{s})$ is readily available: i.e., that for any choice of the decision variable $\mathbf{s}$, the costs of production are known. The team information function is described by the estimates each of the team members has for ε, θ, τ, $\mathbf{p}$, and $\mathbf{w}$, $\hat{\varepsilon}_i$, $\hat{\theta}_i$, $\hat{\tau}_i$, $\hat{\mathbf{p}}_i$ and $\hat{\mathbf{w}}_i$. The assumption that this is a team decision problem implies that all members of the team share the scalar loss function (8) and the function of the GDSS is to obtain the best possible group estimates for ε, θ , τ, and $\mathbf{w}$ based on the individual estimates, i.e., the GDSS provides a <u>group function</u>

$$G: (\hat{\varepsilon}_i;\ \hat{\theta}_i;\ \hat{\tau}_i;\ \hat{\mathbf{p}}_i;\ \hat{\mathbf{w}}_i)(\mathbf{s}) \rightarrow (\hat{\varepsilon};\ \hat{\theta};\ \hat{\tau};\ \hat{\mathbf{p}};\ \hat{\mathbf{w}})(\mathbf{s})\ \forall \mathbf{s} \in \Omega \tag{9}$$

which takes the matrix of individual estimates and computes the group estimate. An optimal group function may be obtained as a consequence of:

<u>Proposition 2</u>. Let $\mathbf{y}$ be a random vector, $\hat{\mathbf{y}}_i$ a matrix of estimates of $\mathbf{y}$, and $\varepsilon_i = \mathbf{y} - \hat{\mathbf{y}}_i$ a matrix of random errors. If the ε_i are unbiased, then $\hat{\mathbf{y}}$, the minimum variance unbiased linear group estimate of $\mathbf{y}$ is

$$\hat{\mathbf{y}} \equiv \frac{\mathrm{Var}^{-1}(\varepsilon_i)\hat{\mathbf{y}}_i}{|\mathrm{Var}^{-1}(\varepsilon_i)|} \tag{10}$$

As discussed in (Pyeatt and Wolfe, 1989) this solves the problem of reconciling expert judgements from a panel of experts, even when the

experts' answers are correlated. It does not, however, solve the problem of expert bias, which is a matter for further research.

Application of Proposition 2 requires that we obtain an estimate of $Var^{-1}(\varepsilon_i)$ based on prior decisions and results. Hence the system must maintain such a database.

IV. City of Quality template for GDSS

The framework selected for collecting the data identified in §2 is based on the work of (Hauser and Clausing, 1988) The original work requires the decision makers responsible for design, development, and production of a system to fill out four "Houses of Quality." Our extension automates the links between the four houses, to produce an integrated system we call the City of Quality (Coq.) Coq:

1) Assists decision makers to establish all requirements. In the notation of §2, Coq reduces the chance that an important target τ_i in (6) above is omitted (i.e. given an incorrect weight $w_i = 0$;)

2) Provides a framework for setting formal weights and targets, rather than leaving the decision process fuzzy and intuitive;

3) Maintains a record of how trade-offs were resolved;

4) Reduces the probability that a customer requirement, identified in (1), fails to be addressed in the final design.

The system that does this is shown in Figure 1, which shows the basic House. The left side of the house, labeled "Customer Attributes," contains the customer requirements; a column is provided for their relative weights. It is designed to encourage a hierarchical approach to the requirement definition, which will be discussed further below. The "attic" (just below the roof) labeled "Engineering Characteristics" in conjunction with the bottom of the house is used to list quantitative, engineering specifications, target values, importance, estimated difficulty and costs. Also provided are spaces for comparison with existing designs.

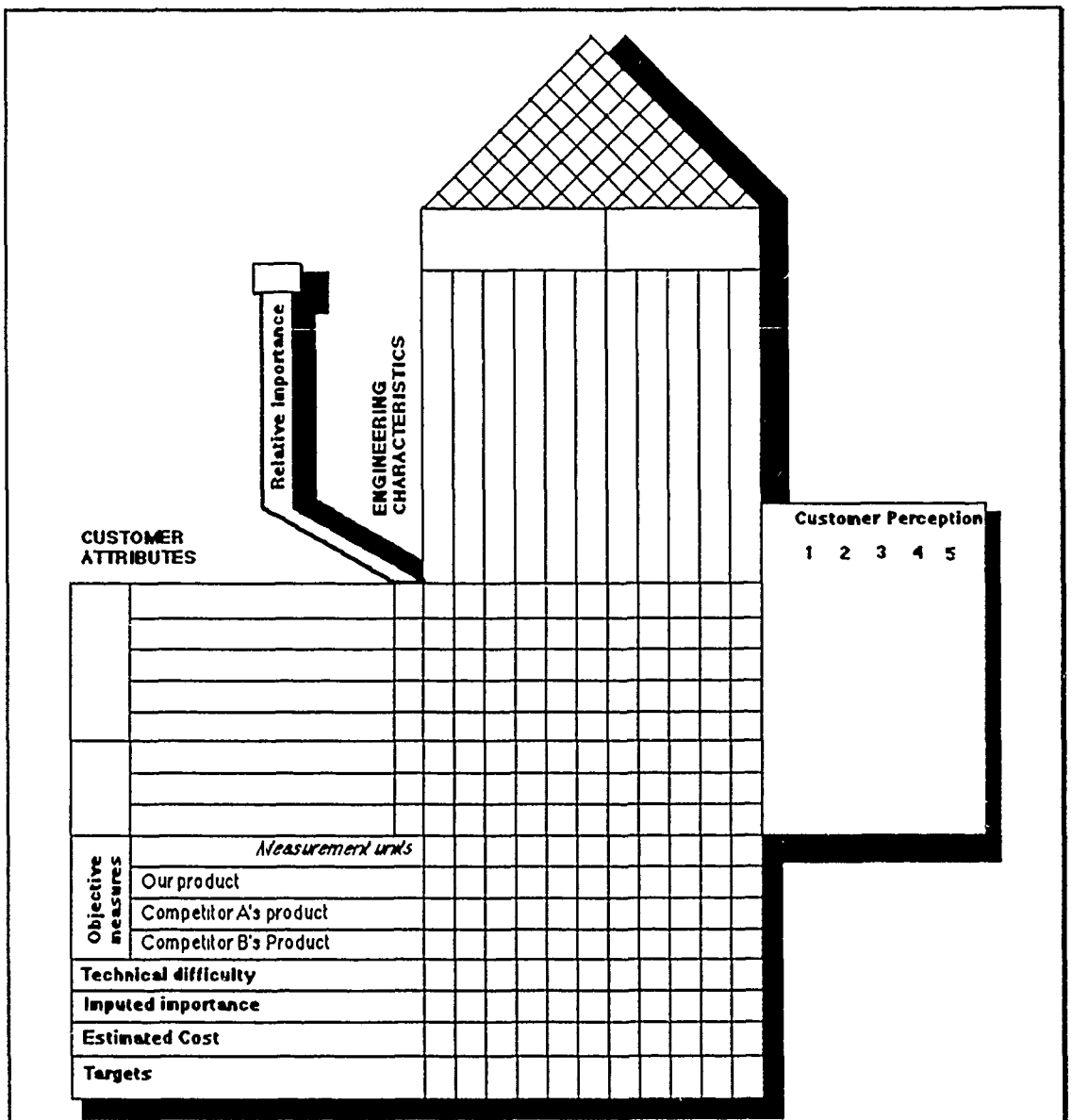


Figure 1. The House of Quality

The central portion of the house provides space to indicate which engineering specifications address (or degrade) a customer requirement. The roof indicates the synergies and conflicts among the engineering specifications. Finally, a graph on the right hand side of the House indicates competitive position.

This decomposes the group decision problem into the various cognitive parts, and provides the support for (1)–(4) above.

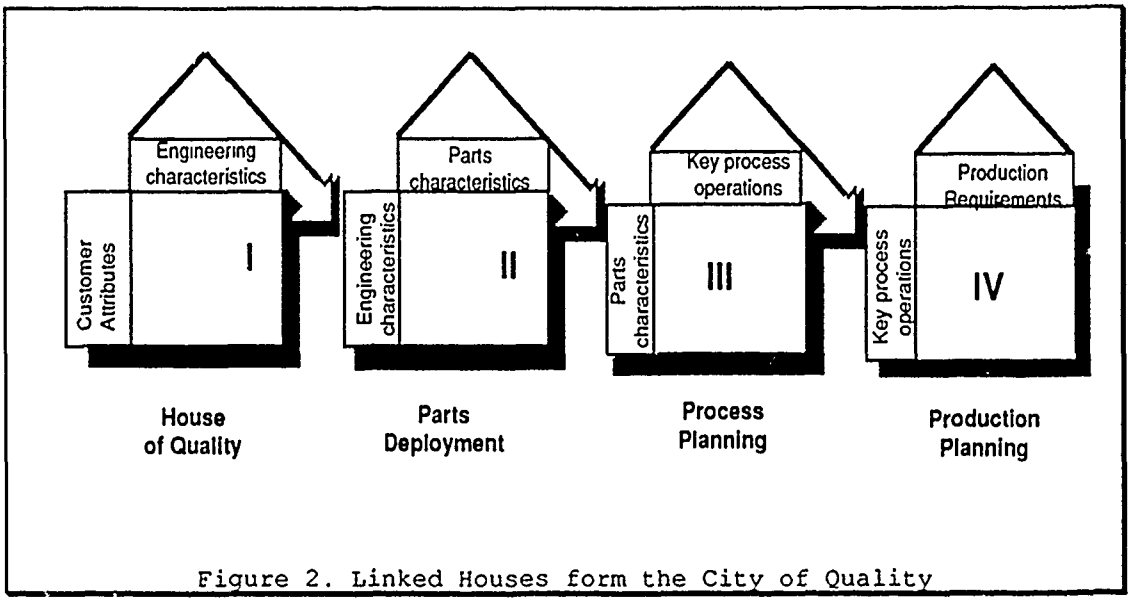


Figure 2. Linked Houses form the City of Quality

Thus, Coq provides an intertemporal GDSS, as well as a framework for trade-off analysis.

V. Hypertext

The concept of hypertext is credited to Vannevar Bush, while the word is credited to Ted Nelson by (Smith and Weiss, 1988) and by (Vaughan, 1988.) Pure hypertext is a non-linear form of writing. Ordinary written documents may be visualized as a linear graph as in Figure 3. Hypertext, on the other hand, allows an arbitrary network as in Figure 4. Topologically speaking, the linear graph of ordinary text can be embedded in one dimensional space, while the network of hypertext requires at least two dimensions, and possibly three.

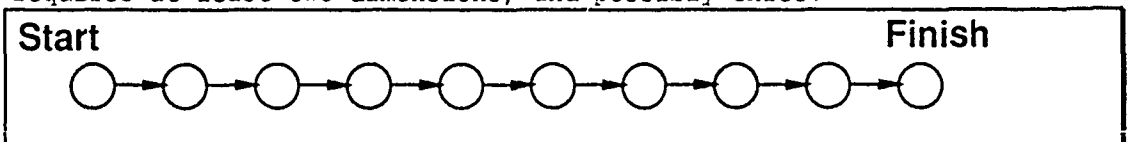


Figure 3. Linear writing.

Each node in the network represents some text, and is, perhaps, best visualized as a page in an encyclopedia or instruction manual. The reader, given a book or other paper document, is forced to move

sequentially through the text, with random access tedious, and the time required for such access interrupting the reader's train of thought.

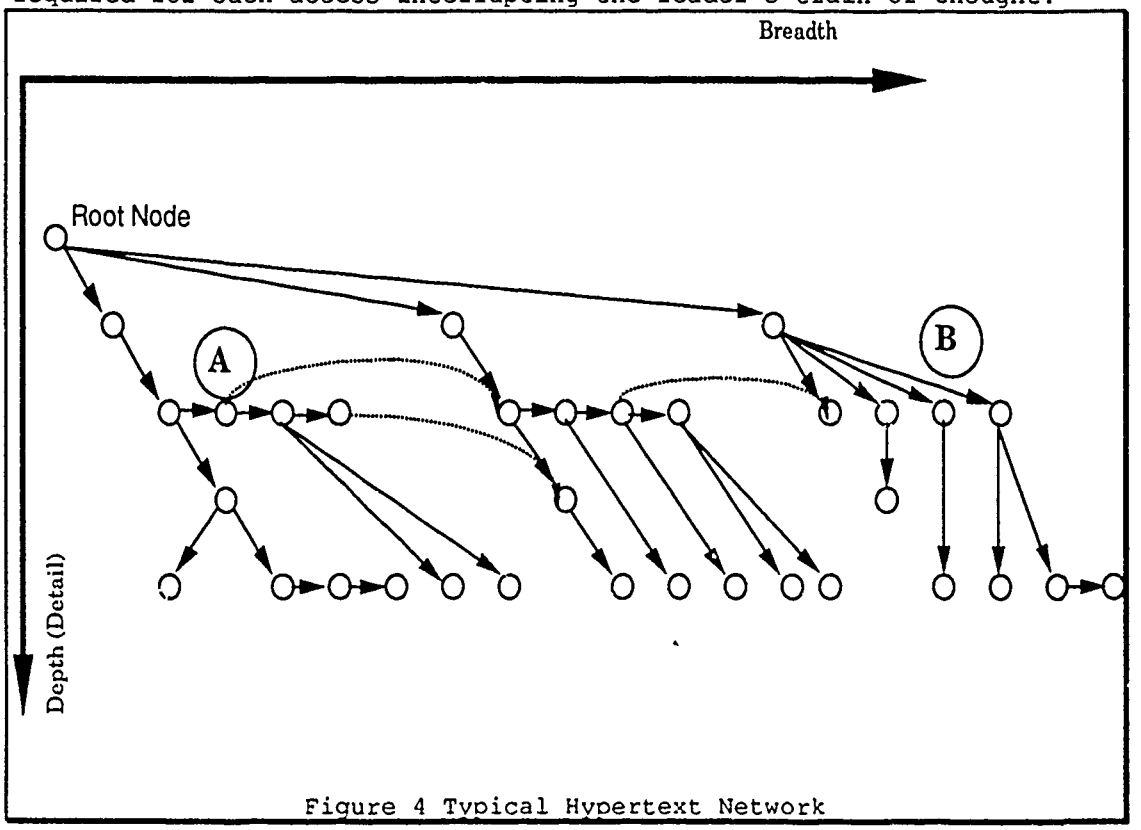


Figure 4 Typical Hypertext Network

Synergy between Hypertext and Coq template

The basic idea of a City of Quality, as shown in Figure 2, suggest some sort of network. From the root, the network branches to the four houses. Less obvious is that the basic House of Quality shown in Figure 1 has a hierarchical decomposition, well suited to a network. In addition, there is interest in encouraging a hierarchical approach to the acquisition of customer attributes and technical specifications, and this is particularly appropriate to the hypertext network: a node is assigned for customer attributes. This has, as subordinate nodes, primary attributes. These nodes have, as their subordinates, nodes for secondary attributes, etc.

Beyond this is the ability to attach notes and comments to any part of a house. The intersection of the row for an attribute and the column for a

specification forms a node. This node has, as children, separate nodes corresponding to each of the decision makers in the group responsible for the design. This nodes then have as children nodes with formulæ used to establish the specification from the attribute and with comments from the decision makers explaining their rationale for trade-offs made. This sort of network, operating interactively, is "readily" implemented in hypertext, but with difficulty using conventional programming.

VI. Preliminary Design of the Coq Hypertext System

A very preliminary prototype of a hypertext system implementing the City of Quality was developed and demonstrated. In addition, a set of rules and documents for structured development of a hypertext document was developed in parallel with the prototype.

A new concept in hypertext, that of a dynamic network, was developed. This involved significant basic research, and new information technologies.

Sophisticated, self-modifying software and recursion are used to make the "knowledge acquisition" process for the parameters required by the Taguchi method as painless as possible for the user.

Since the Coq system is for use by many persons, a facility for maintaining a history of changes, with the identity of persons responsible, is provided. In addition, users may attach comments and view the comments of others.

In addition, once each decision maker has completed cards to provide attributes and technical specifications, a coordinator for the group may then collect the cards electronically or otherwise, and re-distribute them in accordance with the procedures for Delphi or Nominal group techniques. This then replaces paper versions of these processes, eliminates data re-entry, provides a record of how decisions were reached, and, in general, greatly expedites and facilitates the mundane bookkeeping of group decision making.

VII. Summary

The basic mathematical theory of multi-person multi-attribute decision making developed in this paper shows the applicability of the Taguchi formalism. This formalism lends itself to the House of Quality paradigm for "knowledge acquisition" of the parameters that are required by the formalism. Finally, the process of turning qualitative user requirements into a functional system that addresses those requirements is not only amenable to automation, but requires automation in order to minimize the costs of the communications, including intertemporal communications, among the responsible decision makers.

This automation and communication are achieved by the City of Quality, or Coq system, developed using Hypertext methods. Innovative informatics involving self-modifying software were developed. The result is a powerful platform for group decision support of total quality and unified life-cycle engineering.

As a side benefit, for purposes of management of this (and future) hypertext projects, rough guidelines were established for hypertext projects, guidelines that can be extended as hypertext becomes an increasingly important tool for software development and prototyping.

VIII. Recommendations

The design of the Coq system has been described; implementation has been started, but is not yet complete. In developing Coq, it was discovered that hypertext, originally intended to allow an author to prepare a static but multi-dimensional document, is awkward for linking data between different parts of the document. Currently, this is addressed by building complex data structures; however, this solution has an adverse impact on maintainability of the system.

A second limitation of the implementation of hypertext used is based on the expectation of either limited user input, or input by a sophisticated user; hence, the platform has no provision for error trapping. As an example of the impact of this omission, should the user not understand that a numerical weight is needed, and instead provide a verbal description (or make a typo) the error will not be detected until

a calculation is made with the data. The error will break out of Coq and into native Hypercard, where the user must figure out what to do. Since the error message may be delivered long after the error was made, this could prove very frustrating for the novice or casual user.

Partial solutions include developing an algorithm for distinguishing between valid numbers and non-numeric data, or performing an immediate calculation, so at least the error message (perhaps uninformative) will be more closely related to the action which produced it.

A major extension planned that will address both these limitations is to link all cards to a relational database, which will make data transfer relatively painless, as well as providing better security and integrity.

In its current form, the system is limited to sequential multi-user access. This limitation is partly the result of the current hardware platform, which is a stand-alone machine, and partly the result of limitations on the time available for system development. The system can be upgraded to concurrent access once a LAN platform and additional software tools and become available.

In developing the system, a good start was made at developing project management tools for Hypertext projects. These need further testing and development to form a solid foundation for structured development of Hypertext.

The most important direction for further research, however, is to actually field test the system: this will conclusively identify deficiencies, strengths and potentials.

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FINAL REPORT

VARIABLE RESOLUTION IMAGERY FOR FLIGHT SIMULATORS

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VARIABLE RESOLUTION IMAGERY FOR FLIGHT SIMULATORS

by

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ABSTRACT

The design of the human visual system suggests a method for implementing sufficiently high resolution over a wide field-of-view in order to achieve the high fidelity required in flight simulators. Since conventional techniques of image processing are limited to position-invariant operations, we developed the appropriate formalism and techniques which can be applied in nonuniform filtering of images according to a distortion function which approximates the cortical magnification function. Variable resolution images were successfully generated on an IBM-PC, implementing the developed techniques. The images were subsequently transferred to a wide field-of-view display system for psychophysical experiments which will determine, in turn, the optimal distribution of displayed information according to a perceptual criterion. Such experiments have just begun and will continue in the follow-up program.

I. INTRODUCTION:

One of the basic problems encountered in vision and image representation is the high dimensionality of the image space. Since the required computational effort (and cost) increases as some function of the total amount of information specifying and image (e.g., the total number of bits, number of edges, texture density, or any other criterion defining the information), it is desirable to reduce the amount of information by optimally distributing it in accordance with human visual requirements (Kronauer and Zeevi, 1985; Porat and Zeevi, 1988; Zeevi Porat and Geri, 1989). This becomes essential when high-fidelity imaging is required to be generated and displayed over a wide field-of-view, and to be manipulated and/or processed in real time.

Conventional techniques of image processing, which are associated with linear systems theory, are applicable only when the systems operating on (or processing) the image are position-invariant and linear (Oppenheim, Wilsky, and Young, 1983). Under these conditions, it is possible to operate on an image either in the space domain (by convolution) or in the frequency domain (by filtering) in order to condition the image in a desired way. For example, operations in the space domain include smoothing an image in order to improve the signal-to-noise ratio, or extracting edges in order to perform pattern recognition or contour identification. The corresponding operations in the frequency domain would be low-pass and band-pass filtering, respectively. The operation, in the space domain, can be represented formally as:

$$I_{(x,y)} = \int\int I_i(x',y')\cdot k(x-x',y-y')\,dx'\,dy' \tag{1}$$

$$\stackrel{\Delta}{=} I_i(x,y)**k(x,y) \quad ,$$

where $I_i(x,y)$ and $I_o(x,y)$ denote the input and output images, respectively, $k(x,y)$ is the convolution kernel which represents the mask used to operate on the image, and the double asterisk (**) denotes a two-dimensional convolution. In the spatial frequency domain the corresponding expression is obtained by Fourier-transforming Eqn. 1, yielding:

$$L_o(\omega_x, \omega_y) = L_i(\omega_x, \omega_y) \cdot K(\omega_x, \omega_y) \quad , \qquad (2)$$

where L_i and L_o are the spatial frequency spectra of the input and output images, respectively, and $K(\omega_x,\omega_y)$ is the modulation transfer function (MTF) of the system. In the context of the present study, the system is either a low- or band-pass filter as mentioned above.

The computational effort required for generation and real-time manipulation and processing of imagery increases obviously with the size of the image. Consequently, most existing techniques are limited to an image size (i.e., a number of pixels) which cannot accommodate the dual requirements of high-resolution and wide field-of-view confronting the designers of flight simulators.

II. OBJECTIVES OF THE RESEARCH STUDY:

Since image fidelity is in the final analysis determined by the human visual characteristics, it is an intellectual and practical challenge to develop the required techniques which may determine how visual requirements vary over the visual field, and which may facilitate as such the ultimate development of optimal image representation (i.e., images which are matched to visual requirements). The immediate objective of this research program, as was jointly defined with the researchers at AFHRL/OT, was the development of a formalism and associated techniques which could be applied to generate so-called variable resolution images. Unlike conventional images in which the amount of detail is determined by the nature of the visual environment, in the variable resolution image, as the term implies, the resolution varies as a function of the distance from a point which is coincident with the visual axis during its observation.

The longer term objectives, and the results of this research effort will be instrumental in achieving such objectives, are to determine the visual requirements as a function of eccentricity, and to develop efficient techniques for computer generation of images which are matched to visual requirements.

III. POSITION-DEPENDENT IMAGE PROCESSING:

Since we are interested in processing images in a position-dependent fashion similar to that taking place in the visual system, we cannot, for the reasons described above, apply the classical techniques of linear systems theory. Thus, in order to perform image processing with variable resolution, we have to devise techniques which are analogous to filtering, and other linear operations, but which can be applied to position-dependent systems. In order to generate a variable resolution image, it is necessary to operate on a fixed-resolution image with a system whose characteristics vary from point to point. Thus, the kernel, k(x-x',y-y'), shown in Eqn. 1, which is a function only of the differences between pairs of variables along the two spatial coordinates, becomes k(x,x',y,y'), which is, in addition, a function of position in two dimensions. Thus, in this case:

$$l_o(x,y) = \int\int l_i(x,y) \cdot k(x,x',y,y')\,dx'\,dy' \qquad (3)$$

Since Eqn. 3 is not of the form which defines a convolution, the Fourier transform cannot be applied to it, and thus an MTF cannot be defined for the system (filter) which is used to operate on the image. Unlike the convolution operation (Eqn. 1), which can only be performed in the context of linear, position-invariant systems, the integral operation described by Eqn. 3 may transform a bandlimited signal into a signal whose bandwidth is not limited. It is well-known that nonlinear systems introduce frequency components which are not present in the input signal. In the case of systems which are linear and position-invariant, this cannot occur as can be concluded most easily from an examination of the operation in the frequency domain. Since the convolution integral is transformed into the product of the spectrum of the input signal, and that of the MTF of the system, it is clear that no extra frequency components can be introduced. Extra frequency components or bandwidth expansion can also result from a superposition integral of the type shown in Eqn. 3 (which represents a linear but position-varying operation). The addition of frequency components is more often encountered in the case of nonlinear operations (i.e., nonlinear systems). However, this type of bandwidth expansion can also

result from the operation of a system which is linear but position-varying. Consider a signal which is bandlimited and thus satisfies the Nyquist condition. If the signal is transformed by distorting the position axis, the resulting signal no longer satisfies the Nyquist condition in that the signal is no longer bandlimited. The process of spatial axis distortion is analogous in a way to FM modulation of a bandlimited signal which introduces extraneous frequency components (in fact, extends the band to infinity). The distorted signal, therefore, cannot be represented by a discrete set of samples.

If the distortion function is known, there are in principle two ways of representing an image by a discrete set of samples. First, the signal can be restored (the inverse distorted) and then represented like any other bandlimited signal. Image reconstruction is then performed in accordance with the provisions of the Whittaker-Shannon sampling theorem using an interpolation filter in the form of a sinc function (Jerri, 1977). Stated another way, if an image can be projected, by a transformation along the spatial axis, into the space of bandlimited images, then it can be adequately represented by a discrete set of samples. The second way of representing a variable resolution image by a discrete set of samples is to distort and position the interpolation functions (sinc functions) nonuniformly in accordance with the positional distortion function, and then represent the signal even though it is not bandlimited and as such does not satisfy the Nyquist condition.

If the distortion function is not known, or if it is not known whether the image even belongs to the space of locally bandlimited functions, a number of theoretical and practical questions arise. For example, if it isn't known what type of nonuniform processing was used on a given bandlimited image to generate a variable resolution image, how can it be determined whether the latter belongs to any space of locally bandlimited functions and if so to which of the infinite number of such spaces does it belong? Theoretically, in order to determine whether an image belongs to a particular space of locally bandlimited functions, one would have to first apply the nonuniform filter which would project that (or any other) image into that space. If the resultant image is identical to the original one, this implies that the image belonged to the space to start with, since that space itself is a so-called reproducing kernel space (Aronszajn, 1950). Since there are infinitely many such spaces, it is not possible of course to proceed in this way. The alternative, practical, approach is

to devise techniques (such as estimating statistically the rate of zero-crossings from a number of images, or by estimating the effective local bandwidth which characterizes the representation of the image in some combined position-frequency space such as Gabor-space, Wigner distribution, or the complex spectrogram) for estimating a distortion function which optimally matches the nonuniform distribution of information over the image field. Estimation of the optimal distortion function will determine to which of the infinite number of locally bandlimited image spaces does the given image fit best. Several techniques have been proposed by Shlomot and Zeevi (1989) for estimating the distortion function which best represents a given image in that once the corresponding projection to the (well-defined) space of locally bandlimited functions is performed, a minimal least-mean-square error results. In the context of the present study the problems are somewhat simpler because either the exact distortion function, corresponding to a given image, is known or the type of nonuniform processing applied to a bandlimited image is known and as such can be used in estimating the distortion function.

In the context of the present study, we will need to determine how to nonuniformly distribute our sampling points such that a variable resolution image will be transformed into a bandlimited function, and in this process reduce (effectively represent) the image data. Also, starting with a standard (uniform-resolution) image, how can one devise a nonuniform filter which will result in the desired distribution of resolution across the image while at the same time provide a formalism which satisfies certain theoretical constraints related to the proper representation of the image by a set of sampling points, and the reconstruction of the image from that set with no aliasing effects?

Many of the conceptual and practical difficulties described above are avoided in the context of the present study since we wish to generate a variable resolution imagery using a nonuniform filter which corresponds to a distortion function whose exact form is known. Since we are interested in images which will optimally match the processing capabilities of the visual system, the distortion function is simply some derivative of the so-called cortical magnification function (CMF). It should be noted however that published data on CMF are based on stimuli of the simplest primitives (and even with such stimuli the data are scattered over a 2:1 range of CMF), whereas our related study with patches of Gabor

textures indicate that the CMF is related to various stimulus attributes (Geri, Lyon, and Zeevi, 1989). Given this fact it remains to first choose an appropriate form for the kernel of the nonuniform filter, and then to choose its specific structure in accordance with the form of the distortion function reflecting in turn a specific CMF. In order to benefit from the theory and practical implications of the well-established formalism applicable to uniform sampling and reconstruction of bandlimited functions, the filter that we seek should be analogous to a bandpass filter in the sense of being a reproducing kernel as defined earlier. It may be instructive to draw an analogy between the proposed technique for nonuniform processing and the filtering operation which projects images into the space of bandlimited functions, in the case where uniform processing and distribution of information (sampling density) is desired. In that case the image is conditioned by convolving it with a sinc function which projects the image into the space of bandlimited functions (the so-called B^{Ω}-space; see Jerri, 1977). The analogous operation in the case of nonuniform processing (which results in variable resolution images) is the integral operation wherein the kernel is a sinc function whose spatial spread increases as a function of the distance from the center of the image in accordance with the CMF (or any other desired function). This operation projects any image (fixed or variable resolution) into the space of so-called locally bandlimited functions (Horiuchi, 1968; Shlomot and Zeevi, 1989a,b), a space which we denote by B_{γ}^{Ω} and which is the space of functions whose local bandwidth extends over the area of support, Ω, and corresponds to the distortion function, γ.

IV. THE LOCAL BANDWIDTH

The concept of local bandwidth may at first appear to be ill-defined in that it seems to be self-contradictory according to the uncertainty principle. According to this principle, in order to have a limited bandwidth, the function has to be of infinite extent and the information regarding the bandwidth (which determines in turn the resolution in frequency) is determined in relation to the entire extent of the signal. The term local bandwidth implies that the frequency characteristics are derived from local properties, hence the contradiction. It is therefore important to elaborate on this issue. The concept of local bandwidth can best be understood in the context of signal representation in the combined position-frequency space where a different (effective) bandwidth can be specified (within

the limitations imposed by the joint uncertainty; Gabor, 1946) for each position. One interpretation of local bandwidth is that the local properties of the image can be used to determine a transformation of it which will produce a globally bandlimited image (i.e., one that belongs to the space B^{Ω}). Another interpretation of the local bandwidth can be understood in the context of a position-varying MTF analogous to the one introduced by Zadeh (1952) in his analysis of time-varying systems. In analogy to the formalism proposed by Zadeh, the MTF is a function of both position and frequency, and as such, one can formally define the local bandwidth for a given position. Similarly, Horiuchi (1968) proposed that if a signal can be obtained by the inverse Fourier-like operation defined as follows:

$$f(x) = \frac{1}{2\pi}\int_{-\pi W(x)}^{\pi W(x)} F(x,\omega)\cdot e^{j\omega x}\, d\omega \qquad (4)$$

where F(x,ω) is a position-dependent, Fourier-like, transform of f(x), then the signal has a position-varying bandwidth W(x). Shlomot and Zeevi (1989) extended the concept of position-varying bandwidth by showing that any signal which belongs to the space $B_{\forall}^{\Omega}$ is also characterized by such a bandwidth, which is referred to here as a local bandwidth.

Once an analogy is drawn between uniform and nonuniform operations, the concepts of "local bandwidth" and "$B_{\forall}^{\Omega}$-space" (and their application to the generation of variable resolution imagery) become relatively simple and straightforward. It should be stressed that the proposed technique for projecting a given image into $B_{\forall}^{\Omega}$ is the proper way of dealing with the issue of variable resolution in that the resultant local properties are well-defined, and in that such an image can be represented by a (finite) set of samples even though it is not bandlimited and as such does not satisfy the Nyquist condition. Further, such a technique permits additional nonuniform processing in the form of nonuniform pyramids and other schemes (Zeevi, Peterfreund, and Shlomot, 1988; Peterfreund and Zeevi, 1989).

V. SAMPLING OF IMAGES WHICH BELONG TO B_V^Ω-SPACE:

The purpose of development of the variable resolution technique is its eventual application in efficient image representation in flight simulators (and other types of, high-fidelity, wide field-of-view display systems). Thus, for the purpose of image storage and/or manipulation, it is desired to exploit the variable-resolution properties of the image such that the data set can be reduced accordingly. In fact, this is the very reason for generating variable resolution imagery. The question then is, how should the sampling points be nonuniformly distributed over the image field such that they adequately represent the image in the sense that the variable resolution image can be reconstructed from this set of sampling points. Intuitively, it is clear that as the data become more sparsely distributed as a result of variable resolution processing, the required density of sampling points decreases. A sampling theorem for images which belong to B_V^Ω, the space of locally bandlimited function, was first introduced by Clark, Palmer, and Lawrence (1985). This theorem was extended by Shlomot and Zeevi (1989a,b) to allow images to be sampled using other than rectilinear patterns. As stated earlier, Shlomot and Zeevi also showed that the kernel which projects images into B_V^Ω is a reproducing kernel. A formalism for reconstructing an image, f(x), from a properly chosen set (x_n) of nonuniformly distributed samples may be developed as follows.

Considering first the case of uniform sampling, let g(x) belong to the space B^Ω (i.e., assume that the area of support, Ω, defined by its spectrum is limited), and let the periodicity matrix, U, represent the periodic extension of the spectrum, $G(\omega)$, then, g(x) can be reconstructed from its samples along the uniform grid, $x_n = V\,n$, [where the sampling matrix, V, satisfies the condition that the inner-product of U and V equals a constant times the identity matrix, (i.e., $U^tV = 2\pi I$)], according to the following formula:

$$g(x) = |det\ V| \cdot \sum_n g(x) \cdot \phi(x - Vn) \quad , \tag{5}$$

where $\phi(x)$ is defined by,

$$\phi(x) \triangleq \frac{1}{4\pi^2}\int\int_{\Omega} \exp(j\omega' x)\, d\omega \qquad (6)$$

We now extend the above theorem to also include functions which belong to B_{γ}^{Ω}.

If g(x) is a globally bandlimited function (i.e., if $g(x) \in B^{\Omega}$), then f(x) is considered a locally bandlimited function (i.e., $f(x) \in B_{\gamma}^{\Omega}$), if and only if, a distortion function, $\gamma(x)$, exists such that $f(x) = g[\gamma(x)]$ (i.e., such that f(x) can also be expressed as a function of g, and hence is a member of B^{Ω}). Note that if $\gamma(x) = x$, then the two above described spaces become identical. Such a locally bandlimited function can be reconstructed from the properly distributed set of samples, $\{x_n\}$, where $x_n = \gamma^{-1}(Vn)$, according to the following formula:

$$f(x) = g[\gamma(x)] = |det V| \cdot \sum_{n} g(Vn) \cdot \phi[\gamma(x) - Vn]$$

$$= |det V| \cdot \sum_{n} f(x_n) \cdot \phi[\gamma(x) - Vn] \qquad (7)$$

The implementation of the above formalism can be better understood by considering the recontruction of a one-dimensional signal in which case the above Eqn. 8 becomes:

$$f(x) = \sum_{n=-\infty}^{\infty} f(x_n) \cdot sinc[\gamma(x) - n] \quad , \qquad (9)$$

where,

$$sinc(x) \triangleq \sin(\pi x)/\pi x \quad .$$

Substituting $\gamma(x) = W(x) \cdot x$ into Eqn. 9 yields:

$$f(x) = \sum_{n=-\infty}^{\infty} f(x_n) \cdot sinc[W(x) \cdot x - n] \quad , \qquad (10)$$

where W(x) provides a measure of the sampling density and of the local bandwidth. As noted above, if $\gamma(x) = x$, the function f(x) belongs to B^{Ω}. Indeed, in that case, W(x) = 1 and we have uniform sampling density and Eqn. 10 becomes the standard equation of interpolation from a uniformly distributed set of samples.

VI. GENERATION OF VARIABLE RESOLUTION IMAGES:

A locally bandlimited variable resolution image can only be produced using a projection technique, similar to that described by Eqn.3, wherein a (variable) sinc function is used as the integration kernel. Such a technique is computationally intensive and relatively difficult to implement and so we have also implemented an alternative technique which uses an integration kernel in the form of a (variable) pulse function. Although this approach does not conform to any complete formalism, which might otherwise be used to fully characterize the resulting image for the purposes of further processing and representation, it is sufficient for obtaining preliminary psychophysical data which may in turn be used to refine our techniques.

Specifically, the approach we have adopted for generating variable resolution images is a discrete implementation of the operation described by Eqn. 3. Since we desire the final variable resolution image to be radially symmetric (i.e., to contain the same amount of degradation at all points equidistant from the origin or some elliptical function), it is convenient to first transform the image from Cartesian to polar coordinates before applying the various schema for distributing information across the image space. The discrete implementation of the integration operation which results in a variable resolution image can be described as follows:

$$I_o(i,j) = \sum\sum [I_i(m,n) \cdot k(i,j,m,n)] \quad , \qquad (11)$$

where k(i,j,m,n) is a discrete version of the integration kernel. In this implementation of the variable resolution program, the kernel was a simple pulse function. The magnitude of the pulse function was fixed and equal to one, while its width along both the radial and angular coordinates varied as a function of its distance from the center of the image in accordance with various approximations to the CMF derived from threshold psychophysical data. Subsequent to the integration operation, the image was transformed back into the Cartesian coordinate system. A sequence of examples of variable resolution images obtained by performing the nonuniform filtering with three different CMFs are shown in Fig. 1 along with the original image. It should be noted that once such images are projected over a wide field-of-view display system, as they were projected here at AFHRL/OT, they look very similar. However, how similar they appear to an observer has to be determined by careful psychophysical experiments. Such experiments were just begun and will continue as part of the Research Initiation Program.

The second approach brings us back to the question of how to project an image which has nonuniformly distributed information, and which is not bandlimited, into the space of bandlimited functions. Here we start from the end result of the desired variable-resolution image. The image is distorted by a transformation along the spatial axis which projects it into the space of bandlimited functions. Alternatively, a projection filter can be devised, in analogy to the bandlimited filter, which projects the image into the space B_V^0 of locally bandlimited functions. This approach introduces a new formalism which permits a simple transformation from a given distribution of resolution to a desired one. There exists in this case a complete analogy to uniform sampling and reconstruction from a set of samples in the sense that the projection filter is a reproducing filter. In other words, if a given image does not belong to the space of locally bandlimited functions, it will be projected into it. Once it belongs to this space, it will remain there by repeatedly applying the reproducing kernel. Further, there exists an interpolation filter which can be used in reconstruction of the variable resolution image from its set of nonuniformly distributed samples.

VII. RECOMMENDATIONS:

a. The formalism and techniques developed as part of this research effort now afford the evaluation of visual requirements by means of relatively simple psychophysical experiments. The preliminary experiments indicate that definite advantages will result from incorporation of variable resolution imagery into the FOHMD technology. It is therefore recommended that a concerted effort be devoted to immediate evaluation of peripheral visual requirements, with both static and dynamic images. It is further recommended that some of this research will be conducted jointly by the researchers of the CAIP Center, Rutgers University, where a major research effort on foveating systems is currently being conducted, and AFHRL/OT, Williams AFB, where some of the most advanced wide field-of-view image generation and display technology is available.

b. To permit effective execution of the required psychophysical experiments specified in (a), it is recommended that an image workstation interfacing a wide field-of-view display be adopted for sequential representation of images in a way which will permit operator control of frame display time in the range of 30-300 ms and variable interframe interval.

c. In view of the promising preliminary experiments and the adequacy of the developed techniques for careful evaluation of visual requirements, it is recommended that a major R&D program be undertaken with the goal of developing efficient techniques for CGI which exploit the variation of human visual requirements, across the visual field, in an optimal manner (Zeevi, Porat, and Geri, 1989).

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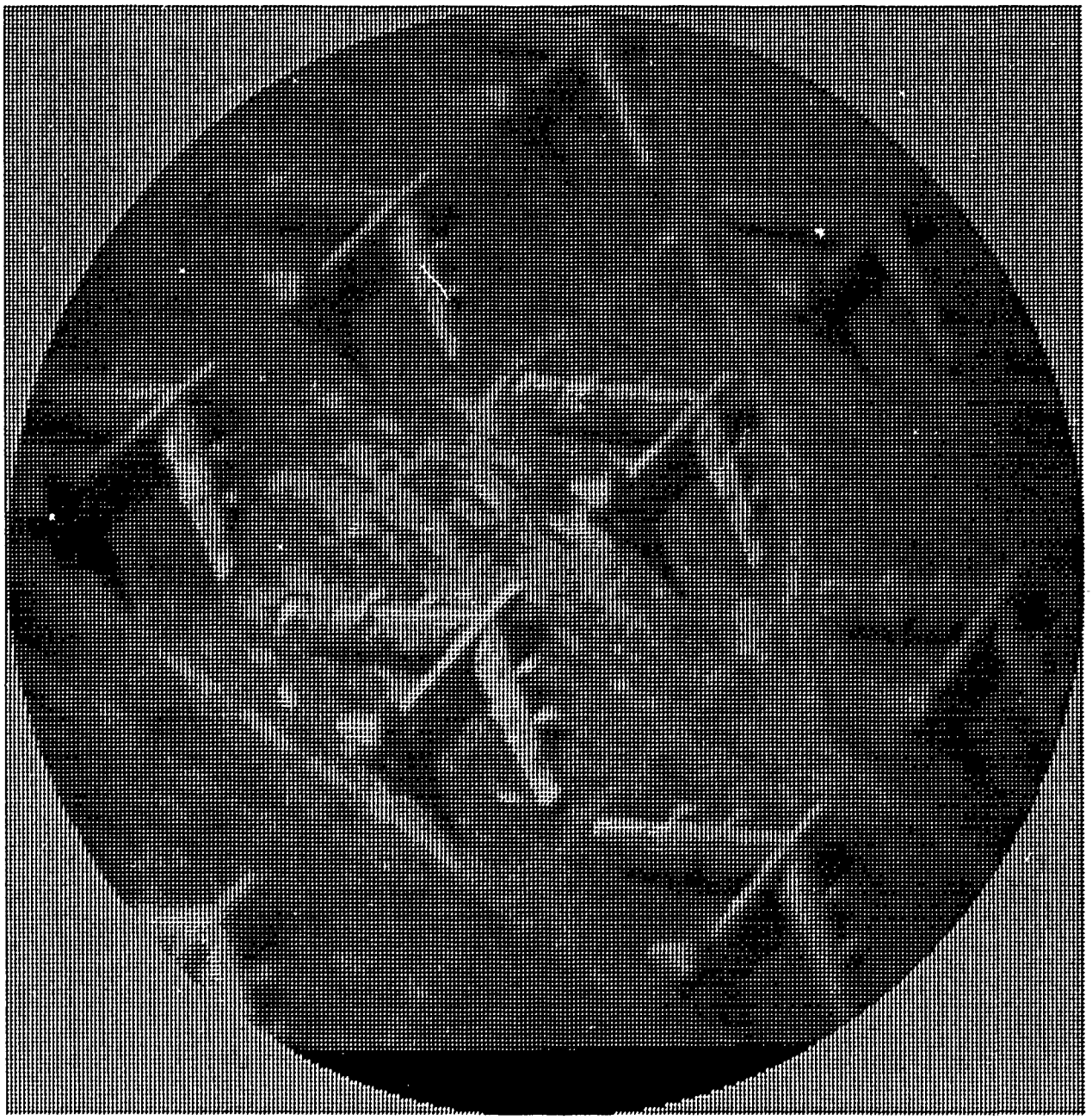
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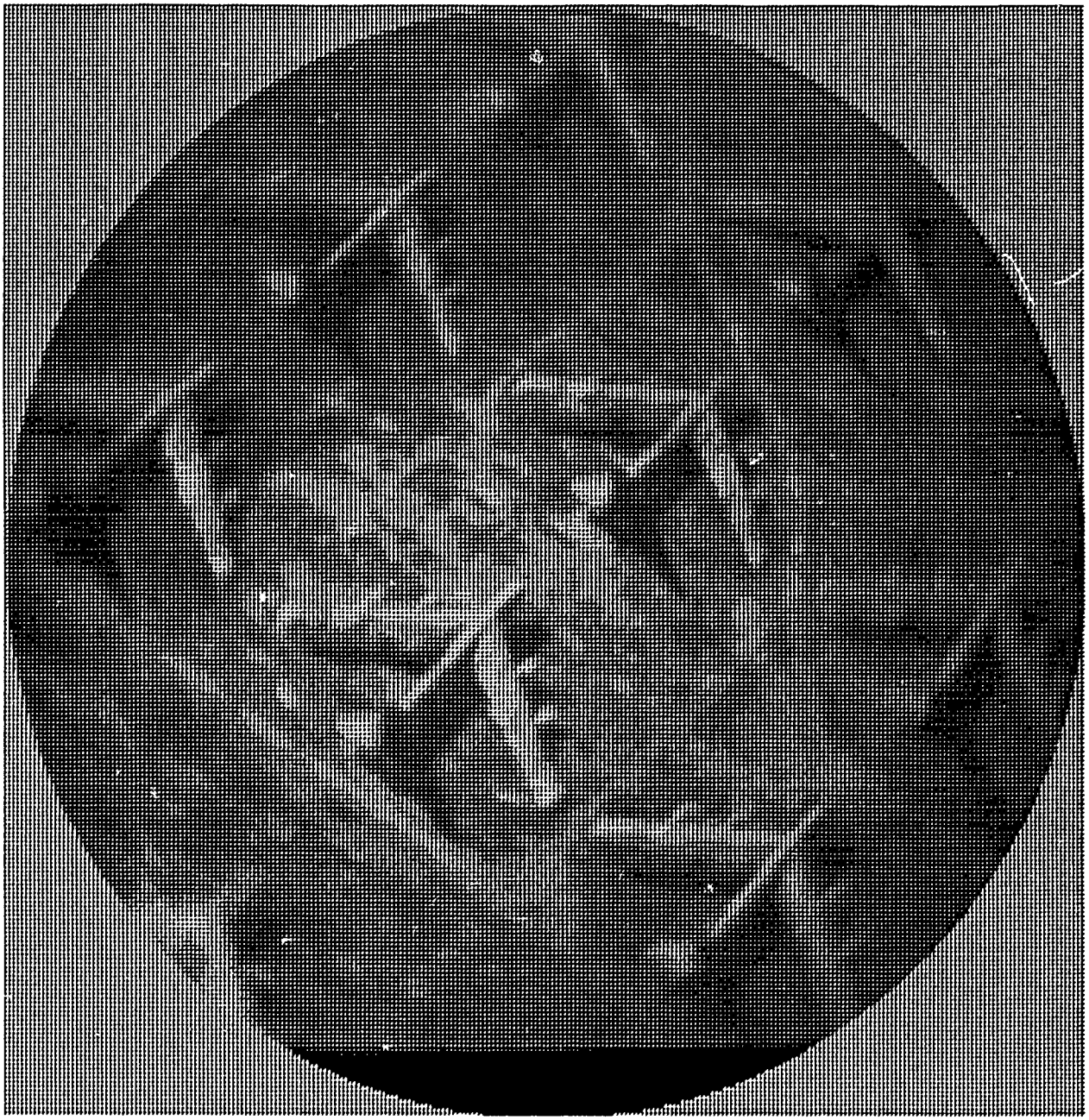


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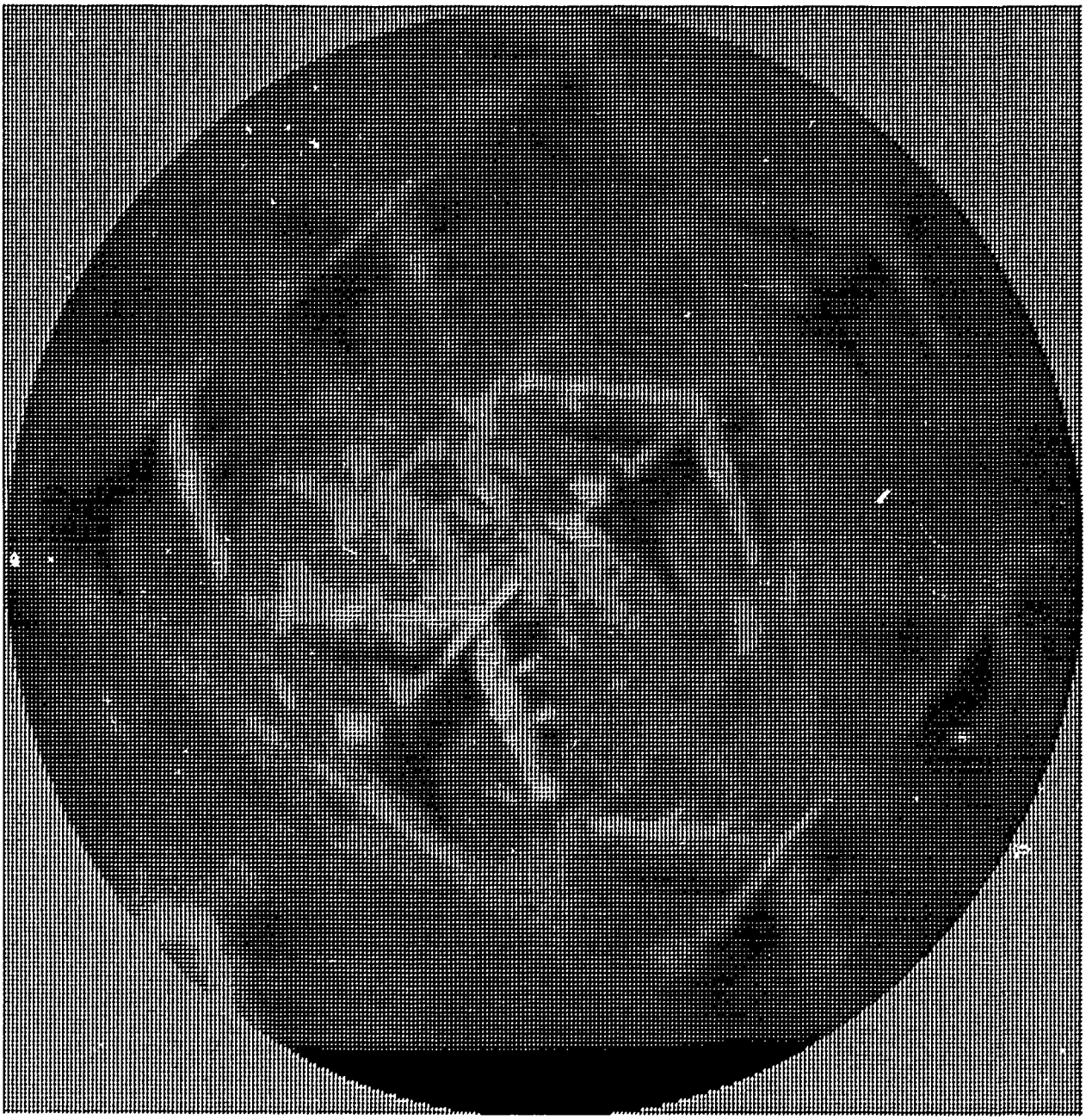
Figure 1. Examples of variable resolution images generated by simple averaging over an area of pxp pixels (in the polar coordinate system) where p increased as a function of the radius according to the CMF: $p = INT\{1+a[1-(1+br)^{-1}]\}$. (A) original, (B) a=2, b=0.04, (C) a=4, b=0.04, (D) a=8, b=0.04



(B)



(C)



(D)

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FINAL REPORT

NEUROCOMPUTING THE STUDENT MODEL IN AN INTELLIGENT TUTORING SYSTEM

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Date:	5 August 1989
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NEUROCOMPUTING THE STUDENT MODEL IN AN INTELLIGENT TUTORING SYSTEM

by

Robert A. Zerwekh

ABSTRACT

Current intelligent tutoring systems (ITSs) share a common architecture, two components of which are the student module that contains information about the student using the tutoring system and an instructional module that consists of pedagogical strategies and plans. The pedagogical plans generated by the instructional module are designed to be individualized for the student who is using the ITS. The student module, therefore, must be constructed so that it models the current student's cognitive states and requirements.

This report critiques current student modeling techniques as computationally too demanding due to very large data bases that attempt to capture all possible errors a student may commit and/or all possible inferences that may be made during a tutoring session. An alternative approach to the student modeling problem is proposed that involves using neural network technology both to model the student and to analyze this model. Follow up research is proposed that will implement this approach in an intelligent tutor for foreign language teaching.

Acknowledgments

I wish to thank the Air Force Systems Command, the Air Force Office of Scientific Research, and the Human Resources Laboratory at Brooks Air Force Base for sponsorship of this research. I would also like to thank Universal Energy Systems for their assistance and support during the past few months.

Many individuals at IDI helped me to have a rewarding and enjoyable research period. I would especially like to thank Lt. Col. Hugh Burns for his insights and his guidance, Major James Parlett for explaining numerous acronyms, Captain Kevin Kline for his sense of humor and discussions about MITT, Wes Regian for taking the time to answer my endless questions, Kurt Steuck for the discussions on knowledge acquisition, and Jim (Frans) Fleming for far too many things to mention.

I. INTRODUCTION:

The Intelligent Systems Division of the Human Resources Laboratory at Brooks Air Force Base investigates principles and techniques of artificial intelligence that can be used in the design and development of intelligent tutoring systems (ITSs). Such tutoring systems provide realistic simulations and hands on training for Air Force personnel for situations or tasks that would be very costly or highly risky if performed on real equipment. Although functioning ITSs are a relatively recent phenomena, enough success has been achieved in their design and deployment that it is possible to identify a system configuration, or architecture, that is common among them all. This architecture consists of five primary components: an expert module that contains expert knowledge about the teaching domain, an instructional module that consists of pedagogical strategies and plans, a student module that contains information about the current student using the tutoring system, a device simulation that provides an instructional environment, and an interface by which student and ITS interact.

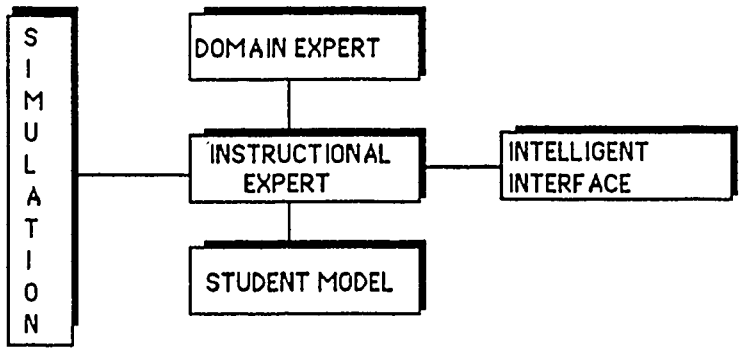


FIGURE 1. INTELLIGENT TUTORING SYSTEM ARCHITECTURE

It is obvious from this architecture that the instructional module figures prominently in an ITS configuration. It is responsible for constructing a

pedagogical plan, or strategy, that will present the lesson material so that it is individualized for the particular student who is currently using the ITS. Consequently, the instructional module must interact with the other primary modules of an ITS, and in particular, with the student module. For if the instructional content is to be tailored to fit the present cognitive states and requirements of a particular student, then it is critical that the instructional module be provided with accurate, informative, and useful information about this student. The process of constructing, or inferring, the student model in an ITS is called diagnosis because the ITS is attempting to uncover a hidden cognitive state (the student's knowledge) from his or her observable behavior (VanLehn, 1988). Thus, a continuing area of research in ITSs is the investigation and development of tools, or techniques, that will efficiently provide a more accurate model of the student.

One of the research areas I had investigated before coming to Brooks Air Force Base pertained to the use of neural networks in representing ambiguous, uncertain knowledge in intelligent systems and on various strategies that could be employed to allow such a system to reason about and learn from its knowledge base. The aim of the research was to acquire a better understanding of the effectiveness and limitations (and reasons underlying those limitations) of how a system based on neural network architecture could learn and reason in an area of knowledge that is characterized by uncertainty.

In addition to my research on neural networks, I have much practical research experience in the design and implementation of computer assisted instructional systems. A colleague in my department (Jim Henry) and myself are the principal architects of a computer based instructional system that teaches second language acquisition to university students. FLIS (Foreign Language Instruction Station) is a frame based system that employs graphics

and text in both English and foreign fonts such as Thai and Burmese. FLIS also provides random access to prerecorded audio speeches using a device called the Instavox. Speeches or words in a foreign language can be recorded using the Instavox and then accessed almost immediately under software control. A typical 'frame' therefore combines interesting and informative visuals with high fidelity human speech.

Both of these research areas contributed to my assignment at the Intelligent Systems Division of the Human Resources Laboratory. Little work has been done on intelligent tutors for foreign language instruction and thus is an area that poses a clear need for research. In addition, no work at all, to my knowledge, has been done with neural networks and intelligent tutoring systems. A clear research need exists to investigate using neural network technology as a tool to help ITSs fulfill their true potential. In particular, neural network technology may prove to provide an excellent technique for performing the diagnosis of the student model.

II. OBJECTIVES OF THE RESEARCH EFFORT:

My research efforts during the Summer Faculty Research Program had two primary objectives. The first was to investigate the feasibility of using neural network technology as either a supplement to, or the replacement of, some primary component (or components) of ITS architecture. Neural networks have been employed in various problem solving environments by a number of researchers, and the successes achieved by some of these investigations warrant further study of this model of computing (see Grossberg, 1988; Rumelhart and McClelland, 1987).

The second objective developed after I began my initial review of ITS literature and had begun to acquire some hands on experience with existing

ITSs. It soon became apparent that diagnosing the student model was a difficult problem, and because of this difficulty, most ITSs have dismally underdeveloped student models. It appeared to be a worthwhile research effort, then, to use a neural network as a tool for modeling the student.

III.

a. The neural network computing model of information processing represents a radical departure from the programmed environment of the conventional serial computer. Instead of relying on algorithms that specify precisely how a problem is to be solved, or in what manner information is to be processed, neural networks produce "transformations in response to their environment" (Hecht-Nielsen, 1987). The basic idea underlying neural networks is that information processing takes place through the actions of a large number of simple processing elements that are called units or neurons. Each unit sends excitatory or inhibitory signals to the other units in the network (Rumelhart & McClelland, 1987).

What distinguishes neural network type processing from serial type conventional computers is its parallel and distributed nature of computation and the absence of algorithms that specify how to reach a solution. Computation is parallel because each processing unit, each neuron, is carrying out its internal computations simultaneously with every other processing unit. Computation is distributed because what the network "knows" does not exist at a single location in memory as in conventional computers. Rather, knowledge exists in the strengths of the connections that exist between the processing units. It is helpful to think of knowledge in this sense as consisting of a pattern of activity among the various processing neurons.

A neural network is not programmed as conventional computers are. Instead of relying on a coded algorithm that describes each step of the information processing activity, a neural network develops its own processing capabilities by being presented with examples of the information processing function it is to emulate. Repeated exposure to such examples causes the network to adapt itself internally such that it eventually is able to perform the desired function. Presenting examples to the network is called training the network. Learning occurs during training in accord with a learning rule which modifies the connection strengths that exist among the processing units. Adjustment of these connection strengths continues until the network is capable of performing the function it is being taught.

There are two fundamental learning paradigms that are employed in neural networks. Supervised learning requires that some external coach be present to indicate to the network what counts as correct output. Typically, during training, facts are fed to the network that consist of some input and the desired output. The network reviews these facts repeatedly while it adjusts the connection strengths among the processing units until presentation of an input produces the correct output. In unsupervised learning the desired output is not explicitly provided. The teaching function is discovered by the network itself. The structure of this internally derived teaching function and the nature of the input patterns determine what features the network will learn to respond to. Such networks are particularly adept at discovering regularities in data.

Although a number of different types of neural networks are currently being explored in research, their architectures can be classified into one of two basic types - feed forward or feedback. In feed forward networks, the processing units receive input only from a previous layer of processing units and send output only to the next layer of units in the network; units in a given

layer do not connect to one another. Feed forward networks employ a variety of learning rules including perceptron, adaline, cognitron, boltzman, counter propagation and back propagation to name a few. These kind of networks are very good at producing generalizations and at pattern matching.

In feed back networks, the processing units can receive input from any other unit, including itself. Since a processing unit can connect to any other unit, these networks typically have only one layer of units. Feed back networks are known by a number of names including Hopfield networks, associative memory networks, bi-directional associative memory (BAM), and content addressable memory (CAM). These kind of networks are quite proficient at reconstructing an internal representation from incomplete and error plagued data.

Researchers have applied neural networks to a variety of problem solving tasks with varying degrees of success. Some of the areas in which neural networks have been used include perceptual completion of familiar patterns, text to speech conversion, image compression, pattern recognition, target classification, label inspection and part sorting, and noise filtering.

b. The parallel and distributed nature of computation that characterizes neural networks has implications that make this model of computing highly attractive for incorporation into the architecture of an ITS. One implication is that it makes the system capable of dealing with very large numbers of constraints such that efficient and tractable computation can be performed. Conventional computers cannot duplicate the non-monotonic reasoning powers of humans. People reason faster when they are able to exploit additional constraints. Yet computers, when laboring under a number of propositions, rules, and

constraints get slower. This soon degenerates into a combinatorial explosion of factors that need to be considered in the reasoning process.

Parallelization and distribution bestow a property of graceful degradation on such systems. Because knowledge is not stored in only one location and since processing is distributed throughout the system, damage to the system or to processing units does not result in the complete loss of knowledge or the inability to continue to function. Instead, damage to the system simply results in some partial reduction of processing power. There is a graceful degradation of the system, not a complete shutdown. It is therefore possible to retrieve and process information even when part of the network has been compromised.

Neural networks share the content-addressability feature of memory with humans. People, with various degrees of success, are often capable of recalling a specific memory based on nearly any attribute of that memory. We are capable of doing this with conventional computers as well, but with very high costs associated with excess memory requirements or multiple index maintenance. Since knowledge resides in the connection strengths of the processing units in a neural network, activating or stimulating one or more of these units leads to the activation of the others in accord with the learned connection strengths. Consequently, we can produce the desired representation or memory merely by providing some feature or combination of features.

In sum, the neurocomputing model of information processing is ideally suited for the kind of processing that is required for an efficient and capable diagnosis of a student's performance and cognitive states. This is particularly true for domains such as language learning where a variety of complex cognitive skills and constraints affecting understanding are at work.

IV.

a. The ITS architecture depicted in figure 1 indicates the central role played by the instructional module in an ITS. Indeed, it can be said to represent the true promise of intelligent tutors. This promise lies in a tutor's ability to dynamically adapt lesson and instructional content and the form instruction should take to meet the changing cognitive states and requirements of the student. The capability to construct individualized lesson plans, however, is logically dependent on receiving accurate information about the particular student who is currently interacting with the ITS. The process of inferring what the student knows, does not know, or is confused about is called diagnosing the student model.

There is some ambiguity in the use of the term diagnosis. In one sense, diagnosis is used to describe the process of examining the information that is already in the student model and then inferring additional things about the student that can be used to update the present student model. In another sense it refers to the process of dissecting the student model to ascertain what the student knows and does not know and using this information in a communicative process with the instructional module in order to design more appropriate teaching strategies to correct misconceptions or provide deeper understanding of various issues. It is perhaps clearer to refer to the first use as inferential-diagnosis and the second as planning-diagnosis.

The presentation of lesson material in an ITS is based upon a teaching plan, or pedagogical strategy, that is designed to achieve certain objectives. One objective, perhaps, may be to provide basic instruction for a new concept that is about to be introduced. Another objective may be to review the student's current understanding. All such teaching plans contain certain expectations

about the student. Specific responses and actions are expected from the student in particular circumstances (Leinhardt and Greeno, 1986). In addition, the student is expected to have a certain level of understanding before the introduction of new concepts or ideas. If the expectations embedded in the teaching plan are not realized, the plan has to be revised in light of the dissonance experienced. Planning-diagnosis can assist in this process. One of its functions should be to test whether the expectations of the teaching strategy are consistent with the current model of the student. Stellan Ohlsson (1986) believes this is important enough to constitute a basic principle for ITS design.

b. VanLehn (1988) states that there have been nine common diagnostic techniques that have been employed in different ITSs. All are concerned with developing a method that will analyze the student model in order to ascertain where a student's knowledge structure differs from the expert's. Instruction can then be directed to help minimize this difference. Anderson's geometry tutor, for instance, uses a nondeterministic interpreter that attempts to put the student's observable behavior in correspondence with a sequence of production rules that are executing in the student model. This correspondence, if discovered, provides an interpretation of the observable behavior (Anderson, Boyle and Yost, 1985). Model tracing, however, is computationally demanding and places rather high demands on the completeness of the student model. Clancey's (1982) GUIDON expert system makes use of inference rules and production rules to provide the system with knowledge of the student's current mental state. The problem with this approach is that rules need to be developed (and subsequently accessed) that pertain to every possible situation that might arise in a tutoring session. Other diagnostic strategies have been employed that

generate possible hypotheses about the student which are then tested against the student's answers (Burton, 1982).

All of the known diagnostic techniques suffer from one of two general problems; some are subject to both types of problems. Some diagnostic techniques require that extremely large data bases or lists be constructed that consist of typical errors students make (so called 'bugs') or that contain inference rules or production rules that attempt to account for every possible scenario that may occur during a specific session. As the size of these data bases increases, the computations necessary to locate the correct 'bug' or rule become more and more demanding. This is the well known combinatorial explosion problem associated with serial type information processing. This problem is particularly acute for domains like language learning that are more complex, less structured, and more ambiguous than domains such as geometry.

Other diagnostic techniques put extreme demands on the speed of the diagnostic algorithms. Present algorithms that attempt to provide true interactive and dynamic diagnosis of the student model are too slow to be of practical use in an ITS. There is a natural desire to want to increase diagnostic speed as one does not want the student to become bored with the tutor or lose any willingness to cooperate. This problem is also related to the first problem, for as the size of bug libraries and production rule libraries increases, the complexity of the algorithm needed to search these libraries to locate the correct information also increases.

There appears to be an implicit assumption behind the process of student modeling that further confounds these diagnostic techniques. The assumption is that the student model should be as fine-grained as possible because good tutoring depends on having a detailed understanding of the student. By 'fine-grained' it is meant that the information in the student model

should be as rich and complete and nearly identical to the actual cognitive states of the individual student. A corollary of this assumption is that ITS instruction should be precisely tailored and individualized for this particular student, at this particular time, with these particular mental states. Perhaps it is time to challenge this assumption.

The current state of the student's mental structures, cannot, of course, be observed directly. The most we can hope for is to have a 'best guess' of the student's mental world. I suggest that researchers in the ITS community accept the truth of this claim and examine new ways of trying to make this guess as best as it can be. The struggle to mirror exactly a student's mind forces our attention to developing larger and larger libraries of bugs, to devising more and more inference and production rules to cover all possible scenarios, and to inventing more complex and brilliant algorithms. Alternatively, if we accept that we can only provide an approximation, or generalization, of a student's mental states, then not only will it be clear that there exists an excellent technique that can readily do this, it will also mean that instruction does not have to be adapted to a particular individual, but to a particular type or kind of individual.

V. RECOMMENDATIONS:

a. The major recommendation of this report is for the ITS community to explore using neural network technology as a tool can that efficiently produce approximations (generalizations) of a student's cognitive states and requirements. Specifically, it would be beneficial theoretically to diagnose the student model in the following fashion. Traditional methods of monitoring student responses during a tutoring session can be used to collect the `raw data' that is used initially to begin the process of student model construction.

This data could then serve as input to a neural network that has previously been trained to recognize certain categories or classes of trainees. Once these representations of classes (categories) of students have been learned by the neural network, a new student using the ITS can be classified almost immediately as representative of a particular type of student based on nearly any attribute or combination of attributes definitive of that type. The instructional module of the ITS could then select teaching strategies and techniques that were appropriate and effective for that kind of student. This type of inferential diagnosis could continue running in the background throughout the course of the tutoring session, providing continuous and efficient updating of the student model with new information.

b. The general strategy outlined above could be implemented as a research and test case using the Foreign Language Instruction Station (FLIS) at my home institution. As I remarked previously, not much work has been done by the ITS community on language acquisition and training despite the fact that the Air Force spends considerable resources on teaching foreign languages to its personnel for overseas assignments. An intelligent tutor for language teaching, therefore, is both a clear need and a readily attainable product given the advanced state of development that the FLIS system currently enjoys.

The theoretical underpinnings of FLIS lie in the comprehension theory of foreign language instruction which contends that language is acquired by supplying the listener with copious supplies of <u>comprehensible</u> input in an environment to which the listener is receptive. Language is acquired, not learned, according to this theory by supplying a listener with second language input that contains structure which is a bit more advanced than the acquirer's current level. Although it may seem paradoxical to contend that we acquire

language only when the input contains structure that is 'a little beyond' where we are now, the answer is that acquirers use more than their linguistic competence to help them understand. They use context, their knowledge of the world, and their extra-linguistic information to help them understand (Krashen, 1982). Thus, in our system, speeches in a foreign language are usually accompanied by pictures on the screen that portray the issue being discussed and so provide informative contextual information.

The FLIS system already has a rather robust student model. The system keeps a record of all important keystrokes as well as all responses to questions posed. It knows how many times a student tried to perform some task, how many times it was missed and whether it was ever performed successfully. It knows which of the various built in aids the student accessed (clues, glossary, dictionary, transcriptions, etc.) and how many times such aids were accessed. In short, it tracks all observable physical activity of the student and so would be classified as an intermediate band width student model (VanLehn, 1988).

The follow up work I propose to do involves building a back propagation (feed forward type) neural network using object oriented programming techniques that are now available in Turbo Pascal. This network would be incorporated into the FLIS system and would serve as the component of the system that performs inferential and planning diagnosis of the student model. Two types of student model construction would be attempted; one using a supervised training procedure of the neural network and the other using unsupervised training.

Supervised training would necessitate the construction of certain "profiles" of second language learners which the network would be trained to recognize. A number of studies in second language and first language acquisition suggest that there are stages that language acquirers go through in

the process of learning to understand a language. The natural order hypothesis holds that acquirers of a given language tend to acquire certain grammatical structures earlier than others. Studies of children acquiring English as a first language, for example, showed a natural order for certain morphemes. Certain markers, such as [ing] and the plural [s] were among the first morphemes acquired. The third person singular [s] and the possessive [s] were typically acquired much later (Krashen,1982).

Other studies of second language acquirers have researched the path that language learners take on route to mastery. Language acquirers tend to make the same kinds of errors during the acquisition process. In acquiring English negation, many individuals pass through a stage in which they place negation outside the sentence as in (Not like it now). A later stage involves placing the negative marker between the subject and the verb as in (I no like this one). And the comprehension theory of language acquisition, on which the FLIS system is based, holds that a necessary condition for an acquirer to move from level i of language competence to level i + 1 of language competence is the provision of comprehensible input that contains i + 1 language structures (Krashen, 1982).

These findings all suggest that it may be possible to build such "profiles" of stages of language acquirers and train a neural network to recognize them. Subsequent student/tutor interaction can then provide the information necessary for the network to classify an acquirer as belonging to a certain classification in an efficient manner. This information could be used to assist in instructional planning by providing language lessons that are appropriate for a particular type of student; e.g., lessons designed to remedy a particular misconception (like reverse negation) or to introduce new vocabulary or new linguistic structures (the possessive [s]).

Additional content that might go into such profiles could include information about affective factors such as motivation, self-confidence, and anxiety level. Another tenet of the comprehension theory is that language acquirers need to have a low or weak "affective filter" to allow the language input a chance to get "in." Clearly, these affective factors play a role in student's receptiveness to language acquisition and should therefore play a role in the student model as well. Historical data about a student can help develop these affective factors. Thus, the system can discover whether students have had previous second language experience, whether they are familiar (and comfortable) with the FLIS system, or whether their previous second language experience, if any, was in a language structurally similar to the one they are currently studying.

The unsupervised training of the neural network would not rely on the provision of any profiles of language learners. Rather, the network would be allowed to construct its own learning function and develop its own classification scheme. This experimental use of the neural network may, in fact, turn out to be the most interesting and promising of the two. The network will learn by interacting with the preliminary data stored in the student model. As it learns, it will form steady states, or energy minimums, that represent critical feature patterns, or prototypes, of the experienced input patterns. It is hypothesized that the network will be able to detect regularities and features that were not part of the constructed profiles but yet represent significant classifications of language learners.

c. The research proposed above represents a new and different approach to the student modeling problem. Any research that promises to open new avenues for understanding cognitive processes should be given serious

consideration, for this is a problem of great complexity, scope, and importance. If the neural network model of computing is capable of modeling the cognitive processes that are involved in understanding language competence, then it appears that there will be a new tool that can be applied to other endeavors and tasks of concern to the Air Force.

Any domains in which similar complex cognitive skills are required would be ideal candidates for modeling through the use of neural networks. In particular, other components of the ITS architecture (the expert and instructional modules) may be improved and streamlined through the use of this technology. Finally, I believe that the investigations proposed here will have important implications for the study of team-oriented activities. In principle there do not appear to be any barriers to instantiating in a neural network the knowledge structures and cognitive states that are characteristic of a group of individuals working together on a common task. Since many of the tasks and activities performed by Air Force officers and recruits are of this team-oriented nature, all possible research which promises to increase our understanding of the dynamics of team work should be pursued.

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FINAL REPORT

STATISTICAL ANALYSES OF DATA PERTAINING TO GROUND WATER CONTAMINATION AND LABORATORY QUALITY CONTROL

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Date:	1 September 1989
Contract No.:	F49620-88-C-0053

STATISTICAL ANALYSES OF DATA PERTAINING TO GROUND WATER CONTAMINATION AND LABORATORY QUALITY CONTROL

by

Barbara Alvin

and

Lisa Newberg

ABSTRACT

Statistics were used to summarize and analyze data sets which were collected in the Installation Restoration Program, a program which is managed by the Air Force Occupational and Environmental Health Laboratory. Different methods of summarizing ground water contamination data were explored. The results of different ways of treating the data were compared.

In addition to comparing methods of treating data on ground water contamination, data from quality control samples were summarized. This data had been collected to monitor the precision and accuracy of the contracting labs which measure the concentrations of analytes of interest. Methods for determining limits on the percent recovery of an analyte and the standard deviation of that percent recovery were explored.

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Our research experience has been greatly enhanced through the support and guidance of a number of people, including our sponsor Philip M. Hunter. We want to thank Richard Anderson and Samuel Hopper for their help in accessing information in IRPIMS. Also, we wish to express our gratitude to Judith Vasil and Major Phil Jung for their time spent in answering questions and providing background information, and for their insight and encouragement.

I. INTRODUCTION:

One of the major tasks of the Environmental Quality Division (EQ) of the Air Force Occupational and Environmental Health Laboratory (AFOEHL) at Brooks Air Force Base is management of the Installation Restoration Program (IRP). The IRP was designed, in part, to analyze and remediate the effect of Air Force operations on the environment. The Installation Restoration Program Information Management System (IRPIMS) is a database for the storage and retrieval of information concerning water and soil contamination and subsequent cleanup. When water and soil samples are collected at sites of possible contamination, the concentrations of different analytes are recorded along with the locations and dates of the samples. Statistical summaries of this data aid in the analysis of the remediation efforts. Also stored in IRPIMS are data pertaining to the quality control programs of the contracting laboratories which analyze the environmental samples. Statistical analysis of these data can be used to compare the performance of the different laboratories with respect to accuracy and precision.

Barbara Alvin's academic and technical expertise is in the area of applied and theoretical statistics. Her background in biostatistics and her interest in the development and application of statistical models to scientific data contributed to her assignment to the EQ Division of AFOEHL. Lisa Newberg is studying for a Master of Science in mathematics and she has an interest in the area of statistics; therefore her assignment to the EQ division is also very appropriate.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The initial goal of the summer project was to provide a meaningful summary of selected subsets of the IRP contaminant data. Summaries related to selected specific contaminants were to be made across all Air Force Installations; in addition, data related to specific contaminants at specific Air Force Installations were to be summarized.

It was decided that every effort would be made to screen all data analyzed to insure its validity. Standard descriptive procedures were to

be used to categorize the observations being summarized. Efforts were to be made to utilize the expertise of the hydrogeologists and chemists who work in the EQ division at AFOEHL, so that all statistical procedures used would be meaningful. It was thought that their knowledge would facilitate the development of statistical models for summarizing the data in the most meaningful manner possible.

During the research period, a decision was made to statistically analyze quality control data which had been recorded by different contracting laboratories. The goal of this analysis was the establishment of control limits for monitoring the accuracy and precision of these laboratories.

It should be noted that any of the analyses which follow are based upon the data which was loaded into IRPIMS at the time of the analyses in the summer of 1989. They are to serve only as examples of how one might choose to analyze this data.

III. Summaries of groundwater contamination data.

A. A variety of requests for information based on the data currently contained in IRPIMS have been made of the EQ division. One request asked for the "top fifty contaminants" of ground water throughout the Air Force. Based on data from thirty-three Air Force Installations which had been loaded into the data base in July, 1989, the inorganic substances and organic compounds in the IRPIMS data base were ordered by the number of Air Force Installations at which they were known to have been measured above the lab detection limit. Lead was number one on this list, having been measured above the lab detection limit at thirty Installations. Toluene, Chromium, Zinc and Benzene were all measured above the lab detection limit at twenty-one Installations and Trichloroethylene (TCE) was measured above the lab detection limit at 20 installations.

The contaminants in the IRPIMS data base were also ordered by the number of National Priority Sites at which they were known to have been measured above the lab detection limit. The federal Superfund law required the EPA to establish a National Priorities List of waste disposal sites that might pose a danger to the public health. There are twelve Air

Force waste sites on the National Priorities List. Five of these sites have some amount of analytical data entered in IRPIMS at this time. Benzene, Chromium, Ethylbenzene, Tetrachloroethylene (PCE) and Lead have each been measured above the lab detection limit at all five of these sites. There are sixteen contaminants which were measured above the lab detection limit at four of these five National Priority Sites.

It should be noted that this method of ordering analytes by the number of bases at which they are found above the lab detection limit does not provide a complete answer to the request for the list of the "top fifty" contaminants, since many of the compounds which appear on the list are naturally occuring compounds; also, for many of the analytes, presence above the lab detection limit does not present a problem. To provide a complete answer to the request for the "top fifty", one needs to look at the levels at which the analytes are present.

B. In order to provide more meaning to the list of the "top fifty", a decision was made to look at the contaminants which fall under the realm of Federal drinking water standards. There are three classes of water standards which apply to the analytes whose measurements are contained in IRPIMS.

Maximum Contaminant Level Goals (MCLGs) are non-enforceable health goals which are set at levels which would result in no known or anticipated adverse health effects with an adequate margin of safety. Maximum Contaminant Levels (MCLs) are enforceable standards and set as close to the MCLGs as is feasible. Interim Primary Drinking Water Standards are MCLs and are enforceable. These Interim standards will be replaced with more permanent MCLs in the future.

For each of those analytes for which an MCL or Interim water standard exists, data was retrieved. This data contains the location and value of all observations of the analyte which were measured to be at or above the limit (MCL or Interim) for that particular analyte. This same data was retrieved for those analytes having MCLGs. However, as these limits are non-enforceable, these analytes were not focused on as much as the others.

One analyte for which an MCL exists is Trichloroethylene. TCE contamination was analyzed from many different statistical viewpoints.

The analyses done on TCE could be repeated on any of the analytes for which MCLs or Interim standards are in place.

TCE was measured at thirty-three Air Force Installations. To get an idea of the TCE contamination at each of these Installations, each observation of TCE was categorized based on where it fell in comparison to the MCL for TCE, 5 uG/L.

At each Air Force Installation the number of TCE observations falling at or below the MCL and the number falling above the MCL were counted. Also counted at each Installation were the number of TCE observations where the lab detection limit was greater than 5 uG/L and the concentration of the sample could not be measured because it was below the lab detection limit. In these cases, it is not known whether the concentration of TCE is above the MCL.

At each Installation, the percentage of observations falling into each of these three categories was calculated. Fourteen of the thirty-three Installations reported TCE measurements above the MCL. At five of these fourteen Installations the percentage of measurements over the MCL was above 30%. These were AFP No. 59 Johnson City and Carswell, Dover, Edwards and Hill Air Force Bases.

There were four Installations where over 10% of the observations could not be classified as above or below the MCL, since the observations fell below the lab detection limit which was above the MCL due to the necessity of diluting the sample which had been analyzed. These were Eglin, Griffiss, and Moody Air Force Bases as well as Minneapolis St. Paul IAP.

The TCE contamination at these thirty-three Air Force Installations was analyzed with a second method. At each Installation, the number of locations where TCE was tested for was counted as well as the number of locations where TCE was measured to be above the MCL, 5 uG/L.

TCE was found to be above the MCL at fourteen out of the thirty-three Installations. For each of these fourteen Installations, a percentage was calculated by dividing the number of locations with results above the MCL by the number of locations where TCE was tested. This percentage was 50% or greater at Carswell, Dover and Edwards Air Force Bases. At AFP No. 59 Johnson City and Hill Air Force Base, TCE was measured to be above the MCL

at 40% and 38% of the testing locations, respectively.

A third analysis done on the TCE data was to find an overall centrality measurement. Rather than take the measurement over all TCE data, only the data from the latest sampling date at each location within each Installation was selected. The median of these most recent observations was calculated for each Installation giving thirty-three numbers in the data set. This eliminated any bias which would have occured as a result of some Installations testing for TCE more frequently than other Installations. The centrality measurement was calculated by taking the median of these Installation medians.

Before the centrality measurement was calculated, though, the data was adjusted to account for those measurements falling below the lab detection limit. There were four different methods used to adjust the data, giving four data sets with thirty-three elements in each.

Table III.1 contains the medians at each Installation, for each of the four methods. Each column in the table corresponds to a particular method. The last row of the table contains the centrality measurement for each of the four methods. Table III.2 contains the minimum and maximum values for each of the four data sets, as well as the 25th, 50th and 75th percentiles.

In the first method, zero was entered for all those observations falling below the lab detection limit. Using this method, 75% of the Installation medians were 0.0. Therefore, the centrality measurement was also 0.0. The maximum value median value under method one was 8.4 uG/L.

In method II the value of all those observations which fell below the lab detection limit was changed to the value of the lab detection limit. While method I gave the lowest possible value, 0.0, to these observations, method II assigned them the highest possible value. The centrality measurement under method II was 0.6 uG/L. Twenty-five percent of the median values were below 0.12 and 25% of the median values were above 2.0. The maximum median value for this data set was 10 uG/L.

Method III was a compromise between methods I and II . Those observations which measured below the lab detection limit were entered as (lab detection limit)/2. Note that the centrality measurement calculated for this data, 0.4075, is between the lower and higher centrality

TABLE III.1 Centrality Measurements for TCE Based on Medians of Last Recorded Observation at Each Location within an Installation

AIR FORCE INSTALLATION	NUMBER OF LOCATIONS	MEDIAN METH. I	MEDIAN METH. II	MEDIAN METH. III	MEDIAN METH. IV
AFP No. 59 Johnson City	4	3.0	4.0	3.5	8.5
Arnold AFS	9	0.0	0.12	0.06	.
Beale AFB	29	0.0	0.1	0.05	0.2
Bergstrom AFB	2	0.4	0.415	0.4075	0.8
Chanute AFB	23	0.0	2.0	1.0	.
Charleston AFB	48	0.0	0.12	0.06	0.51
Carswell AFB	42	5.7	5.7	5.7	193.0
Des Moines Map	12	0.0	3.0	1.5	.
Dobbins AFB	3	0.0	1.0	0.5	.
Dover AFB	16	8.4	8.4	8.4	14.0
Duluth IAP	36	0.0	0.6	0.3	12.2
Edwards AFB	51	4.3	4.3	4.3	69.0
Eglin AFB	42	0.0	10.0	0.5	15.8
Eielson AFB	12	0.0	0.6	0.3	.
Griffiss AFB	19	0.0	10.0	5.0	.
Hancock Field	6	0.0	0.00012	0.00006	.
Hill AFB	69	0.4	2.0	1.1	20.0
Kelly AFB	68	0.0	0.98	0.68	16.2
K.I. Sawyer AFB	50	0.0	0.2	0.1	2.85
Loring AFB	11	0.0	2.0	1.0	5.65
MacDill AFB	71	0.0	0.2	0.1	.
March AFB	10	0.0	0.5	0.25	9.0
Mather AFB	4	0.0	0.5	0.25	5.7
McEntire ANGB	16	0.0	0.00012	0.00006	0.0012
Minneapolis St. Paul IAP	14	0.0	2.0	1.0	.
Minot AFB	81	0.0	2.0	1.0	.
Moody AFB	17	0.0	1.25	1.0	1.95
Norton AFB	81	0.0	0.12	0.06	25.0
Pease AFB	17	0.0	2.0	1.0	2.3
Point Arena	3	0.0	0.5	0.25	.
Reese AFB	6	0.0	0.12	0.06	.
Robins AFB	58	0.0	0.12	0.06	.
Tyndall AFB	36	0.0	0.05	0.025	0.255
CENTRALITY MEASUREMENT		0.0	0.06	0.4075	7.1

TABLE III.2 SUMMARY OF THE DISTRIBUTION OF THE MEDIANS FOR TCE CONCENTRATIONS FOUND IN TABLE III.1

	MIN	25TH PERCENTILE	50TH PERCENTILE	75TH PERCENTILE	MAX
Method one	0.0	0.0	0.0	0.0	8.4
Method two	0.00012	0.12	0.6	2.0	10.0
Method three	0.00006	0.06	0.4075	1.0	8.4
Method four	0.0012	1.0875	7.1	16.1	193.0

measurements calculated with methods one and two. Under method three, 25% of the median values were below 0.06, 75% of the median values were below 1.0 and the maximum median value was 8.4 uG/L.

The fourth method was to consider only those observations falling above the lab detection limit. The centrality measurement for this method is understandably higher, 7.1 uG/L. The minimum and maximum values were 0.0012 and 193. Twenty-five percent of the values were below 1.0875 and 75% of the values were below 16.1 uG/L. This method gives an accurate centrality measurement for those observations which were measured to be above the lab detection limit, but gives too high a number for an overall centrality measurement.

It should be noted that in another analysis a median was calculated over all the TCE data in the IRPIMS base, rather than just the most recent samples. The restrictions on this data were that each measurement be positive and above the lab detection limit. The value of this median, 9.3, is even higher than the centrality measurement from method four due to the more frequent sampling of TCE at Installations where the TCE concentration is high. By taking only the latest observations from each Installation, and taking the median of the Installation medians, this bias would be eliminated.

IV. Analysis of Quality Control Data

The second major objective of the summer was the establishment of a method for evaluating the performance of contracting laboratories with respect to the accuracy and precision of their chemical analyses of water samples. It is standard practice for these laboratories to generate quality control data in order to check on their own accuracy and precision.

A. One method laboratories use for checking their accuracy is to spike a sample of reagent water with a known amount of a given analyte and to then analyze that sample in order to determine what the measured amount of the analyte will be. The percent recovery of the measurement is defined to be 100% times the measured concentration of the sample divided by the true

concentration of the sample.

$$\text{Percent Recovery} = \frac{\text{(measured concentration) } 100\%}{\text{(true concentration)}} .$$

For these types of control samples, known as blank spikes, the desired percent recovery is 100%. Therefore, one wants the mean percent recovery of all of a laboratory's samples of blank spikes for a particular method and a particular analyte to be as close to 100% as possible. In addition to that goal, one wants the standard deviation of percent recovery to be as small as possible. A question of the chemists at AFOEHL is how close to 100% recovery should be expected and how small can they expect the standard deviation of percent recoveries to be for the different labs for different specific analytes which are analyzed by the different specific methods.

The ideal method of answering these questions would be to first analyze a large collection of blank spikes in a very careful and controlled fashion for each method and each analyte of interest, and then to calculate the mean percent recovery and the standard deviation of the percent recovery for that collection of blank spikes. Then, for any collection of blank spikes from a given lab, one could statistically test to see if the standard deviation of the population of percent recoveries is larger than the standard deviation of the population of percent recoveries in the controlled laboratory setting. If the standard deviation of percent recoveries is not too large, then one could proceed to test to see if the mean of percent recovery is "close enough" to 100%, where "close enough" would be determined from the results of the controlled analysis of blank spikes. No data from such an ideal experiment is available at this time for answering these questions; therefore the data from the IRPIMS data base was used to give first approximations to the answers to these questions.

The data on blank spikes, which were in the IRPIMS data base in July, 1989, for purgeable halocarbons analyzed with methods 601 and 602, are

TABLE IV.1 PERCENT RECOVERY OF BLANK SPIKES WITH METHODS 601 AND 602

ANALYTE	N	MEAN %RECOVERY	S.D. %RECOVERY	# OF OB. PER DISTINCT LAB
I. Analytical method 601				
Chloroform	9	98.00	15.7639	(1,8)
1,1-Dichloroethylene	2	99.05	10.5359	(1,1)
1,2-Dichloroethylene	11	101.918	23.2115	(1,1,9)
1,3-Dichloropropylene	2	91.1	6.9297	(1,1)
Methylene Chloride	8	99.45	21.2083	(1,7)
1,1,1-Trichloroethane	17	98.6471	15.2886	(1,6,10)
Trichloroethylene	25	98.79	13.855	(2,1,22)
Carbon Tetrachloride	3	94.367	9.209	(3)
1,2-Dichlorobenzene	7	94.7143	14.7842	(7)
1,3-Dichloropropylene	2	97.00	7.0711	(2)
Trichloroflouromethane	2	113.00	18.3848	(2)
1,3-Dichlorobenzene	1	115.00	.	(1)
1,4-Dichlorobenzene	1	107.00	.	(1)
1,2-Dichloropropane	1	88.20	.	(1)
PCA	1	114.00	.	(1)
II. Analytical Method 602				
Benzene	11	94.85	12.3979	(1,1,1,8)
Toluene	16	91.9331	22.5176	(1,1,6,8)
1,2-Dichlorobenzene	2	85.00	5.6569	(1,1)
Ethylbenzene	16	94.52	13.9702	(1,1,7,7)
1,3-Dichlorobenzene	1	86.00	.	(1)
1,4-Dichlorobenzene	1	87.00	.	(1)

summarized in table IV.1. Summary statistics on the data are reported for different analytes within the two methods. The first column of the table gives the name of the analyte. The number of observations, N, is listed in the second column. The mean of percent recovery and the standard deviation of percent recovery are contained in the third and fourth columns. Listed in the fifth column are the number of observations coming from each distinct lab. This is an important factor to look at since observations of an analyte which come from more than one lab will exhibit inter-laboratory variance as well as intra-laboratory variance. Listed below those analytes with observations coming from more than one lab are those analytes with multiple observations from just one lab, and listed last are those analytes with just one observation from one lab.

The values in this table give a first approximation to the control limits which OEHL may want to place on percent recovery and the standard deviation of percent recovery for blank spikes which are analyzed using methods 601 and 602.

B. Another method used by laboratories for checking their accuracy is the spiking of environmental samples with analytes which would not usually be found in the sample. For these types of control samples, known as surrogate spikes, the desired percent recovery is again 100%. However, the environmental samples may be contaminated with other analytes which may interfere with the percent recovery on the surrogate spikes; therefore, the laboratories may not be as concerned with low mean percent recoveries for surrogate spikes as they would be with low mean percent recoveries for blank spikes. But, there is still an interest in determining limits which might be placed on acceptable percent recoveries and an acceptable standard deviation of percent recoveries for surrogate spikes. Again, these might best be determined in a controlled setting where researchers who were analyzing water for contaminants were being as careful as possible. Again, this is not possible. But with enough data points from enough different laboratories it becomes possible to at least see "how well a laboratory can perform" with respect to percent recovery and the standard deviation on percent recovery.

Data points were retrieved from IRPIMS for water samples which were

spiked with nitrobenzene-D5 (NO2BZD5). The medians, the means and the standard deviations on percent recovery of NO2BZD5 were calculated for the four laboratories for which data was available in IRPIMS on water samples spiked with NO2BZD5. They are presented table IV.2.

Table IV.2 Summary statistics on percent recovery for samples spiked with nitrobenzene-D5

Laboratory (LABCODE)	N	median % Recov.	mean % Recov.	st. dev. % Recov.
Ecology and Environment (ECEN)	244	63.0	63.80	20.13
Ch2m Hill (CHM)	16	63.5	62.70	10.70
Princeton Testing Labs (PTL)	23	39.0	38.22	7.89
Savannah Laboratories and Environmental Testing (SLES)	17	67.0	72.59	20.71

The usual analysis of variance (ANOVA), based on the observations coming from four normal populations with equal variances, was performed; due to the difference in variances of observations from the different laboratories, this was backed up with the nonparametric Kruskal-Wallis test. The results of both analyses indicate that the percent recovery on observations from laboratory PTL were significantly lower than the percent recovery on the observations from the other three laboratories ($p < .001$).

F tests were used to test for the equality of variances on percent recovery for the different laboratories. Without adjusting for multiple comparisons, observations from laboratories PTL and CHM exhibited significantly less variation ($p<.005$) than did observations of percent recovery from laboratories ECEN and SLES. And, even after the conservative adjustment of the significance values by the Bonferroni method (due to the six possible paired laboratory comparisons), the sample variances of observations from laboratories PTL and CHM were significantly less than those from the other two laboratories ($p < .03$).

If there are no outside factors which would explain the lower percent recovery for laboratory PTL, one would conclude that this lab is not as accurate as the other three laboratories which are represented in the above analysis. And without outside explanatory factors, one would conclude that among the three laboratories with a mean percent recovery above 60%, lab CHM is more accurate than the other two since its variance (and hence standard deviation) of percent recovery is significantly lower.

C. One method which laboratories use to check their precision is to split environmental samples of water into two or more separate subsamples which they then analyze separately. Ideally, the two separate measured concentrations should be equal since one would assume that the concentration of an analyte in water is uniform. However, the laboratory methods of measuring the concentration of an analyte in water result in unavoidable variation in the measured amount of the analyte. The question asked by the chemists at OEHL is what types of bounds should be placed on this variation? The ideal method for answering this question is analagous to the ideal method for answering the questions concerning the accuracy when working with blank spikes and surrogate spikes. But, again, data from such an ideal experiment is not available for analysis.

Neither is there much data currently available in IRPIMS for studying the precision of laboratories. However, a small collection of pairs of measurements of tetrachloroethylene (PCE), which were made using EPA method 601 on environmental samples and their laboratory replicates and a small collection of pairs of measurements of trichloroethylene (TCE), which were again made using method 601, were studied. Scatterplots were

made where the measurement of PCE in an environmental sample of water was plotted against the absolute difference between the measured value of PCE in the environmental sample and the measured value in a laboratory replicate. The same process was repeated with the paired measurements on TCE. There was not enough data to draw any definite conclusions, but, for both PCE and TCE, it appears that the variance in the absolute difference between the measurements in a pair increases as concentration of the analyte increases.

V. RECOMMENDATIONS:

A. Data summaries:

From a statistician's point of view, IRPIMS, the data base where data from the IRP is stored, is a well designed information management system. Recorded along with the measured concentrations of the different analytes are the locations and dates of the samples. There are also places for site information as well as other related variables. Full advantage should be taken of the information which is available in the data base. To analyze the data without taking into account the fact that the concentrations of given analytes which occur in the data base are from different populations (different locations within different Air Force Installations on different dates) is statistically incorrect and could seriously bias any summary statistics which might be calculated. Therefore, I would strongly recommend that anyone involved in statistical analysis of data in IRPIMS become familiar with the structure of the data base. Different methods of dealing with concentrations of an analyte which are below a laboratory detection limit should be explored. In order to present a complete picture of the concentrations of analytes across the Air Force, one should present a variety of data summaries where non-detectable concentrations of the analytes are treated differently.

B. Establishment of limits on percent recovery and its standard deviation:

Much work remains to be done with respect to establishing bounds on percent recovery and its standard deviation for blank spikes and surrogate spikes. Similar bounds need to be found for matrix spikes which are samples of environmental water to which different analytes of interest are added. These differ from surrogate spikes of environmental water in the sense that the analyte which is being added to the water sample may already be present in the sample.

One approach for establishing these bounds on percent recovery and its standard deviation would be to begin with the steps which were outlined in section IV.B for analyzing the performance of labs ECEN, CHM, PTL and SLES with respect to the measurement of the surrogate spikes where NO2BZD5 was added to samples of water. Namely, the mean percent recovery and the standard deviation of percent recovery for these labs should be calculated. A nonparametric analysis of variance (such as Kruskal-Wallis) should be used to determine whether the labs perform significantly differently with respect to percent recovery. If two or more labs perform "better" than the other labs with respect to mean percent recovery and if their variance on percent recovery is not significantly higher, then the mean percent recovery of those better labs could be used as a target percent recovery for all labs. Then, that target percent recovery and the pooled standard deviation on percent recovery could be used in the following manner to set limits on percent recovery.

Let M = mean percent recovery for the "better labs",

Sp = pooled standard deviation on percent recovery for k "better" labs which are under consideration,

N(1) = # water samples from lab #1 (of k labs under consideration),

.

.

and N(k) = # water samples from lab #k.

Let $v = N(1) + N(2) + \ldots + N(k) - k$.

Let $(\alpha)100\%$ be the percent of time one is willing to make the error of saying the analytical process is inaccurate when it actually is accurate. Let $t(v,\alpha)$ represent an upper percentage point of the t distribution with v degrees of freedom.

Then, if $M < 100$, set the lower limit on percent recovery (for single observations) at

$$L = M - t(v,\alpha)Sp .$$

Since an ideal target on percent recovery is 100%, set the upper limit on percent recovery at $200 - L$ (i.e. at the same distance above 100 that L is below 100).

If $M > 100$, set the upper limit on percent recovery (for single observations) at

$$U = M + t(v, \alpha)Sp,$$

and set the lower limit at $200 - U$.

With respect to precision, bounds need to be placed on the standard deviation of percent recovery. Sp, the pooled standard deviation of two or more labs with good percent recovery and a smaller standard deviation, could be used as a target standard deviation. If there are again k such labs with N(1), N(2) . . . , and N(k) observations, then a limit on the standard deviation of percent recovery for n observations could be established in the following manner.

Again, let $v = N(1) + N(2) + \ldots + N(k) - k$. Then set the uppper limit on the standard deviation of n observations at $Sp\sqrt{F(n-1,v,\alpha)}$, where $F(n-1,v,\alpha)$ is an upper percentage point of the F distribution with n-1 numerator degrees of freedom and v denominator degrees of freedom.

The limits on percent recovery and its standard deviation should not be set without first consulting with chemists who might be able to explain why one lab may be able to perform more accurately and precisely than another lab. The chemists should also be involved in setting the significance level, α , which is used in setting these bounds.

Not enough data is currently available for setting up these control limits. When more data has been loaded into IRPIMS, an effort should also be made to see if the control limits on percent recovery and its standard deviation should change as the concentration being spiked changes.

The above outline pertains mainly to surrogate spikes and blank spikes. The establishment of control limits for matrix spikes might also depend upon the background level of the analyte of interest. The application of linear models to the data, when it becomes available, would help to determine this.

More data needs to be loaded into the data base before laboratory duplicates can be used to study the precision of the contracting laboratories.

In addition to using the methods which are suggested above for establishing limits on percent recovery and its standard deviation, additional efforts should be made to theoretically study the relationship between lab detection limits, method detection limits and the changes in precision and accuracy which occur as the true concentration increases above the lab detection limit.

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FINAL REPORT

Design of an Automated Radiofrequency Radiation Measurement System (ARRMS)

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DESIGN OF AN AUTOMATED RADIOFREQUENCY RADIATION MEASUREMENT SYSTEM (ARRMS)

Stewart J. Maurer

ABSTRACT

In order to increase the measurement capability of the electromagnetic radiation consultant branch of the Air Force Occupational and Environmental Health Laboratory (AFOEHL), a design using a spectrum analyzer, power meters and a wide selection of antennas is proposed. The frequency coverage is from 50kHz to 22MHz and incorporates GPIB/IEEE 488 programmable equipment which will allow for a highly automated system. An antenna mount using a stepping motor is proposed which will give three axis data by means of rotation on a central axis. Prices and suppliers of the components are given.

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Lt. Colonel Edward Maher provided me with support, encouragement, and a truly enjoyable working atmosphere. Lts. Noel Montgomery and Steve Rademacher were invaluable for the logistic support and the information provided on Rf emitter surveys and present AFOEHL assessment methods. I want to especially thank the Officers, NCO's and support staff at AFOEHL for their help in making available the excellent computer and technical support facilities at AFOEHL and Brooks Air Force Base. I am most appreciative of the friendship and fellowship given to me during my stay in San Antonio by those at AFOEHL.

INTRODUCTION

The United States Air Force Occupational and Environmental Health Laboratory Radiation Consultant Branch (AFOEHL/RZC) located at Brooks Air Force Base, Texas 78235, (512) 536 3486, provides laboratory and field consultation in support of the USAF world wide radio frequency (RF) radiation protection program. This mission is only part of the nonionizing and ionizing radiation protection program provided by the AFOEHL/RZ Division. The RF service includes the management of potential RF hazardous sources; providing response teams and equipment in support of investigations and reconstruction of alleged or suspected overexposures; coordination of medical evaluations for personnel known to have been overexposed; maintenance of an emitter or source data base which stores radiation information regarding personnel hazards; management of the RF exposure repository and measurements of operational as well as research and development (R&D) RF systems.

The present method by which RF fields are measured is with light weight and portable broadband isotropic electric and magnetic field probes and meters of the types made by the Narda and Holaday companies. While these probes have been adequate for measuring a number of RF field source configurations they are limited and can produce erroneous readings when multiple frequency sources are present. The USAF standard for exposure to RF radiation is given by AFOSH Standard 161-9, 12 Feb 87, which defines permissible exposure standards for personnel. When applied, it is necessary to know

frequency as well as electric and magnetic field levels. In the past, outside agencies were called in to make measurements which could not be done with the probes on hand.

The purpose of this paper is to investigate a more sophisticated and flexible measurement system which will greatly extend the capability of AFOEHL/RZ to perform its mission.

DESIGN CONSIDERATIONS

The greatest limitation of the existing system used by AFOEHL/RZ is that detailed information as to the actual RF fields and frequencies cannot be determined by direct measurements. Frequency information must be determined by a review of the radiation systems in the area. Further errors introduced by stray and out-of-band pickup by the broadband isotropic probes are not directly detectable and can only be assumed as present or not. One of the best ways to evaluate the RF environment is to use a spectrum analyzer and a variety of broadband antennas. The spectrum analyzer along with gain and/or antenna factors can yield accurate electric and magnetic field strengths from which RF power can be determined for a large number of cases, (certainly for all the cases the broadband isotropic probes can measure and many more). If the frequencies covered by such a system includes all the RF sources contributing to the measurement point then a proper field survey can be made.

No one system can meet all present and future measurements of fields from all the sources used by the Air Force. Appendix A gives a partial listing of USAF RF sources from

which design specification may be determined. These and considerations of keeping costs to within budget, meeting the permissible exposure levels (PELs), and the large amount of experience that AFOEHL/RZ has had in making surveys at worldwide RF installations have contributed to practical design specifications. They are as follows.

FREQUENCY RANGE: 100 KHz to 21 GHz
PULSE WIDTH: 0.1 MICROSECONDS to continuous wave (CW)
PEAK ELECTRIC FIELD: 6.3 VOLT/METER to 200 KILOVOLT/METER
PEAK MAGNETIC FIELD: 0.016 to 500 AMP/METER

DUTY CYCLE: .01% TO 1% (10^{-4} TO 10^{-2})

Further considerations are that the system be portable for use in the field. When required, an isolation space to protect personnel and equipment from hazardous RF fields must be provided while measurements are being taken. Remote and/or automatic control of measuring and recording equipment are necessary. Antenna factors which relate voltages to field strengths for different frequencies should be stored in a data bank to be used in the automatic calculation of field strengths. Similarly insertion losses of any intervening cables or devices should be stored and used when true field levels are computed. Average power, weighted average power according to the Air Force PELs, peak power, peak electric and magnetic fields should be able to be measured or calculated and then stored in a data base. Care must be taken so that the system's receiving antennas, the antenna mounts, and their connecting cables not interact with the RF fields and thereby introduce measurement errors.

In order to keep developmental costs down and to plan for the present and future needs of the RF exposure assessment group at AFOEHL, the design of a fully automated system as described above is not seen as an immediate goal but one which should be approached in steps. Automation can be achieved by designing individual measurement procedures and programs each performing a specific measurement or data processing goal, and then tied together under a single menu driven program. In this manner the system will have immediate though limited usefulness. In time the system will produce more and more functions. This will give AFOEHL the ability to modify the systems capability as its needs grow or change. The steps in the design are as follows:

1. Decide which measurements are needed.
2. Choose equipment which will fulfill the present needs and at the same time have sufficient flexibility to meet a host of future needs.
3. Field test the equipment in manual mode in order to learn algorithms needed for automation.
4. Write simple programs in modular form for the various field tests.
5. Integrate into larger programs for multifunctional testing.
6. Design menu driven automated system.

PROPOSED HARDWARE AND SYSTEM CONSIDERATIONS

Figure 1 shows a block diagram of the proposed hardware for the basic system. All hardware has been chosen with programmable operation. The Hewlett Packard HP70000 series spectrum analyzer with its modular features allows the system to be configured according to the anticipated measurements required. That is, components not needed can be left at base thus saving on weight. At the same time the shipping cases do not have to

be altered when more components are needed. Further the HP70000 series has tracking generators and preselectors which cover the entire band from below 50 kHz to over 21 GHz. The modular spectrum analyzer and associated components provided by the HP 70000 series spectrum analyzer insures minimum size and simplifies both RF connections as well as the programmable control using IEEE 488 or its equivalent HP-IB bus. This system can be modified or have components added in the future with a minimum of complications.

HP 71201A 50 kHz to 22 GHz SPECTRUM ANALYZER $47,975.00

WHICH INCLUDES:
HP70255A B&W GRAPHICS
HP70900 LOCAL OSCILLATOR
HP70902A IF SECTION
HP70905B RF SECTION
HP70600A PRESELECTOR 0-22 GHz
HP70001A MAIN FRAME
INCLUDES OPTION 908 - RACK MOUNTING KIT FOR MAINFRAME

The above consists of a complete spectrum analyzer. That plus an antenna and mount is all that is needed for measurements. The addition of a power meter and some switches will give another degree of measurements.

The following items are not urgent and consist of items which will have possible future use in the system. They all fit in a single main frame.

HP70001A MAIN FRAME $5,500.00
OPTION 908 RACK MOUNTING KIT $35.00
HP70100A POWER METER $2,500.00
The power sensors below will work with this meter.
HP70300A TRACKING GENERATOR 20 Hz TO 2.9 GHz $10,350.00
HP70301A TRACKING GENERATOR 2.7 TO 18 GHz $25,000.00
HP70700A DIGITIZER $7,650.00

Subtotal $51,035.00

The following programmable switches and attenuators are essential for configuring a flexible system.

2-HP33311 SPDT PROGRAMMABLE MICROWAVE SWITCHES $2,300.00
2-HP8497K PROGRAMMABLE ATTENUATOR $3,200.00
HP11713A ATTENUATOR/SWITCH DRIVER $1,700.00

Subtotal $7,200.00

The following power meter and sensors offer direct power measurements. Figure 2 shows a simple setup for use in the field. AFOEHL already owns a number of the Holaday HI3320 data loggers. The system shown can be used for long term monitoring of RF fields. It can also use the continous 6 min. averaging capability of the data logger. All the equipment shown is battery operated.

HP438A POWER METER $5,100.00

Dual channel digital meter allows two simultaneous power inputs to be recorded. Duty cycle range yet to be determined.

HP435B POWER METER $1,500.00

Analog power meter which with Option 001 .....$100.00 an installed rechargable battery, a portable data logger, and an antenna will allow for a simple portable system for accurate Rf power measurements. Duty cycle range yet to be determined.

THERMOCOUPLE SENSORS FOR THE HP POWER METERS
10 MHZ to 18 GHz
HP8481H - 0.1 to 3000 milli Watts $825.00
HP8484A - 0.1 nano to 10 micro Watts $900.00
100 kHz TO 4.2 GHz
HP8482A - 0.001 to 100 milli Watts $650.00

Subtotal $9,075.00

For all the power sensors it is important that they initially have a large attenuator in front of them for protection.

HP8340B SIGNAL GENERATOR 0.01 TO 26.5 GHz $31,888.00*

For use at base. This is a precision synthesized signal generator. It is HP-IB programmable with digital sweep capability

*This a special price due to purchase contract with Kelly AFB. NATIONAL STOCK number 6625-01-258-3140 contract F41608-88-D-0017 with Kelly AFB. Expiration date Jan 91 ($31,888).

MS DOS PROCESSOR WITH TWO 3.5 INCH DISK DRIVES

Must be an AT PC with a coprocessor to work with the viper card listed below.

VDT and Computer estimated cost $8,000.00
HP-2225A THINKJET PRINTER WITH HP-IB INTERFACE.....$495.00
2-HP82300C HPBASIC PROCESSOR SYSTEM $4,590.00
(VIPER CARD) CONTAINS ROCKY MT. BASIC SOFTWARE, HP-IB CONNECTOR

The C subscript is not shown on the data sheet from the 1989 HP Catalog. (Kevin Smith of HP (512-491 1255) will send the specs for this unit). This unit contains more RAM, works at a higher speed and has better documentation than the B unit. Both the PC controller and the VDT must be transportable and able to meet MIL-T-28800C standards for mechanical shock. By using two 3.5" drives and no hard disk, sensitive data will not be left in the computer and transportability requirements should be more easily met. Perhaps a laptop with provision for the Viper card would be a good choice. The computer and printer should include rack mounting capabilities.

COMPUTER ESTIMATED SUBTOTAL $13,085

ANTENNAS

ELECTRIC FIELD 10KHz to 750 MHzs

EFS-2, made by Nano-fast (416 West Erie, Chicago, IL 60610,

(312)943-4223, Carl Vesper) is a spherical electric field sensor with integral optical analog transmission system. Also called a fiber-optic isolated spherical dipole (FOISD). It consists of two 4.7-inch diameter hemispheres separated by a small gap. An electric field is sensed and converted by battery (3-hour rechargable) run electronics inside to an optical signal. This signal is sent on 100ft of fiber optic cable to a receiver which converts the signal to a precision electrical replica of the original which can be sent to a spectrum analyzer or power meter. Cost for the sphere, 100 ft of fiber optics, and the transponder-controller is $26,900. The cost of a Teflon gimbal which allows the sphere to be oriented, mounted on a tripod, etc. is $1,975 for a total of $28,875. (Peak detection and IEEE-48 capability can be added at a later date for $6,800 and $4,775 respectively) $28,875.

ELECTRIC FIELD 20 MHz TO 1 GHz

EMCO MODEL 3121C Adjustable Element Dipole Antenna Set contains an adjustable dipole antenna, four compensated baluns, clamp with a dielectric rod for mounting on a tripod screw (1/4 by 20), ruler and carrying case. It covers piece wise the frequency range listed and comes calibrated for precision measurements. Model C has a type N connector. This antenna system will be useful as a back up to the more versatile FOISD antenna and can be used to check E field calibrations. The system was designed for EMI and Tempest testing. Technical contacts for EMCO are Jim Psencik, product design engineer and Jack Sargant, sales engineer (800-253-3761,

P.O. Box 1546 Austin Texas 78767)

Cost $2,450.00

ELECTRIC FIELD 10kHz to 32 MHz

EATON 41" Passive Rod Antenna Model 92197-3 (also need 94592-1 rod antenna coupler and 92199-3 ground plane). Made by EATON (Jim Wisdom & Associates 800 E. Arapaho, Richardson, Texas 75081 (214)470-9093 sales rep for Brooks AFB) (Larry Toller product engineer (213)822-3061). The antenna rod is an effective halfmeter and is telescoping. The antenna covers the range in 8 switched bands either manually or programmable when a cable is added. This setup will provide additional calibration and checks for the frequencies below 32 MHz.**

Cost $1,793.00

MAGNETIC FIELD 10 kHz to 1 GHz

EATON 3" Loop Probe Model 90799-2 (with N female connector). While the antenna factor for this loop is linear from 122 db down to 20 db over the frequency range shown it is not known if it starts to pick up E fields. It should be useful over a significant portion of the lower frequency range. **

Cost $260.00

MAGNETIC FIELD 1kHz to 30 MHz

EATON Model 96020 12" diameter loop set antenna. 1/4 by 20 thread in bases for tripod mounting. One is an active antenna with a gain of 30 or better, while the other is a passive antenna. Both are made shielded and are made of aluminum. Cost is $2,990. EMCO has an identical set and is listed under Model 6505 and costs $2,925. (Jack Sargant says that

EMCO manufactures them). EMCO Cost $2,925.00

** GSA Contract # GS 00F03424 Group 66 Part 2 Section H From 8-1-88 thru 7-3-91 For Eaton.

HIGH FREQUENCY ANTENNAS

OMNI DIRECTIONAL BICONICAL: This antenna acts like a dipole with it maintaining a cosine variation perpendicular to its central axis over a broader frequency than the dipole. In order to use this class of antennas to measure the E-field components for later vector addition, the cosine variation must be maintained. This represents the upper frequency limit of their use for our design. The antenna pattern is approximately uniform around its central axis.

4-40 GHz

Watkins-Johnson Model Number WJ-48972 (WJ-8549-11 old no.) is vertically polarized with deviation from omni of ($_+$)2 dB and has 0 dB gain. Its dimensionns are 4.5" high and maximum diameter of 7.6" and has a 1.7mm female connector. This antenna will receive up to 40 GHz, but is expected to maintain the cosine variation at least to the 22 GHz neede for this specs.

Cost minus calibration $2,570.00

See below for calibration costs.

1-26 GHz

Watkins-Johnson Model Number WJ-48962 (WJ-8549 old no.) is vertically polarized with 0 dbi gain and has a deviation from omni $^+$ 1 dB. Its dimensions are 7.25" high and 11.4" maximum diameter and has a 3mm female connector.

Cost minus calibration $2,512.00

See below for calibration costs.

0.5-18 GHz

Watkins-Jenkins Model WJ-48971 (WJ-8549-11 old no.) is vertically polarized with a gain of 2 to 4 dbi and a deviation from omni of $^{+}$ 2 dB. Its dimensions are 9" high and a maximum diameter of 19" and uses a 3mm female connector.

Cost minus calibration ...$4,250.00

To calibrate the above three antennas from .5 to 22 GHz, with 2 evaluations in elevation and one in azimuth at each frequency point the cost is $2,600. Considerable savings is effected when calibrations for several antennas are done together at Watkins-Johnson. Contact at WJ is Brian Horowitz, design engineer, (2524 North First St., San Jose, CA 95131; (408)435-1400).

Calibration cost...$2,600.00

DIRECTIONAL LOG PERIODIC Greater accuracy can be had with this class of antennas provided the signals are coming from only one direction. Where possible dual polarized antennas will be chosen; this will allow fast sequential readings of horizontal and vertical polarization measurements to be taken. In using these, care must be given to the possibility of strong reflections or multipaths which may not be fully detected if the log periodic antennas are pointing towards the transmitting antenna.

0.5 TO 12 GHz

WATKINS-JOHNSON WJ-48210 encapsulated dual polarized antenna with 20 dB inter-channel isolation. 12.5" high 8.5" diameter.

Has 2 3mm Female connectors.

Estimated cost with calibration is $4,000.00

1 to 18 GHz

Watkins-Johnson WJ 48195 printed circuit log periodic antenna is compact and relatively inexpensive. If calibration is done at the same time as other WJ antennas this cost will be minimized. Cost for the antenna minus calibration is $466. Recommended is the purchase of two of the antennas ($932) as they can be mounted at right angles to each other for simultaneous vertical and horizontal polarization measurements.

Cost $932.00

ESTIMATED ANTENNAS COST $55,207.00

ANTENNAS MOUNTS

MAXWELL ELECTRONICS, MODEL SMC-202B-1,
STEPPING MOTOR AND CONTROLLER WITH
GPIB/IEEE-488 BUS $1,870.00

ANTENNA MOTOR CASING AND FABRICATION (See Fig.3 for three axis measurements using only rotation). Allow approximately $3,000.00

WOODEN TRIPOD Estimated cost..... $500.00

Estimated subtotal $5,500.00

CABLES

High frequency cables are needed which will perform reliably in the field up to the 22 GHz range. These are not inexpensive but are well worth the price. The Gore company makes a GORE-TEX cable which can have considerable flex and not change its chracteristics in the GHz range. This is not true for the Teflon filled cables. Further the Gore cables come

with a universal fitting on to which a variety of connectors (N, 3mm, 2.7mm, etc.) can be attached with a wrench and thus form a versatile yet low loss and low reflection interconnection system.

2-50' cables, 3-3' cables

COST ALLOWANCE $5,000.00

HP-IB CABLES

1-HP10833A 1 METER LONG (3.3 ft.)	....$80.00
1-HP10833B 2 METERS LONG (6.6 ft.)	....$90.00
2-HP10833C 4 METERS LONG (13.2 ft.)	....$200.00
Subtotal	....$370.00

These cables can be piggybacked both on the equipment for daisy chaining or off the equipment to make a longer cable.

ISOLATION TENT

Swift Textile Metalizing Corp. manufactures heavy marquisette (like window screen) metalized nylon fabric -- $16.50/yard (1-9 yards) 36" wide. This material which allow for air flow can be used to line tents or equipment boxes for RF isolation. The firm also makes a heavy rip stop material which in itself has sufficient strength to use as a tent material. The cost of this material is $33.14 per yard in 36" widths. 60 yards of this material would just cover a rectangular box surface 9'*12'*7' (including the floor). This would cost less than $990.00

SHIPPING BOXES

Metalized or metallic boxes are neede to ship equipment. It is desirable that equipment can be both shipped and operated without

removal from cases. Metalized nylon fabric can be used to protect and isolate equipment from RF while operating.

It is recommended that a HP operating case with removable castors be used for the two main frames of the 70000 series spectrum analyzer. Thus either one or two mainframe racks may be mounted in the case. Power meters with rack adapters may be substituted for one of the main frames.

HP9211-1242	.....$1,800.00
Castor kit HP1490-0913	.....$210.00
Rack adapters	
HP5061-9672 for single power meter	..... $39.00
HP5061-9694	.....$200.00
HP5061-9674 for side by side rack mount	..... $50.00
Estimated extra boxes cost 5 for $300/box	.....$1,500.00
Estimated subtotal	...$3,599.00
Total estimated cost for system	$230,924.00

COMMENTS

The equipment above is certainly more than enough to start implementation of a RF radiation measurement system. Some items can be purchased at a later date. The systems described should be put together and tested extensively in the field. Considerable computer programming along with the field testing is envisioned. Operation manuals should also be written.

Training for USAF personnel is vital. At present the duties of the RF personnell is such that x-ray and ionizing radiation field measurements take a considerable part of their available time. This leaves little time to gain the necessary experience in the sophisticated realm of RF measurements. I strongly recommend full time effort by a person with an Electrical Engineering

background and ideally that person should have experience in electromagnetic theory and antennas. Either a full time civilian or an officer with this background would be ideal.

Both Ed Mantiply, Chief of RF division of the Environmental Protection Agency (US EPA, P.O.Box 98517, Las Vegas, NV 89193 phone (702)798-2476) and James Miller, Chief of Engineering Division (1839th Engineering Installation Group, Keeesler AFB, Ms., 39534 AUTOVON 88-868-4329, (601)377-4329) offered RF personnel at AFOEHL the opportunity to accompany and/or use their facilities for RF surveys. Week-long stays with either of these groups will provide excellent training for the systems proposed in this study.

A

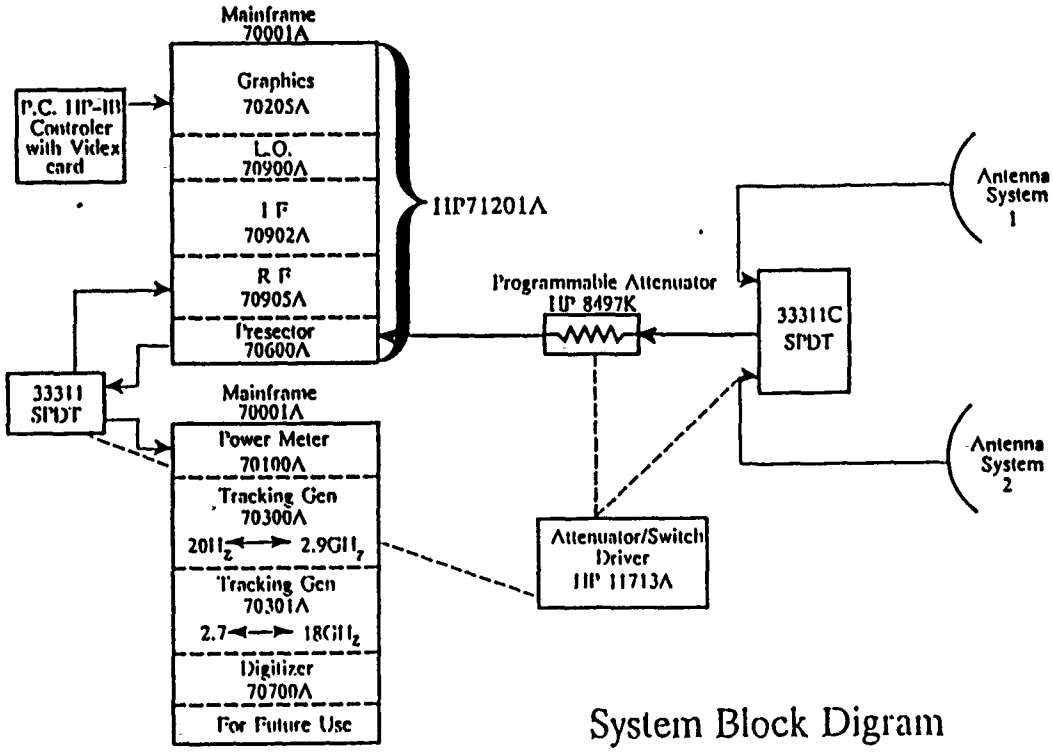
Operational Parameters of MST-T1A

MST-T1

Antenna	Transmitter	Frequency (GHz)	Pulse Width (usec)	PRF (PPS)	Max Duty cycle	Power Peak	(kW) Avg	Gain (dB)
	1	.65-.775	0.4-1.7	750-2250	0.006	50	0.3	--
	2	.775-.92	0.4-8	260-2070	0.006	50	0.3	--
1	5	2.1-2.7	1.2-4	271-303	0.002	20	0.04	37
	7	2.9-3.1	0.5-2.5	375-1700	0.002	250	0.5	39
	IFF	1.03	0.8	220-1000	0.005	0.4	0.002	21
	2	.775-.92	0.4-8	260-2070	0.006	50	0.3	--
	6	2.75-4.05	0.2-1	1000-2400	0.024	1	0.002	36
	8	3.9-4.1	0.25-0.5	917-1000	0.001	500	0.5	36
2A	11	6.2-7.0	0.25-1.0	385-1126	0.001	500	0.5	42
	12	6.7-7.4	0.25-1.0	1000-2825	0.001	100	0.1	42
	14	7.75-8.5	0.2- 0.7	1000-2000	0.001	280	0.28	43
	3	1.02-1.1			CW		0.03	25
	4	1.02-1.28	0.5-1	1000-1050	0.005	5	0.025	25
	9	4.9-5.1	0.35-1	813-1000	0.001	750	0.75	38
	10	4.9-5.1	0.35-1	813-1000	0.001	750	0.75	38
2B	13	6.5-7.15			CW		0.2	42
	15	7.75-8.5	0.2-0.7	917-1000	0.001	300	0.3	43
	16	8.5-9.6	0.2-1.7	1000-2500	0.001	200	0.2	43
	17	8.5-9.6	0.2-1.1	973-1000	0.001	200	0.2	43
	18	11.9-13.2	0.2-1	275-5000	0.001	100	0.1	43
3A	19	14.5-15.4	0.2-0.6	1000-2825	0.001	100	0.1	43
3B	Beacon	8.8-9.5	0.5-2.35	220-1000	0.001	63	0.063	36

List of Typical AF Emitters

Nomenclature	Platform	Frequency	PW	PRF	Peak Power
618T	VARIOUS	2-30 MHZ	CW	CW	400W
AN/APA-165	F-4D	10GHZ	CW	CW	300
AN/APG-63	F-15	MICROWAVE			
AN/APG-68	F-16	MICROWAVE			
AN/APM-358	SHOP	10.9-22G			0.15
AN/APM-427	SHOP	775-14.8G	.1-.4U	6-250K	.1-129W
AN/APN-59	C-130,141	9.3-9.4G	.35-2.3	.18K-2K	50-70KW
AN/APN-221	HH-53	13.3G	CW	CW	50mW
AN/APN-224	A-10,B-1B	4.2-4.3G	0.2-.2	17K-23K	5W
AN/APN-226	ACFT	16.2G	.2-.4	10-4.5K	200W
AN/APQ-120	F-4	8.6-9.5G	.4-2	330-1060	160KW
AN/APQ-122	C-130	9.5G	0.2	2K-4K	75K
AN/APQ-128	F-111	16.7-17G	0.2	4045	30K
AN/ARC-164	VARIOUS	225-400	CW	CW	10W
AN/ARC-165	GROUND	2-30	CW	CW	225W
AN/ARC-186	VARIOUS	30-87	CW	CW	40mW
ARSR-1E	GROUND	1300M	2.0	360	4.9MW
AN/FPS-108	GROUND	1215M	1500	30-400	4KW
AN/FPS-115	(PAVE PAWS)	420-450M	DUTY CYCLE 11-25%		580KW
AN/FPS-116	GROUND	2700M	2	360	3.4MW
AN/FPS-118	(OTH-B)	5.7-28.4MHZ			
AN/GPN-20	GROUND	2.7-2.9G	0.8	2080	550KW
MST-T1	GROUND(MUTES)	6[illegible]00M	SEE ATCHD REPT		



System Block Digram

Figure 1. System using a Spectrum Analyzer or power meter.

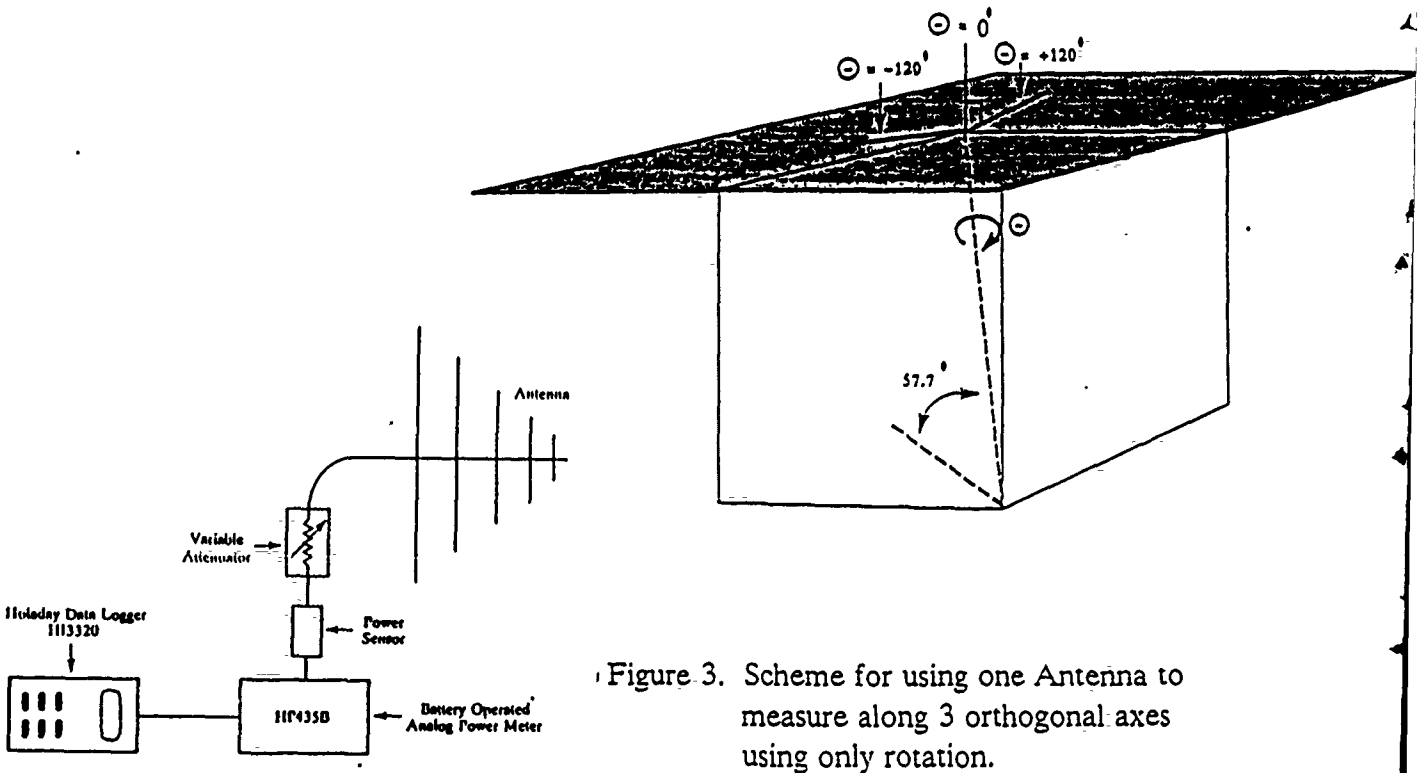


Figure 2. Highly portable system for power measurements.

Figure 3. Scheme for using one Antenna to measure along 3 orthogonal axes using only rotation.

1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

GRADUATE STUDENT RESEARCH PROGRAM

Sponsored by the

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Conducted by the

Universal Energy Systems, Inc.

FINAL REPORT

CONSTRUCTION AND USE OF AN IDENTIFICATION MANUAL FOR IDENTIFYING FIBROUS MATERIAL BY SCANNING ELECTRON MICROSCOPY WITH ATTACHED X-RAY ANALYZER

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Date:	31 Aug 89
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CONSTRUCTION AND USE OF AN IDENTIFICATION MANUAL FOR IDENTIFYING FIBROUS MATERIAL BY SCANNING ELECTRON MICROSCOPY WITH ATTACHED X-RAY ANALYZER

by

Edmund C. Shearer

ABSTRACT

The air asbestos analysis laboratory at Brooks Air Force Base often needs to identify fibrous material that may or may not be asbestos. In order to help improve the laboratory's ability to identify such fibers, a pictorial manual using scanning electron microscopy was assembled. The manual includes 75 different fibrous and particulate samples which might be commonly found in air asbestos analyses. The manual consists of 250X and 2000X printed micrographs, a brief description of the sample, particle and fiber size measurements where appropriate, and qualitative and quantitative elemental analyses performed by x-ray energy dispersion spectrometry. Because of the closeness in elemental compositions of different samples seen by this laboratory, considerable work was done to document the reliability of the x-ray analysis unit which was attached to the scanning electron microscope.

Acknowledgements

It is more than appropriate to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsoring this research program. All the administrative efforts of Universal Energy Systems are appreciated for making physical and administrative burdens a minimum. Their contributions greatly enhanced the enjoyment of this experience.

The personnel of the Occupational and Environmental Health Laboratory (OEHL) at Brooks Air Force Base made this a rewarding and enriching experience. A special thanks goes to the personnel in the Particle Analysis section of the Analytical Services Division of OEHL. Russell Lundy, Jo Mullen, Doris Tessmer, and Lt. Mark Dibben all took a real interest in the development of the project, and contributed to in many ways. They also took a real interest in making me feel comfortable while away from home.

In addition to contributing in these same ways, Kenneth T. Roberson provided me with skills, encouragement, and a share of his experience without which the goals of this project would not have been accomplished. Mr. Roberson made my work far more pleasant and simple by taking care of a lot of little things, in a unique way, which frequently slow down the pace of a project such as this. His help and interest are especially appreciated.

I. INTRODUCTION:

The air asbestos analysis section of the Analytical Services Division of the Occupational and Environmental Health Laboratory analyzes several thousand air filters annually for their fiber content according to NIOSH Method 7400. This method calls for using phase contrast microscopy to count the fibers on an air filter using carefully defined criteria.

NIOSH Method 7400 calls for counting fibers on air filters without distinguishing them as asbestos or non-asbestos fibers. Situations arise where this laboratory needs to identify the fibers on the filters as asbestos or some other fibrous material as a service to its constituency. One potential way of providing this service is to supplement the fiber count analysis with scanning electron microscopy using an SEM which is equipped with an x-ray analyzer. The combination of an electron micrograph with an elemental composition as determined by energy dispersive x-ray analysis can frequently identify fibers as asbestos or nonasbestos, and often yield a reasonable answer as to the true identity of the fiber.

My interest in atmospheric environments and air analyses in a variety of settings, including the indoor environment, contributed to my being selected to help this laboratory better prepare itself to identify fibers found on air filters.

II. OBJECTIVES OF THE RESEARCH EFFORT:

In order to use the scanning electron microscope (SEM) and its associated x-ray analyzer for particle and fiber identification, standard samples of a large number of specimens need to be on file for direct comparison. The primary objective of this work was to obtain and prepare specimens for examination under the SEM, obtain electron micrographs and energy dispersion x-ray analyses (EDXA) for each of them, and assemble the information in a notebook for ready comparison with unknown samples. A further objective was to preserve the specimens for further examination if needed at a future time.

Because this laboratory primarily analyses samples for asbestos, special emphasis was placed on asbestos and corresponding non-asbestos minerals, and on substances which might easily be mistaken for asbestos. Because elemental composition becomes a key factor in deciding the identity of many species, special studies were performed to determine the reliability of elemental compositions obtained by this technique.

Additional specimens were prepared of substances which might commonly be found on air filters received by this laboratory. A few samples were examined because of their availability while obtaining other needed samples.

III. EQUIPMENT AND PROCEDURES:

A. Equipment:

The scanning electron microscope used in this study was an Amray Model 1820 equipped with a data entry keyboard and an air-cooled turbomolecular vacuum pump. It was an automatic imaging SEM, and included digital image storage and processing. It was capable of dot mapping of elemental locations.

The x-ray analysis unit was a Tracor Northern Model 5500 Micro Z-II microanalyis system equipped with a standard beryllium window detector. The detector was not sufficiently sensitive to elements with atomic numbers below sodium to include them in the elemental analyses.

An Anatech LTD Hummer VI Sputtering System was used to metal coat the specimens before introduction into the scanning electron microscope. A gold-palladium alloy was used as the coating metal. The coating was about 85% gold by weight.

B. Procedures:

Samples were obtained in a variety of ways. Many of them were commercially available samples from R. P. Cargille Laboratories,

Inc. A few were already available in the laboratory. Several natural and synthetic fibers were obtained from local retail stores. These were not used unless clearly labelled as to fiber content. Hair samples were obtained from laboratory personnel in the case of human hair, from pets and animals owned by laboratory personnel, and from experimental animal stock at the School of Aerospace Medicine at Brooks Air Force Base.

The following procedure was used to mount the samples for the SEM. Fibrous samples such as hair and silk were cut into short pieces no longer than about 0.5 cm with a pair of sissors, making sure a large number of different fibers were included. The cut pieces were collected on a piece of cleaned, waxed, black paper, and dusted onto an SEM stub which had been precoated with carbon paint. A second coat of carbon paint was applied to the stub just before the sample was dusted onto the stub to adhere the sample to the stub. Crystalline materials were applied to the SEM stubs using a similar dusting technique. Those particulates which contained large particles were first crushed into a powder with the handle of a pair of stainless steel tweezers. After the carbon paint dried, the stubs were inverted and thumped to allow any loose material to fall off.

Each of the specimens was metal coated using the equipment described above. The stubs were placed in the coating chamber and

the system was evacuated to about 30 millitorr. Argon was leaked into the system to maintain a pressure of 80 millitorr. High voltage was applied such that a 10 milliamp current flow was established. A discharge was created which sputtered a Au/Pd alloy onto the surface of the specimens. Coating was allowed to continue until a layer 15 nanometers thick was deposited.

After each sample was placed into the SEM and the system evacuated, a search of the field was made until important features were located which would yield an informative photograph. Three pictures were taken at magnifications of 250X and 2000X using Polaroid Type 53 film. Size measurements were made when appropriate, such as the widths of hairs and fibers. Measurements were normally made at 10 different sites in the sample, selected as randomly as possible.

At least two energy dispersive x-ray analyses were done on each sample to determine elemental composition of elements heavier than argon. In order to better relate compositions to chemical formulas, elemental compositions were reported in atom percentages. If the samples consisted almost entirely of elements below sodium in atomic number, no more than two spectra were recorded unless they were significantly different. When a number of elements heavier than sodium were present, three or more spectra were taken for improved statistical results. More than three were taken when there was a variation in elemental composition as noted by relative peak

heights. On samples with more than one element heavier than sodium present in substantial concentration, the quantitative analysis software program "SQ" of the Tracor-Northern system was activated to yield a quantitative analysis for those elements. Different spectra were taken from different locations within the sample.

Based on previous work, the specimen stubs were processed at a tilt of 38.5° in order to produce the most intense x-ray pattern sensed by the x-ray detector. A detector slide position of 45.0 mm proved most advantageous in obtaining a useful EDXA spectrum in less than five minutes of analysis time.

Several special elemental analyses were performed to determine the validity of the elemental compositions. The biotite specimen was used because it contained several hevy elements and gave very consistent results. Using the biotite sample, a large number of analyses were taken at different sample sites within the sample to yield a measure of reproducibilty from site to site. Several analyses were obtained at the same analysis sight to yield a measure of reproducibility at the same sight.

Another study was done in which a particular sample sight was selected and analyzed at different rotational orientations to the electron beam. Because this particular crystal face lay at a 25° angle to the surface of the stub, the geometry of the crystal face

changed with regard to the electron beam and the x-ray detector.

IV. RESULTS AND DISCUSSION:

An identification notebook was assembled which contains the data collected for seventy-five different specimens. The specimens are listed and categorized in Table I. The following information was provided for each specimen which appears in the identification notebook.

A. Category: The categories used are presented in Table I.

B. Specimen: This section provides the common name of the specimen, and in the case of vegetable fibers, the botanical name.

C. Chemical Composition: In the case of minerals whose composition is fairly well defined, the chemical composition was given. Compositions were not provided for synthetic fibers, vegetable fibers, or animal fibers. For minerals which exhibit a characteristic range of composition, that range was reported.

D. Specimen Source: The source of each specimen used in the study was reported as part of the documentation.

E. About the Specimen: This section presented an introduction to

TABLE I

LIST OF SPECIMENS

A. Synthetic Fibers

1. Graphite
2. Graphite with Binder
3. Fiberglass
4. Glass Wool
5. Mineral Wool
6. Acrylic (Orlon)
7. Nylon Monofilament
8. Nylon Twine
9. Polyester
10. Dacron
11. Polyethylene Braided Rope
12. Polypropylene Braided Rope
13. Polypropylene Twine

B. Vegetable Fibers

1. Cotton
2. Mercerized Cotton
3. Flax
4. Hemp
5. Jute
6. Ramie
7. Non-Coniferous Wood--Mechanical
8. Coniferous Wood--Sulfite
9. Rag
10. Esparto
11. Manila
12. Sisal
13. Coastal Bermuda Grass

C. Animal Fibers

1. Silks
   a. Viscose Silk
   b. Acetate Silk
   c. Cuprammonium Silk
   d. Mulberry Silk
   e. Wild Silk
   f. Turmeric Viscose Silk
   g. Litmus Silk
2. Animal Hair
   a. Dog Hair (German Shepherd)
   b. Dog Hair (Small, Long-haired Mixed Breed)
   c. Cat Hair
   d. Horse Hair
   e. Wool
   f. Mohair
   g. Rat Hair
   h. Mouse Hair
   i. Hamster Hair
   j. Guinea Pig Hair
   k. Rhesus Monkey Hair

TABLE I

LIST OF SPECIMENS, Concluded

3. Human Hair
   a. Caucasian Hair
   b. Negroid Hair
   c. Oriental Hair
4. Other Animal Fibers
   a. Spider Web
   b. Chicken Feather
   c. Peacock Feather

D. Asbestiform and Corresponding Nonasbestiform Minerals
1. Chrysotile
2. Crocidolite
3. Amosite
4. Anthophyllite
5. Actinolite
6. Fibrous Tremolite
7. Nonfibrous Tremolite
8. Serpentine
9. Riebeckite
10. Antigorite
11. Grunerite
12. Cummingtonite

E. Other Minerals
1. Wollastonite
2. Calcite
3. Barite
4. Gypsum
5. Hornblende
6. Talc
7. Quartz
8. Biotite
9. Vermiculite

F. Miscellaneous
1. Rice Starch (5% Potato)
2. Diatomaceous Earth
3. Viscose Rayon
4. Perlite

that specimen as found in numerous reference works.

F. Physical Description: This section was used to describe features of the specimen that were considered important to this study. It included such topics as morphological details, size measurements when appropriate, and general appearance.

G. EDXA Spectrum: A sample spectrum was presented for each specimen as printed out by the software of the x-ray analysis system.

H. Peak Listing: This entry was a listing of the elemental peaks appearing on the EDXA spectrum. Also provided on the listing were x-ray energies and peak areas for the elements found in the sample.

I. Discussion of EDXA: This section was used to call attention to major features of the spectrum, particularly elemental compositions, the presence of artifacts, background, and peak misidentifications by the software program accompanying the x-ray analysis unit.

J. Scanning Electron Micrographs: Polaroid pictures taken at 250X and 2000X magnifications were presented for each specimen.

K. References: References were listed pointing to literature from which additional information about the specimen could be found as it

related to the study at hand.

The fiber diameters (or widths in the case of flat fibers or fibers with oval cross sections) of a large number of specimens examined are presented in Table II. Two significants points need to be made in regard to Table II. First, samples of a particular specimen type obtained from a different source might yield significantly different measurements. This is particularly true of synthetic fibers. Secondly, only a very few specimens had fiber diameters small enough to be comparable to asbestos fibers. Of the non-mineral specimens, small fibrils which could become separated from wild silk, spider webs, some vegetable fibers, polyethylene, and polypropylene had diameters characteristic of harmful asbestos fibers. These should be easily distinguishable from asbestos because they have either a cellulose content or a low melting point.

Of the minerals examined which did not belong to the asbestiform and corresponding non-asbestiform category, only talc had a fibrous component that looked like asbestos. These talc fibers could be distinguished from asbestos because they had a morphology different from chrysotile, and a composition similar to chrysotile but different from the other asbestiform minerals. The only other mineral specimens to have any fibrous-looking component were wollastonite and diatomaceous earth. However, their elemental compositions were different from all asbestiform minerals.

TABLE II

DIAMETERS (WIDTHS) OF SEVERAL FIBERS IN MICRONS

Specimen	Range	Average	Standard Deviation
Synthetic Fibers			
Graphite	7.0 - 7.9	7.5	0.3
Fiberglass	5.0 - 8.0	6.5	0.8
Mineral Wool	0.8 - 5.8	2.4	1.4
Acrylic	18.3 - 21.3	19.7	1.1
Nylon Monofilament	250 - 268	263	6
Polyester	23.0 - 28.1	25.9	2.2
Polyethylene Rope	251 - 335	287	27
Polypropylene Rope	237 - 281	258	16
Vegetable Fibers			
Cotton	7.5 - 36.9	19.3	8.1
Mercerized Cotton	6.5 - 15.8	12.3	3.4
Flax	7.6 - 16.8	10.7	3.1
Hemp	10.5 - 39.7	28.2	8.4
Jute	6.9 - 31.9	18.6	6.9
Sisal	8.3 - 25.1	16.9	4.6
Animal Fibers			
Viscose Silk	19.0 - 28.3	22.6	3.2
Acetate Silk	5.7 - 16.6	11.7	3.4
Wild Silk	19.1 - 37.3	28.3	4.9
Large Dog Hair	31.6 - 45.2	40.3	5.2
Cat Hair	9.8 - 15.0	11.8	1.6
Wool	22.8 - 40.8	29.7	6.3
Mouse Hair	9.3 - 15.0	11.9	1.9
Caucasian Hair	17.2 - 78.0	56.3	19.7
Asbestos and Talc Fibers			
Chrysotile	0.27 - 0.98	0.48	0.25
Amosite	0.14 - 2.32	0.78	0.64
Fibrous Tremolite	0.44 - 0.86	0.60	0.15
Talc	0.19 - 2.83	1.20	0.82

There were several problems associated with the elemental composition determinations reported from the energy dispersion x-ray analyses. The best and the worst of the results are reported in Table III. Elemental composition analyses on the biotite specimen were very consistent for a large number of different particles examined on the same SEM stub. On the other hand, different particles from the vermiculite sample were very inconsistent, presumably because the elemental makeup of the particles varied considerably. The most disturbing elemental composition was obtained from the gypsum sample, a sample which should report an atom percent of 50% each for calcium and for sulfur. Both the elemental composition and standard deviation of multiple determinations were far from satisfactory for no apparent reason. While the standard deviation of the barite analyses was much better, the elemental composition results were not.

The orientation of the crystal face on the SEM stub is important. Figure 1 presents the elemental composition of the same face of a biotite crystal as the crystal is rotated through 360° with respect to the electron beam. Because the crystal was at a 25° angle to the surface of the stub, the geometry of the face with respect to the electron beam and the x-ray detector continually changed. The change in reported elemental compositions was significant at some orientations. The same phenomenon was also observed with other specimens.

TABLE III

SAMPLE ELEMENTAL COMPOSITIONS

A. Biotite - $K_2(Mg,Fe^{2+})_{6-4}(Fe^{3+},Al,Ti)_{0-2}[Si_{6-5}Al_{2-3}O_{20}](OH,F)_4$

(Average of 5 Spectra)

Element	Range, Atom %	Average, Atom %
Fe	8.8 - 9.6	9.2 +/- 0.3
Si	44.1 - 45.4	44.7 +/- 0.5
Mg	17.7 - 18.8	17.9 +/- 0.5
Al	14.0 - 14.8	14.5 +/- 0.3
K	9.6 - 10.4	9.8 +/- 0.3
Ti	1.1 - 1.3	1.2 +/- 0.1

B. Vermiculite $-(Mg,Ca)_{0.7}(Mg,Fe^{3+},Al)_6[Al,Si)_8O_{20}](OH)_4 \cdot 8H_2O$

(Average of 5 Spectra)

Element	Range, Atom %	Average, Atom %
Fe	0.1 - 6.5	3.2 +/- 2.7
Si	45.4 - 65.2	54.7 +/- 7.6
Mg	0.3 - 29.2	18.9 +/- 12.3
Al	2.6 - 22.5	14.9 +/- 6.1
Ca	0.1 - 7.2	1.8 +/- 3.0
K	0.4 - 15.2	5.4 +/- 6.0

C. Barite - $BaSO_4$ (Average of 3 Spectra)

Element	Range, Atom %	Average, Atom %
Ba	38.9 - 42.6	41.3 +/- 2.1
S	57.4 - 61.1	58.7 +/- 2.1

D. Gypsum - $CaSO_4 \cdot 2H_2O$ (Average of 4 Spectra)

Element	Range, Atom %	Average, Atom %
Ca	50.2 - 76.0	60.6 +/- 12.4
S	24.0 - 49.8	39.4 +/- 12.4

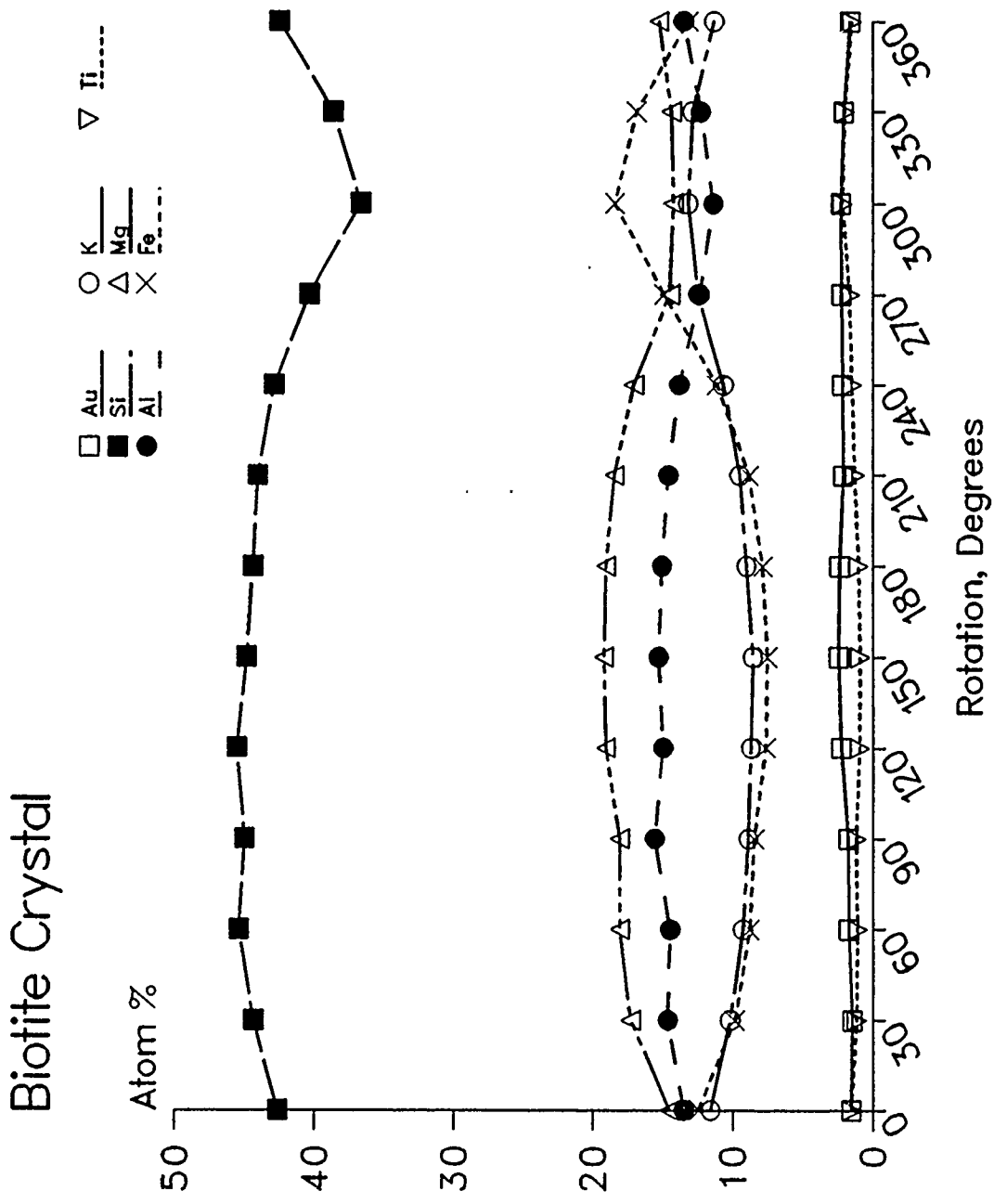


FIGURE 1: Elemental Composition of Biotite by EDXA as a Function of Crystal Face Rotation.

Not infrequently some peak identifications given by the x-ray analysis system were faulty. The most common misidentifications were Nb instead of Au, Tl instead of Cl, Rb and Au instead of Si, and Cl instead of Pd when Cl was also present.

V. RECOMMENDATIONS:

A scanning electron microscope equipped an energy dispersion x-ray analyzer presents a practical method of identifying particulate and fibrous material commonly found in atmospheric environments. When using this equipment, a number of procedures need to be followed in order to assure the greatest accuracy.

1. Electron micrographs need to be obtained at more than one magnification.

2. Several EDXA spectra should be obtained in order to establish the elemental composition of the sample with reasonable accuracy. Such a procedure will aid in determining whether or not the composition of the material is constant.

3. EDXA spectra should be taken from surfaces which are parallel to the SEM stub surface. This will help prevent variations in elemental compositions because the geometry of the specimen surface to the x-ray detector and the electron beam remains constant.

4. When examining fibrous material, measurements for diameters (or widths) should be taken from several fibers so that a statistical presentation of fiber size may be used in comparison to standards.

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FINAL REPORT

BIOLOGICAL ANALYSIS OF THREE PONDS AT PETERSON AFB, COLORADO SPRINGS, CO

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BIOLOGICAL ANALYSIS OF THREE PONDS AT PETERSON AFB, COLORADO SPRINGS, CO

by

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ABSTRACT

A series of three man-made ponds on the golf course at Peterson AFB, Colorado Springs, CO were analyzed to determine their current ecological status and future potential for recreational fishing. Biological analysis consisted of collection, enumeration and identification of organism from the water column and sediment from three sampling sites at each pond. The ponds were evaluated on the basis of species diversity and the types of species present. Chemical analysis of water and sediments for toxicants was also performed.

The results indicate that ponds 1 and 2 are in excellent ecological condition and should be able to maintain stocked game fish which are safe for human consumption. Pond 3 cannot be recommended for stocking with fish in its current condition. Low species diversity and the presence of pollution indicator species suggests that this pond is being stressed by an unknown pollutant. The most like source is a storm drain which may be chronic source of pollutants for this pond.

ACKNOWLEDGMENTS

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We wish to extend our personal thanks to some specific individuals at AFOEHL who made this research effort rewarding and enjoyable. Lt. Col. Bob Binovi provided leadership, support, encouragement and some funny stories. The assistance of Lt. Becky Bartine and Sgts. Rolon and Hernandez was invaluable in solving many problems. A special thanks and salute to Sgt. Carol Wilson who worked and put up with us on a day-to-day basis. She was an unending source of assistance, support and supplies.

I. INTRODUCTION

A series of three man-made, 1-2 acre ponds at Peterson AFB in Colorado Springs, CO have been impacted by an unknown chemical or suite of chemicals which resulted in a fish kill and an apparent decrease in the invertebrate and plant populations in one of the ponds, designated pond 3. The remaining two ponds (ponds 1 and 2) have been impacted to a lesser extent because of pumping of water from pond 3 into these two ponds. The Air Force is particularly concerned about the ecological health of pond 3 because the Air Force would like to utilize the pond as a recreational fishing pond for the base and as a source of water for watering the base golf course.

My research and the interests of my accompanying graduate student have been in the area of invertebrate ecology of marine organisms. We have both completed graduate research on the physiological and behavioral ecology of marine crustacea with regards to feeding on various prey organisms. We are both broadly trained in all fields of ecology and were thus assigned to the Ecology Section of the Occupational and Environmental Health Laboratory.

II. OBJECTIVES OF THE RESEARCH EFFORT

The short-term goals of this research were to 1) try to determine the physical factors or toxicant which lead to

the original biological impact, 2) determine if these ponds are now capable of maintaining a fish population and, 3) determine if fish taken from these ponds are and will be safe for human consumption. From a long-term perspective we hoped to be able to 1) suggest preventive measures that will maintain the water quality of the ponds so that game fish may be safely stocked and so this pond can be used to supply water for the golf course, and 2) suggest ways to bring these ponds back to a natural ecological state with a self-sustaining population of game fish.

III. GENERAL RESEARCH APPROACH

Our initial approach to evaluating these goals was wide-ranging because of the unknown nature of the toxicants. Our preliminary suspected toxicant was Ansulite 3% AFFF Freeze Protected (a fire extinguishing agent) which was accidentally spilled into pond 3 shortly before the first fish kill. Unfortunately, we could not be certain that this chemical is the source of the problem because; 1) AFFF should not persist very long and yet a subsequent restocking resulted in a second fish kill, and 2) pond 3 receives the drainage from the entire base which confounds the problem of targeting a specific toxicant.

All sampling was conducted during the period 6-8 June 1989. Three sampling sites were established in each pond: station C was near the deepest point of each pond; station B was located where the water depth equaled the depth of

the photic zone; station A was approximately 1 m from the shoreline. The biological health of all three ponds was evaluated at the population level (Warren, 1971) by qualitatively and quantitatively sampling the water column and the benthos (bottom sediment) for invertebrates, vertebrates and plants. The water column was sampled for plants and animals with plankton nets, seines and water bottles. Benthic samples were taken along transects with grab samplers for macrobenthos and cores for meiobenthos and the infauna preserved in the field. Since there is a gradient to the impact, with pond 2 being slightly impacted and pond 1 apparently not being impacted at all, we will use pond 1 as a control for comparing species composition. The usual set of physical measurements (temperature, pH, secchi disk depth, nutrient levels, etc.) were taken at each pond.

In order to determine possible toxic chemical levels in all 3 ponds, both water and sediment samples were analyzed for a series of possible toxicants (hydrocarbons, heavy metals, pesticides, herbicides, etc.). Fish were similarly evaluated for toxic chemicals to determine if they are safe for human consumption.

IV. METHODS AND RESULTS

A. Physical characteristics

All three ponds were located on the golf course at

Peterson AFB, Colorado Springs, CO (Fig. 1). The circumference of each pond was measured with a tape and the pH, temperature and dissolved oxygen levels were measured at various locations and depths with probes. The depth was measured by using a weighted rope and the photic zone (depth of light penetration) measured by using a white, water sampling bottle. The results are summarized in Table 1 below.

TABLE 1 - PHYSICAL CHARACTERISTICS OF 3 PONDS

	POND #1	POND #2	POND #3
TEMPERATURE (^{O}C)	14	14	15
pH (range)	7.8-8.2	7.1-7.6	6.2-6.5
Dissolved Oxygen (surface/depth)	9.0/9.7	9.6/10.0	6.7/6.9
Circumference (m)	384.6	303.9	360.0
Deepest Point (m)	3.9	1.8	1.65
Depth of Photic Zone (m)	1.35	0.67	0.90
Estimated Shoreline Plant Cover (%)	80	70	0

Ponds 1 and 2 had mechanical aerators in operation at the time of sampling and water was being pumped into each. Ponds 1 and 2 also had moderate amounts of vascular plant detritus (mainly tree leaves) along the shoreline. The general water quality of ponds 1 and 2 appeared to be good to excellent. Pond 3 had no aerator and was receiving an inflow of 242,000 gallons/day from an open channel storm

drain as measured by an ISCO 2780 flow meter (Lt. Col. Binovi, pers. comm.). The decaying, floating bodies of 30-50 Necturus sp. (mudpuppies) were observed along the shoreline of pond 3. Also, pond 3 had no observable submerged aquatic vegetation and no aquatic shoreline macrophytes. General water quality of pond 3 was poor.

B. Phytoplankton Composition

Replicate phytoplankton samples were collected at stations C and B in all ponds (Fig. 2) by filling a 2 liter bottle with water, 0.5 m under the water surface. The samples were immediately preserved with Lugol's fixative (Wetzel and Likens, 1979). Three 1 ml subsamples were counted from each sample using a Sedgwick-Rafter counting cell under 100x magnification. The phytoplankton were identified to the genus level and the results summarized in Table 2. The diversity of species at each station in each pond was calculated by using the Shannon-Wiener species diversity index (H') (Shannon and Wiener, 1963).

This data clearly indicates that pond 3 was unable to support a phytoplankton community. This lack of primary producers is strong evidence that this pond was stressed. Comparison of the Shannon-Wiener diversity indices also indicates that ponds 1 and 2 have healthy, diverse and large phytoplankton communities which probably result in a fairly high primary productivity which can support higher trophic levels. The differences in species composition

between ponds 1 and 2 may be due in part because of the greater depth of pond 1 and the deeper photic zone. The generally reduced numbers of organisms collected at station C can be attributed to the aerators which probably reduced the number of delicate species.

Table 2 - Phytoplankton Species Composition
(mean no./ml)

	POND 1		POND 2		POND 3	
Genus	Sta. B	Sta. C	Sta. B	Sta. C	Sta. B	Sta. C
Anacystis	4.5	0.25	11.25	3.5	0.0	0.0
Acanthocystis	4.4	1.5	1.25	0.75	0.0	0.0
Asterionella	4.7	0.75	21.5	12.0	0.0	0.0
Ceratium	0.25	0.5	1.0	1.0	0.0	0.0
Closterium	0.25	0.0	0.25	0.0	0.0	0.0
Cocconeis	19.4	11.25	16.5	5.25	0.0	0.0
Coelastrum	0.25	12.88	14.5	20.25	0.0	0.0
Cosmarium	1.5	5.0	69.5	69.0	0.0	0.0
Cymbella	31.0	9.0	39.63	4.75	0.0	0.0
Dictyosphaerium	147.5	78.8	419.75	337.5	0.0	0.0
Fragilaria	355.4	195.0	525.25	416.75	1.25	1.75
Gloeobotrys	12.0	7.0	24.75	20.5	0.0	0.0
Nephrocytium	69.75	18.5	287.0	379.25	1.25	0.25
Oocystis	6.0	2.0	7.0	9.75	0.0	0.0
Pediastrum	36.75	6.0	116.75	84.25	0.0	0.0
Scenedesmus	168.25	83.25	177.5	149.25	0.0	0.0
Sphaerocystis	58.75	125.25	119.75	190.75	0.0	0.25
Staurastrum	341.75	341.75	276.5	239.5	1.0	0.75
Synedra	32.75	56.25	6.75	0.5	0.0	0.0
Unknown diatoms	254.75	63.0	54.25	17.3	1.5	1.75
Shannon-Wiener Diversity Index	2.12	2.07	2.21	2.12	1.37	0.61

C. Zooplankton Composition

Replicate zooplankton samples were collected at stations C and B in all ponds (Fig. 2) by taking vertical tows from the pond bottom to the pond surface using a 153 micron mesh, 0.5 m diameter plankton net. Since a flow

meter was not available these samples are not quantitative and species composition can only be compared on a relative basis. The samples were fixed with 5% buffered formalin and then stained with rose bengal to facilitate sample enumeration. A Hansen-Stempel pipet was used to withdraw three 1 ml subsamples from each replicate sample. The animals in the sample were enumerated using a dissecting microscope under 100x magnification. Identification was to the lowest taxonomic group using Pennack (1953) for species keys. Since these samples were qualitative it was not possible to calculate a species diversity index.

These results (Table 3) show a similar trend to those seen in the phytoplankton composition table. Ponds 1 and 2 have a relatively greater species diversity than pond 3. The rotifer species are almost nonexistent in pond 3, probably because these species are sensitive to poor quality water conditions. The low diversity of species in pond 3 is typical of systems which are under stress from either physical conditions or pollutants. There is a shift in species dominance between ponds 1 and 2, but the relative diversity of species remains the same. The shift may be due to the decreased depth of pond 2 which results in a decrease in feeding area and increased competition amongst species.

Table 3 - Zooplankton Species Composition
(mean percentage of total)

Organism Name	POND 1 Sta. B	POND 1 Sta. C	POND 2 Sta. B	POND 2 Sta. C	POND 3 Sta. B	POND 3 Sta. C
CRUSTACEA:						
Bosmina coregoni	4.6	3.3	22.4	25.9	0.0	0.0
Bosmina longirostris	4.4	4.3	8.2	9.5	0.29	0.5
copepidites	4.4	3.8	2.4	1.4	1.2	1.4
Cyclops sp.	7.2	7.6	13.8	12.6	2.3	3.5
Daphnia pulex	10.4	10.0	7.1	6.3	18.6	17.3
Diaptomus sp.	0.23	0.11	0.0	0.3	0.0	0.0
nauplii	29.4	31.3	16.8	17.3	77.2	77.1
ROTIFERA:						
Brachionus plicatilis	0.06	0.05	0.0	0.15	0.0	0.0
Keratella cochlearis	35.2	37.0	28.7	24.4	0.15	0.25
Keratella quadrata	3.9	2.8	1.3	1.9	0.29	0.0

Note: Totals do not equal 100 because of rounding.

D. Benthos Composition

Replicate benthic samples were collected at stations A, B and C in all ponds (Fig. 2). Meiobenthic infauna (defined as larger than 64 microns and smaller than 125 microns) were collected by taking 5.07 cm^2 cores of the sediment. Macrobenthic infauna were collected by taking a composite sample of three 5.07 cm^2 cores. All of these samples were preserved with 5% formalin and later stained with rose bengal to facilitate the counting of organisms. Before identification and enumeration, the meiofauna samples were sieved through a 125 and 64 micron sieve and

the material retained on the 64 micron sieve was examined. Macrobenthic samples were only sieved through a 125 micron sieve. Organisms were identified to the lowest possible taxonomic group by use of a dissecting microscope with a magnification of 100X. Since these samples were quantitative, the diversity of species at each station in each pond was calculated by using the Shannon-Wiener species diversity index (H'). The results for the meiofauna are summarized in Tables 4, 5 and 6.

The Shannon-Wiener species diversity index for the meiofauna populations of ponds 1, 2 and 3 is; 1.5, 1.4 and 1.1 respectively. Pond 3 has a lower species diversity again, but the difference is not as great. This is somewhat expected since the sediment is a more stable environment and benthic populations are buffered against any rapid physical changes in the water column. The greatest difference in ponds in seen at station C where pond 3 has sharply reduced numbers of organisms. Observations in the field indicated that the sediment at this site was almost completely anaerobic. The species composition and dominant species varies widely between the ponds. This again can be attributed to the relatively stable environment of the benthos which leads to the establishment of relatively constant biological communities with patchy distributions.

Table 4 - Meiofauna Composition of Sampling Station A
(mean number/core)

Organism Name	Pond 1	Pond 2	Pond 3
Tobrillus sp. (nematode)	38.5	18.5	14.2
Stauroneis sp. (benthic diatom)	22.6	0.0	0.0
Nitzchia sp. (benthic diatom)	4.5	52.6	0.0
Contracted Rotifera	16.8	11.1	20.0
Desmids (green algae)	5.9	58.2	38.4
Planaria sp. (flatworm)	4.8	2.3	4.3
Crustacea nauplii	7.7	6.2	3.8
Chaetonotus sp. (gastrotrich)	0.0	2.9	0.0

Table 5 - Meiofauna Composition of Sampling Station B
(mean number/core)

Organism Name	Pond 1	Pond 2	Pond 3
Tobrillus sp. (nematode)	74.3	21.6	18.0
Stauroneis sp. (benthic diatom)	283.4	0.0	0.0
Contracted Rotifera	15.8	16.9	13.6
Desmids	4.8	3.3	17.3
Bdelloidae rotifers	0.0	1.2	3.5
Planaria sp.	1.7	10.9	0.8

Table 6 - Meiofauna Composition of Sampling Station C
(mean number/core)

Organism Name	Pond 1	Pond 2	Pond 3
Tobrillus sp. (nematode)	12.6	110.4	5.9
Stauroneis sp. (benthic diatom)	48.7	0.0	0.0
Nitzchia sp. (benthic diatom)	5.8	62.1	0.0
Contracted Rotifera	3.0	3.5	1.6
Desmids (green algae)	0.0	0.0	6.2
Nematoda - unidentified	8.1	19.0	4.5
Chaetonotus sp. (gastrotrich)	21.8	24.7	2.9
Tardigrada	5.2	17.3	2.1

The data collected for the macrobenthic populations is summarized in Tables 7, 8 and 9. The Shannon-Wiener species diversity index for the macrobenthic populations of ponds 1, 2 and 3 is; 1.75, 1.9 and 1.4.

Table 7 - Macrofauna Composition of Sampling Station A (mean #/core)

Organism Name	Pond 1	Pond 2	Pond 3
Actinolaiminiae sp. (nematode)	13.4	8.5	1.3
Tobrillus sp. (nematode)	42.3	20.7	11.5
Naidium breviseta (oligochaete)	14.3	0.0	0.0
Metriocnemus knobi (insect larva)	14.6	12.8	0.0
Chironomus tentans (insect larva)	0.0	0.0	5.5
Macrocyclops albidus (crustacean)	2.3	2.9	6.6
Pleuroxus aduncus (crustacean)	0.0	0.0	43.1
Musculium sp. (bivalve)	1.2	3.2	0.0
Candona sp. (ostracod)	6.9	10.3	0.0
Planaria sp. (flatworm)	4.0	11.1	1.5
Harpacticoid copepods	0.0	0.0	6.2
nauplii	0.7	2.1	5.4
Desmids (green algae)	1.6	24.6	2.3

Table 8 - Macrofauna Composition of Sampling Station B (mean #/core)

Organism Name	Pond 1	Pond 2	Pond 3
Actinolaiminiae sp. (nematode)	3.4	1.8	0.0
Tobrillus sp. (nematode)	29.4	18.2	45.8
Naidium breviseta (oligochaete)	8.9	9.1	0.0
Lumbriculus inconstans (oligochaete)	0.0	0.0	44.9
Metriocnemus knobi (insect larva)	4.1	8.9	0.0
Chironomus tentans (insect larva)	0.0	0.0	11.5
Macrocyclops albidus (crustacean)	0.0	0.0	4.1
Musculium sp. (bivalve)	2.7	3.2	0.0
Candona (ostracod)	3.6	2.9	0.0
Planaria sp. (flatworm)	1.9	21.2	3.2
Attheyella sp. (crustacea)	1.6	1.1	0.0
Desmids (green algae)	0.0	2.6	3.5

Table 9 - Macrofauna Composition of Sampling Station C (mean #/core)

Organism Name	Pond 1	Pond 2	Pond 3
Actinolaiminiae sp. (nematode)	6.6	2.3	0.0
Tobrillus sp. (nematode)	78.9	98.2	49.1
Naidium breviseta (oligochaete)	16.5	8.4	0.0
Lumbriculus inconstans (oligochaete)	0.0	0.0	29.6
Metriocnemus knobi (insect larva)	0.0	3.7	0.0
Chironomus tentans (insect larva)	0.0	0.0	4.7
Macrocyclops albidus (crustacean)	0.0	0.0	6.4
Musculium sp. (bivalve)	1.2	2.3	0.0
Nematoda - unidentified	16.8	3.8	4.3

Once again the species diversity of pond 3 is the lowest indicating that the conditions of this pond were not as good as those of ponds 1 and 2. N. breviseta, M. knobi and Musculium are all organisms which occur only in well oxygenated, high quality aquatic systems. They were absent from pond 3 and replaced by low oxygen tolerant species (L. inconstans and C. tentans) which occupy the same niche.

E. Fish Composition

The fish and macroinvertebrate populations of the shoreline waters of all three ponds were sampled by pulling and 10 foot long, 0.5 inch mesh seine along the banks. The only fish caught by this method were Pimephales promelas (fathead minnows) from ponds 1 and 2; no fish were caught in pond 3. A total of 636 minnows were measured for their standard length and minnows from both ponds had similar length frequency distributions and mean standard length of 38.7 mm.

Also caught in ponds 1 and 2 were Cambarus bartoni (crayfish) which had a mean carapace length of 44.5 mm. The only organisms seined from pond 3 were leeches (Class: Hirudinea), snails and a large aquatic beetle (Hydrophilus sp.)

F. Chemical Analysis

Both water and sediment samples were taken from each pond and the storm drain input to pond 3 for chemical analysis by USAFOEHL/SA for: total organic carbon (TOC),

nitrates, orthophosphates, oil and grease, and MBAS surfactants. An additional group analysis referred to as E.P. Toxicity was done on water and sediment samples for each pond. E.P. Toxicity analyzes for pesticides and a group of biologically active heavy metals. Also, trout (sampled by volunteers using long line sampling methods) and fathead minnows were analyzed for mercury and PCB's as recommended by the E.P.A. For the sake of brevity I have only reported on significant results.

The only analysis to produce detectable results for the fish flesh was for the PCB Aroclor 1254 which was present in 0.07 and 0.11 ug/gram concentrations in both the minnow and trout from pond 2. The E.P. Toxicity analysis of the sediments from pond 3 indicated the the metals Barium, Cadmium, Lead and Selenium were all present in higher concentrations than ponds 1 and 2. While none of these levels are currently dangerous, there should be concern as to finding the source for these toxicants.

V. RECOMMENDATIONS

The ecological condition of ponds 1 and 2 appear to be excellent based on these findings and they should continue to provide an excellent area to stock with game fish. I cannot recommend that pond 3 be used for recreational fishing in its current condition. It's ecological condition is questionable as indicated by its low species diversity levels and the presence of pollution indicator

species. The primary problem with utilizing pond 3 as a game fishing area is the presence of the storm drain. The presence of the drain means that there is the constant potential for an ecological disaster on a small scale. The drain is a constant source of water of unknown quality. If any pollutant is accidentally spilled anywhere on the base it has a good chance of entering this drain and pond 3. Also, the drain is a source of chronic pollution which may take years to manifest itself. The fact that low levels of some PCB's are detected in fish and the sediments have higher levels of some biologically active metals should cause concern. While these levels are not currently dangerous the sources of these pollutants need to be determined and minimized before a problem arises. In order to utilize pond 3 for fishing the storm drain should be diverted to some other area before the pond can be prepared to accept fish. The current practice of using water from pond 3 to fill ponds 1 and 2 should also be curtailed in order to keep these ponds in top condition.

One caveat of this study is that all of the samples analyzed (both chemical and biological) were collected over a 2 day period and may not reflect year round conditions. This study should be continued with periodic sampling so that any temporal variability can be observed. This is particularly true of any pollution study in which there may be a chronic, low-level addition of pollutants.

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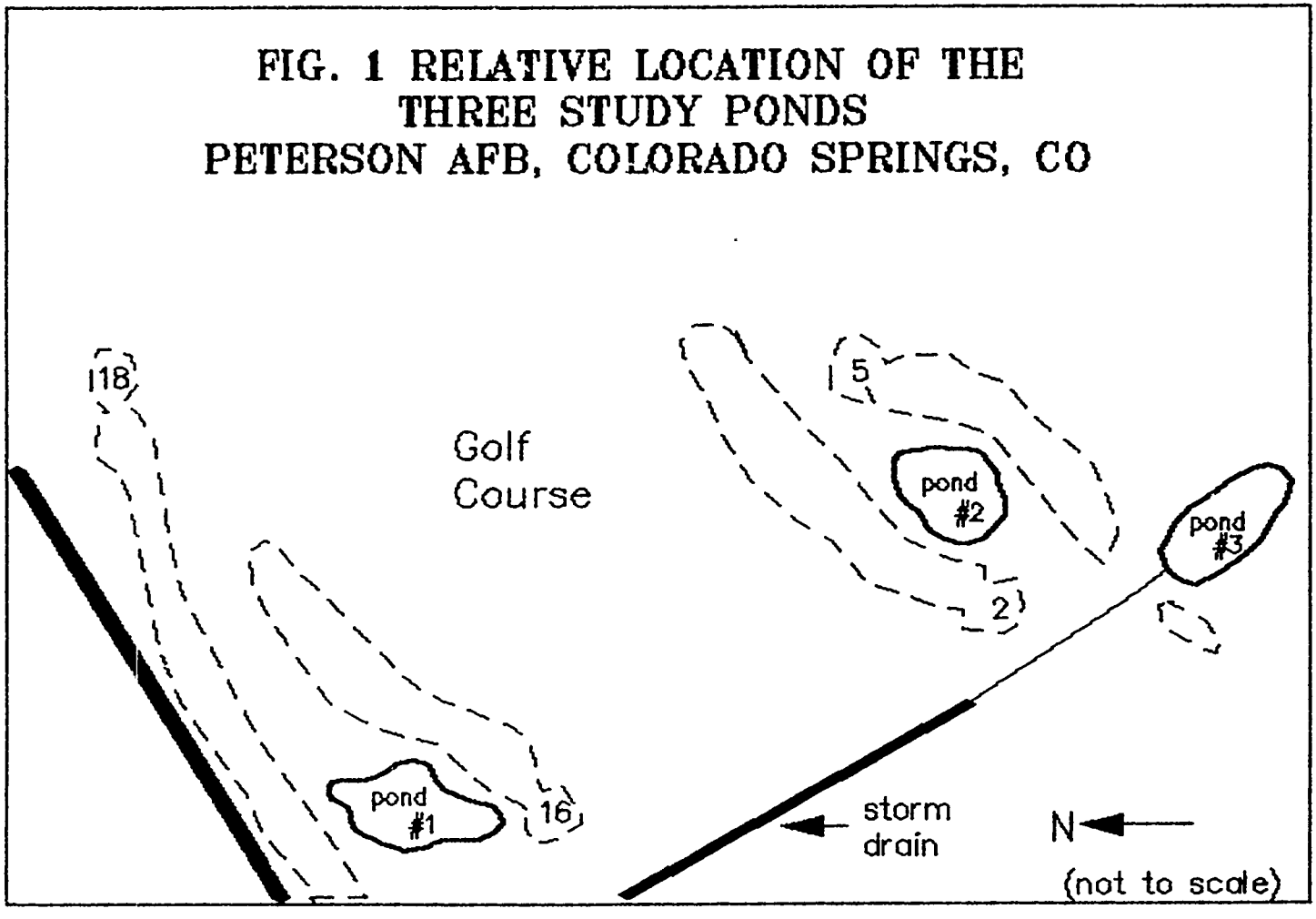
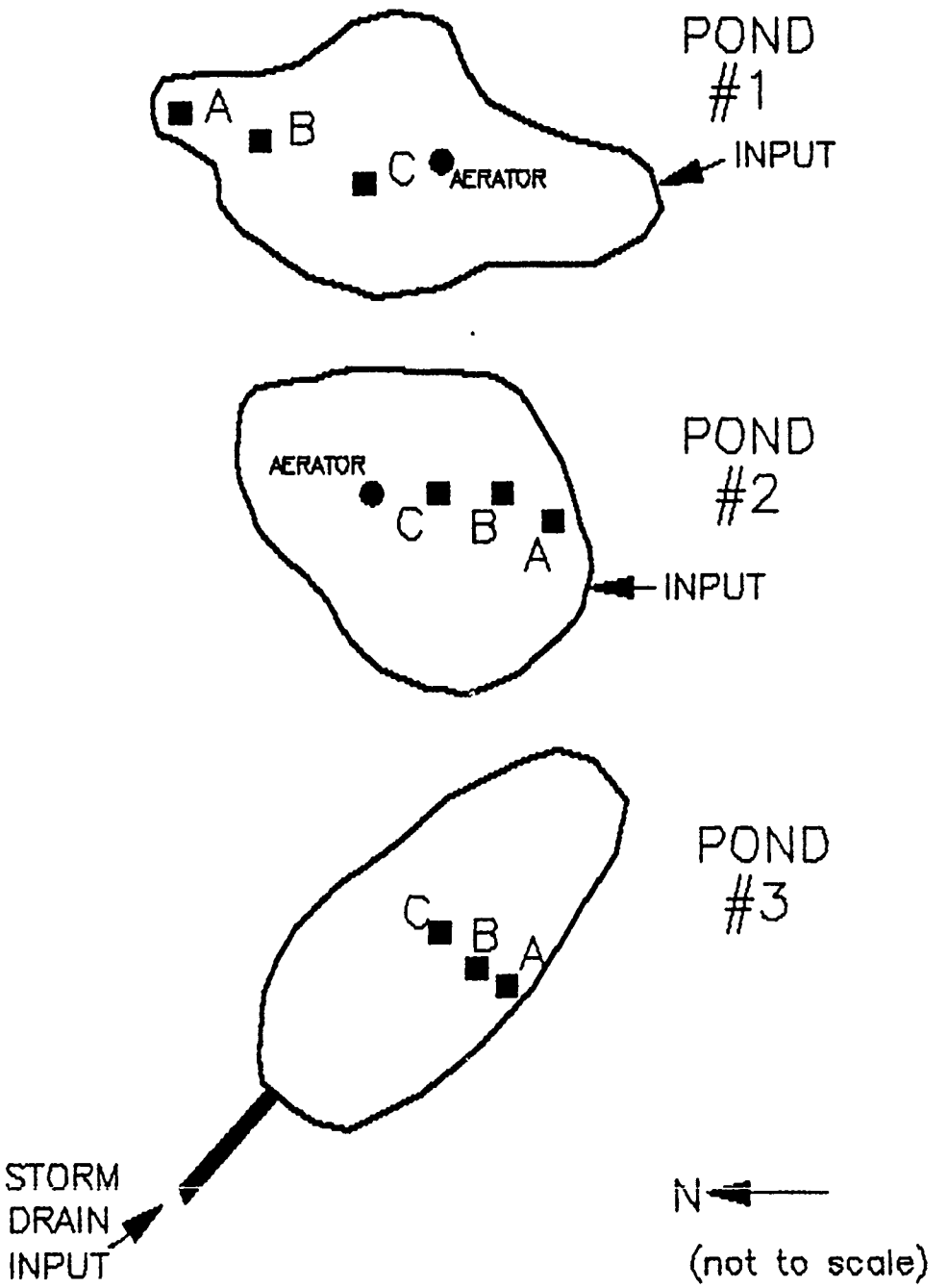

FIG. 1 RELATIVE LOCATION OF THE
THREE STUDY PONDS
PETERSON AFB, COLORADO SPRINGS, CO
18
Golf
Course
5
pond
#2
pond
#3
2
pond
#1
16
storm
drain
N
(not to scale)

FIG. 2 SAMPLING SITES



1989 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

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FINAL REPORT

Convergence Properties of the Occurrence/Exposure Rate

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Convergence Properties of the Occurrence/Exposure Rate

by

Thomas Abraham

ABSTRACT

In this paper, we make an attempt to determine the degree of convergence to the limiting properties of the *Occurrence/Exposure Ratio by resorting to computer generated samples in the context of a Poisson or exponential death process that has a constant death rate.

* Refer to the page on Objectives of the Research Effort.

ACKNOWLEDGEMENTS

I wish to express my appreciation to UES for giving me a great opportunity to work under a coherent system such as the one I have seen at Brooks Air Force Base. - Thanks to the support rendered by AFOSR -

Next, my thanks goes to Dr. Burton who kept overseeing our effort very closely. I like to commend Mr. Mitchell's facility which was quite conducive to the proper functioning of my activities. Especially, I like to thank Dr. Richard Albanese and his crew for making me feel at home, and enabling me to precipitate something shapely from my effort. In this context, Lt. Erik Nielsen's name is to be highlighted for helping me during my initial visit, and arranging for my housing. Also my thanks are due to Dr. Ram Tripathi for his assistance in my attempt to comprehend the problem. Dr. Michalek is to be thanked in turn for allowing Dr. Tripathi to expend his time for the purpose. Again, I like to thank the authors for their profound publication on which I had a chance to work with. Finally, my home institution, Saint Paul's College Lawrenceville, Virginia deserves to be thanked for recommending me to this program.

I. INTRODUCTION:

I was quite excited in my selection as a Research Fellow with UES. In the mean time, I had great apprehensions about the kind of research I will be doing because of my inexperience in research work per se. Now, I realize that with proper guidance and routine skill, one can contribute significantly to the objectives of the AFOSR if one is expressly committed to the purpose.

I ventured on statistics because of my impression that it is an area where a lot of research is being conducted. In fact, I was not quite ready to initiate on the project that I did. However, my consistent effort coupled with the ingenuity of my sponsor, Dr. Richard A. Albanese, brought forth some fruitful effects.

My success could partially be attributed to my background education in mathematics, and the teaching of it for the past twenty six years. Also my computer programming exposure helped me quite a bit. This laboratory is involved in investigating the theory of Radiation effects, and I hope I can contribute my share in its realization.

II. OBJECTIVES OF THE RESEARCH EFFORT:

We have decided to work on a paper published by Z. D. Bai, P. R. Krishnaiah, and Y. Q. Yin on occurrence/exposure measure usually confronted in medical research[1]. If n patients are observed for T years, the ratio of the number of people died during this period to the total number of years each of them is exposed to risk is called the Occurrence/Exposure Ratio. According to the authors, the random variable obtained from this ratio is asymptotically normal. My research colleague was very curious in applying this result to samples to determine the smallest size in which the rule was satisfied.

We joined hands in an effort to unravel the mystery with the aid of the computer.

III.

Suppose an experiment is conducted for a fixed period of time, say, T years and n patients are observed during this period. Also, let Xi denote the total time the ith patient is exposed to risk. Then the Occurrence/Exposure Rate is given by:

$$R_n = V_n/U_n \quad \text{where}$$

$$V_n = Z_1 + Z_2 + \ldots + Z_n \quad \text{and} \quad U_n = Y_1 + Y_2 + \ldots + Y_n$$

$$\text{such that} \quad Z_i = \begin{cases} 1 & \text{if } X_i \leq T \\ 0 & \text{if } X_i > T \end{cases} \quad \text{and} \quad Y_i = \begin{cases} X_i & \text{if } X_i \leq T \\ T & \text{if } X_i > T \end{cases}$$

The authors establish asymptotic normality for the random variable Rn as follows:

$$\text{let } r = \lim_{n \to \infty} R_n = \lim_{n \to \infty} V_n/U_n = \frac{\lim_{n \to \infty} V_n/n}{\lim_{n \to \infty} U_n/n} = \frac{q}{u}$$

where q is the expectation of death and u is the expectation of the time of death.

Let $W_i = uZ_i - qY_i$

$$\text{If } \xi_n = \sqrt{n}(R_n - r) = \sqrt{n}(V_n/U_n - q/u)$$

$$= \frac{n}{uU_n}(uV_n - qU_n)$$

$$= \frac{\sqrt{n}}{uU_n}\frac{1}{\sqrt{n}}\left(u\sum_{i=1}^{n} Z_i - q\sum_{i=1}^{n} Y_i\right)$$

$$= \frac{n}{uU_n}\sum_{i=1}^{n} W_i/\sqrt{n}$$

Here $\{Wi\}$ is a sequence of bounded *i . i . d random variables with mean given by:

$$E(Wi) = E(uZi - qYi)$$

$$= uE(Zi - qE(Yi)$$

$$= uq - qu = 0$$

So, by Central Limit Theorem $\sum_{i=1}^{n} Wi/\sqrt{n}$ is asymptotically distributed as normal with mean zero and variance σ^2 where

$$\sigma^2 = E(Wi^2) = E(uZi - qYi)^2 \quad \ldots\ldots (1)$$

Since $\frac{n}{uUn} \rightarrow \frac{1}{u^2}$, ξ_n is asymptotically distributed as normal with mean zero and variance σ^2/u^4

The statistic η_n obtained by multiplying ξ_n by u^2/σ, the standard deviation of ξ_n, we have:

$$\eta_n = \frac{u^2}{\sigma} \xi_n = \frac{nu}{Un} \frac{1}{\sqrt{n}\,\sigma} \sum_{i=1}^{n} Wi$$

The random variable η_n is asymptotically distributed as normal with mean zero and variance one.

* means mutually independant and identically distributed

IV.

We wanted to test the validity of these results in III in the context of a Poisson or exponential death process. The time T is divided into maxk intervals of time ΔT. If ρ is the probability of death in each of these intervals, the Occurrence/Exposure Rate r is calculated as follows:

$$r = \frac{\text{Expected deaths}}{\text{Time observed}}$$

$$= \frac{\sum_{k=1}^{maxk} (1-\rho)^{k-1}}{\sum_{k=1}^{maxk} (1-\rho)^{k-1}\rho\left[(k-1)\Delta T + T/2\right] + T(1-\rho)^{maxk}}$$

$$= \frac{1-(1-\rho)^{maxk}}{\rho\Delta T \overset{*}{\sum}(1-\rho)^{k-1}(k-\frac{1}{2}) + T(1-\rho)^{maxk}}$$

$$= \frac{1-(1-\rho)^{maxk}}{\rho T \sum k(1-\rho)^{k-1} - \rho\Delta T/2 \sum (1-\rho)^{k-1} + T(1-\rho)^{maxk}} \quad \ldots\ldots\ldots(1)$$

* means summation k = 1 to k = maxk

Let $s = \sum k(1-\rho)^{k-1}$

$$= (1-\rho)^0 + 2(1-\rho)^1 + \ldots + \text{maxk}(1-\rho)^{\text{maxk}-1}$$

$$s(1-\rho) = (1-\rho) + 2(1-\rho)^2 + \ldots + \text{maxk}(1-\rho)^{\text{maxk}}$$

Subtracting, we have:

$$s\rho = 1 + (1-\rho) + (1-\rho)^2 + \ldots + (1-\rho)^{\text{maxk}-1} - \text{maxk}(1-\rho)^{\text{maxk}}$$

$$= \frac{1-(1-\rho)^{\text{maxk}}}{\rho} - \text{maxk}(1-\rho)^{\text{maxk}}$$

Substituting for s in equation (1), we have:

$$r = \frac{\left\{1-(1-\rho)^{\text{maxk}}\right\}}{\frac{\Delta T}{\rho}\left\{1-(1-\rho)^{\text{maxk}}\right\} - T(1-\rho)^{\text{maxk}} - \frac{\Delta T}{2}\left\{1-(1-\rho)^{\text{maxk}}\right\} + T(1-\rho)^{\text{maxk}}}$$

$$r = \frac{1}{\frac{\Delta T}{\rho} - \frac{\Delta T}{2}} \quad \text{if} \quad 1 - (1 - \rho)^{maxk} \neq 0$$

Simplifying:

$$r = \frac{2}{\Delta T(1 - \rho)} \qquad \ldots\ldots\ldots\ldots(2)$$

This compact form could not have been obtained but for the strenuous effort of Dr. Richard Albanese.

An expression for σ^2 has been found in section III:

$$\sigma^2 = E(uZi - qYi)^2 \qquad \text{(equation (1) of III)}$$

$$= E(uZi - urYi)^2 \qquad (r = q/u)$$

$$= u^2 E(Zi - rYi)^2$$

$$\frac{\sigma^2}{u^2} = E(Zi - rYi)^2$$

$$= r^2T^2(1-\rho)^{\max k} + \sum (1 - r\left[(k-1)\Delta T + T/2\right])^2 (1-\rho)^{k-1}\rho$$

$$= r^2T^2(1-\rho)^{\max k} + \sum (1 - r\Delta T\left[k - \tfrac{1}{2}\right])^2 (1-\rho)^{k-1}\rho$$

$$= r^2T^2 + (1 + r\Delta T + r^2\Delta T^2)\sum (1-\rho)^{k-1}\rho$$

$$- r\Delta T(2 + r\Delta T)\sum k(1-\rho)^{k-1}\rho$$

$$+ r^2\Delta T^2 \sum k^2(1-\rho)^{k-1}\rho \qquad \ldots\ldots(3)$$

Let $S = \sum k^2(1-\rho)^{k-1}$

$$= (1-\rho)^0 + 4(1-\rho) + 9(1-\rho)^2 + \ldots + (maxk)^2(1-\rho)^{maxk-1}$$

$$S(1-\rho) = (1-\rho) + 4(1-\rho)^2 + \ldots + maxk(1-\rho)^{maxk}$$

Subtracting:

$$S\rho = 1 + 3(1-\rho) + 5(1-\rho)^2 + \ldots + (2maxk-1)(1-\rho)^{maxk-1} - (maxk)^2(1-\rho)^{maxk}$$

$$= \sum (2k-1)(1-\rho)^{k-1} - (maxk)^2(1-\rho)^{maxk}$$

$$= 2\sum k(1-\rho)^{k-1} - \sum (1-\rho)^{k-1} - (maxk)^2(1-\rho)^{maxk}$$

$$\sum (1-\rho)^{k-1}\rho = 1 - (1-\rho)^{maxk} = q$$

$$\sum k(1-\rho)^{k-1}\rho = \frac{1-(1-\rho)^{maxk}}{\rho} - maxk(1-\rho)^{maxk}$$

$$= \frac{q}{\rho} - maxk(1-\rho)^{maxk}$$

$$\sum k^2(1-\rho)^{k-1}\rho = \frac{2}{\rho}\left[\frac{q}{\rho} - maxk(1-\rho)^{maxk}\right] - \frac{q}{\rho} - (maxk)^2(1-\rho)^{maxk}$$

Substituting the above in .....(3) we have:

$$\frac{\sigma^2}{u^2} = r^2T^2(1-\rho)^{maxk} + (r\Delta T/2 + 1)^2 q$$

$$- r\Delta T(2 + r\Delta T)\left[\frac{q}{\rho} - maxk(1-\rho)^{maxk}\right]$$

$$+ r^2\Delta T^2\left\{\frac{2}{\rho}\left[\frac{q}{\rho} - maxk(1-\rho)^{maxk}\right] - \frac{q}{\rho}\right.$$

$$\left. - (maxk)^2(1-\rho)^{maxk}\right\}$$

$$= \left(\frac{r\Delta T}{2} + 1\right)^2 q + 2r^2\Delta T^2\frac{q}{\rho}\left[\frac{1}{\rho} - 1\right]$$

$$+ 2r\Delta T\left[maxk(1-\rho)^{maxk} - \frac{q}{\rho}\right]$$

$$+ r^2\Delta T^2\left[1 - \frac{2}{\rho}\right]maxk(1-\rho)^{maxk} .....(4)$$

These values of r and σ^2/u^2 from equations (2) and (4) are used against a computer generated values of Rn to examine how closely the corresponding random variable η_n approximate to the standard normal distribution with mean zero and variance one. The data collected is displayed in Table I. Also, a selected few from Table I are graphically plotted, and appended as Figure I to envision the amount of deviation from the standard normal curve. Again, the program that generated the data in Table I is attached as Appendix I.

V. FINDINGS:

Upon careful examination of the collected data, it is our determination that for sample size up to 500, the distributions are not quite close enough to normal.

A tendency of skewedness to the right that we have observed may be due to the imperfect randomness of the random number generator of the computer.

VI. RECOMMENDATIONS:

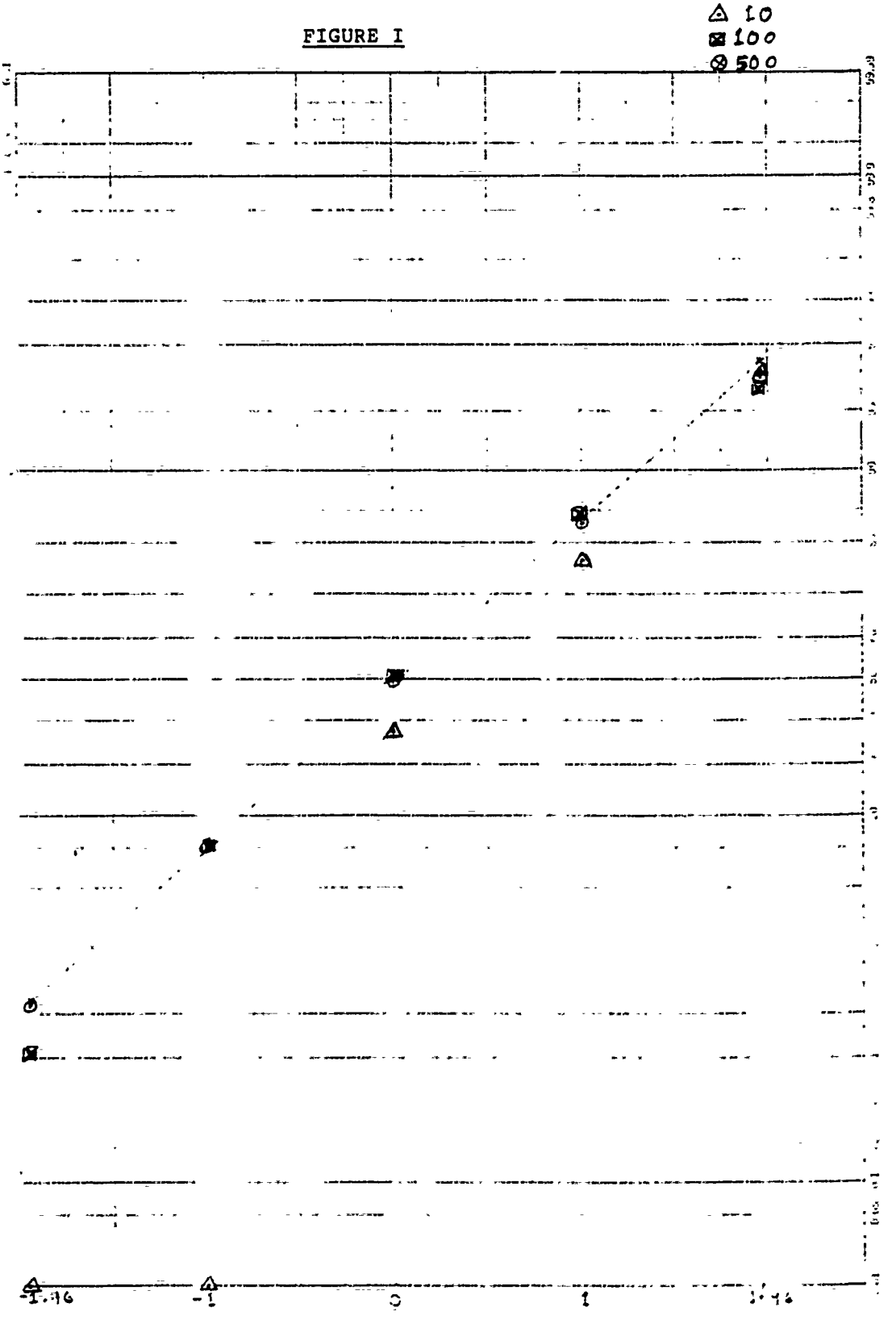
It is our recommendation that the normal approximation may be made when the sample size is large enough as 500 or more. However, when the sample size is small, we cannot rely upon this technique.

TABLE I

Table showing η_{η} less than -1.96, -1.00, 0.00, 1.00, and 1.96 respectively when ρ = .001

No. of subjects	No. of iterations	cm1.96	cm1.00	ccc	cp1.00	cp1.96
10	1,000	0.000	0.000	0.369	0.769	0.955
20	1,000	0.000	0.141	0.428	0.899	0.971
30	20,000	0.000	0.213	0.444	0.848	0.943
50	5,000	0.006	0.133	0.471	0.797	0.969
60	5,000	0.022	0.177	0.491	0.815	0.955
90	5,000	0.006	0.133	0.513	0.858	0.965
100	5,000	0.011	0.153	0.525	0.846	0.962
200	1,000	0.017	0.143	0.509	0.856	0.966
300	5,000	0.015	0.147	0.495	0.829	0.966
400	1,000	0.016	0.175	0.494	0.839	0.965
1,000	500	0.020	0.156	0.536	0.836	0.960
500	1,000	0.024	0.152	0.504	0.832	0.965
		when ρ = .1				
30	2,000	0.002	0.117	0.485	0.803	0.942
40	5,000	0.005	0.116	0.482	0.803	0.947
50	10,000	0.007	0.118	0.487	0.808	0.947
60	10,000	0.008	0.133	0.484	0.814	0.948
500	50	0.02	0.120	0.480	0.820	0.920
200	1,000	0.016	0.150	0.488	0.822	0.960
300	1,000	0.019	0.154	0.489	0.835	0.970
400	500	0.006	0.122	0.472	0.820	0.974

FIGURE I



LIST

APPENDIX I

TARA2 1-AUG-1989 08:54

```
10 randomize
12 input "number of subjects", n
14 input "iterations", maxll
25 dim z(n)
30 dim y(n)
40 T = 20
45 maxk = 100
50 deltat = T/maxk
60 ro = .001
62 cml.96 = 0
64 cml = 0
66 ccc = 0
68 cpl = 0
70 cpl.96 = 0
80 r = (2 * ro)/(deltat * (2 - ro))
100 q = 1 - (1 - ro)^maxk
110 u = q/r
115 sl = r * deltat
120 s2 = maxk * (1 - ro )^maxk
130 ss = ((sl/2 + 1)^2) * q + 2 * sl^2 * (q/ro) * (1/ro - 1)
140 sss = ss + 2 * sl * (s2 - (q/ro))+sl^2*(1-(2/ro))*s2
150 sigma2 = sss * u^2
160 sigma = sqr(sigma2)
170 for ll = 1 to maxll
180 ndth = n * q
190 for i = 1 to n
200 z(i) = 0
210 y(i) = 0
215 next i
218 Vn = 0
220 Un = 0
230 tm = 0
240 for i = 1 to n
250 if z(i) = 1 then 330
260 x = rnd
270 if x<= ro then 280 else 310
280 y(i) = tm + deltat/2
290 z(i) = 1
300 goto 330
310 y(i) = T
320 z(i) = 0
330 next i
340 tm = tm + deltat
350 if tm > T then 360 else 240
360 for i = 1 to n
370 Vn = Vn + z(i)
380 Un = Un + y(i)
390 next i
400 Rn = Vn/Un
410 eta = sqr(n) * (Rn - r)
420 nu = u^2 * eta/sigma
430 if nu <= -1.96 then cml.96 = cml.96 + 1
440 if nu <= -1.00 then cml    = cml    + 1
450 if nu <=  0.00 then ccc    = ccc    + 1
460 if nu <=  1.00 then cpl    = cpl    + 1
470 if nu <=  1.96 then cpl.96 = cpl.96 + 1
480 if nu < -1.8 then c1 = c1 + 1
490 if nu >= -1.8 and nu < -1.4 then c2 =    + 1
500 if nu >= -1.4 and nu < -1 then c3 = c3 + 1
510 if nu >= -1 and nu < -.6 then c4 = c4 + 1
520 if nu >= -.6 and nu < -.2 then c5 = c5 + 1
530 if nu >= -.2 and nu < .2 then c6 = c6 + 1
540 if nu >= .2 and nu < .6 then c7 = c7 + 1
```

```
550 if nu >= .6 and nu < 1 then c8 = c8 + 1
560 if nu >= 1 and nu < 1.4 then c9 = c9 + 1
570 if nu >= 1.4 and nu < 1.8 then c10 = c10 + 1
580 if nu >= 1.8 then c11 = c11 + 1
590 next 11
600 print  c1/n,c2/n,c3/n,c4/n
610 print  c5/n,c6/n,c7/n,c8/n
620 print  c9/n,c10/n,c11/n
630 print "cm1.96", cm1.96/max11
640 print "cm1", cm1/max11
650 print "ccc", ccc/max11
670 print "cp1", cp1/max11
680 print "cp1.96", cp1.96/max11
690 std1 = (cp1 -cm1)/max11
700 std2 = (cp1.96 - cm1.96)/max11
710 print std1
720 print std2

Ready
```

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FINAL REPORT

Transmission Electron Microscopy of Mouse Macrophase RAW 264.7 Cells treated with Lipopolysaccharide, 3-Amino Tyrosine, and RFR

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Transmission Electron Microscopy of Mouse Macrophage RAW 264.7 Cells treated with Lipopolysaccharide, 3-Amino Tyrosine, and RFR

by

Robert V. Blystone

ABSTRACT

The mouse macrophage cell line known as RAW 264.7 was examined by means of transmission electron microscopy. These cells were being used in a protocol that was designed to investigate the effects of RFR on the cells after treatment with lipopolysaccharide and 3-amino tyrosine. The initial fine structure examination revealed the presence of probable viral particles of C-type morphology. Previous reports indicated that although the cell line was established through transformation with Abelson murine leukemia virus, that viral shedding did not occur in this transformed mouse macrophage line. The original cell treatment protocol was separated into its parts and subsequent treated cells were examined in the TEM. Based on this initial TEM examination, lipopolysaccharide is suspected of inducing the expression of the Abelson murine leukemia virus by the RAW 264.7 cells. The virus was observed in all three morphological states: forming, budding, and shed. The possible induction of a C-type virus from the RAW 264.7 genome with an endotoxin has far reaching implications.

Acknowledgements

I very much appreciate the opportunity afforded me by the Air Force Systems Command and the Air Force Office of Scientific Research. Having been in academic administration for a while, the chance to get my research back on track has been most beneficial.

The summer program managed by UES is far better operated and responsive than my previous experience in a similar program 13 years ago. I found very helpful the personal meeting with Dr. R. Burton, chief science officer for USAF/SAM, and the Thursday Seminar program that he established for the UES faculty fellows and graduate students.

I was most fortunate to have two USAF/SAM Radiation Sciences scientists working with me. Jim Merritt provided me with a lab away from home and signatures when I needed them. Johnathan Kiel helped me put the pieces together and provided the treated cells and the background information. To both, I extend my gratitude.

Many others in RZP provided me with assistance. Jill Parker answered many questions. Dave Simmons literally opened doors all over the base. John Awls cultured the cells. Stan Carter helped me with the "gophering." Frank Chamness, Brenda Cobb, and Mel Frei provided the good cheer and information on how to access the resources of the base.

Others at the base were very helpful. Col. Davis provided me access to the VSP EM facilities. Minnie Butcher helped me get going with the initial stages of microtomy and microscopy. And Joe and Ann in the Strughold Library helped with several important computer searches and answered a myriad of reference questions. Access to this kind of staff support is a great strength of this program.

I. INTRODUCTION:

Radiation Sciences, Physics Division, at USAF/SAM has several ongoing projects which could take advantage of transmission electron microscopy. I have 21 years of experience in biological electron microscopy. It was appropriate that I would be assigned to RZP to assist in these two ongoing projects in need of electron microscopy.

Dr. Johnathan Kiel at USAF/SAM RZP is currently investigating the possible effects of RFR on cells in culture. RAW 264.7 mouse macrophage cells are the cells of choice for this study. To better explore the possible effects of RFR on these cells, they are pretreated with lipopolysaccharide (LPS) and 3-amino tyrosine (3-AT) before exposure to RFR. Such treatment and subsequent RFR treatment have produced cells that have altered growth characteristics. Light microscopy suggested that structural differences might exist after treatment of the cells. It was thought that electron microscopy might provide additional and more comprehensive information as to what is happening in the cell.

I have a background in all stages of microscopy: electron, scanning, and light. Some years ago, in conjunction with the Thorman Cancer Lab at Trinity University, I looked at various types of cultured cells in the transmission electron microscope (TEM). My more recent research employed the use of "whole" tissue; however, I have been contemplating projects that would have me looking at cell cultures again. Knowing of Dr. Kiel's interest in looking at treated RAW cells in the TEM, the opportunity of re-establishing skills in this area presented itself to me.

Additionally, Mr. Jim Merritt at the RZP field source and Dr. Melvin Frei working at the same location have been examining RFR effects on rats. Their results have suggested that the blood/brain barrier and the lungs of these RFR treated animals should be examined by

electron microscopy. I have had considerable experience over the last ten years in looking at the fine structure of the lung. I have also briefly examined brain cortex and hypothalamus but not the blood/brain barrier. It was hoped that I could perform an initial TEM examination of RFR treated animals at the two tissue sites mentioned.

On my arrival at Brooks AFB, the high energy microwave source needed for the rat study was not operational. It was determined that I should work on the RAW cell project first, until such time as the RFR generator was in operation again. The initial results with the RAW cells were so promising that the tissue work was put off to allow more time to work on the RAW cell project. Therefore, the summer project has focused almost entirely on the TEM examination of treated RAW 264.7 cells.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Inasmuch as no fine structure had been collected in either proposed project, the main objective was merely to gather some base line data about the structure of the rat blood/brain barrier and lung and of the treated RAW cells. Once collected this data might suggest further focus for an EM study.

Dr. Kiel and I decided to examine the cultured cells as in situ monolayers. Working to this end, I had developed a technique earlier for preparing cells for in situ observation. The technique employed the use of 8-well culture slides. These culture slides need only a small cell seeding which could be obtained with no additional input from the ongoing culture work in Dr. Kiel's lab. Previous work suggested that the cells should be given several days to reach a monolayer stage. We chose four days of culture time after seeding. I selected an EM chemical preparation scheme that had been effective on Chimp Lung Cells

that I had examined previously. After two sample runs, the technique was yielding useful results.

Viral particles were seen among the cells. These C-type virus were totally unexpected. A new question arose: was the presence of the virus a result of contamination or induced by some aspect of the treated cell protocol? New cultures were readied that broke the original treatment scheme into its three parts: treatment with LPS only, treatment with 3-AT only, and treatment with RFR only. Cells which had received no treatment of any kind had not been included in the original experimental group. These untreated cells were added. Several weeks were involved in producing these permutations of the original experiment. Given the interest in confirming the viral presence in the cell cultures and what might be causing their production, the rat tissue study was abandoned after only one control preparation.

The electron microscopy of these additional samples suggested that LPS may be inducing the expression of the viral genome. The mechanisms of such action were unknown to me. The experiment had moved beyond a basic examination of the cell's fine structure. At this point I stopped the EM work in order to familiarize myself with new aspects of the project. These aspects included the structure and function of TNF, LPS and 3-AT; viral transformation; and viral gene expression. Through literature searches on these topics, I was collecting information on how to best procede in the fine structural examination of the RAW cells in the presence of a expressible gene. The focus of what was originally a three or four week study into the basic ultrastructure of treated RAW cells turned into what was the mechanism(s) that could induce the production of a virus in the RAW cells. A simple short project evolved into a very complicated and longer range research effort.

III. PHASE ONE: RAW CELL STRUCTURE

Mouse macrophage RAW 264.7 cells are being used in a USAF/SAM protocol as part of an investigation into the possible effects of RFR on cells. These cells were selected for the study for several specific reasons. RAW 264.7 cells are reported to be excellent producers of Tumor Necrosis Factor (TNF) (Remick et al., 1988). The production of TNF provides a marker by which possible RFR effects on protein synthesis can be detected. Another factor encouraging the use of these cells is the report that these transformed cells do not shed viral particles (Raschke et al., 1978). Viral production would be competitive with other protein synthesis and make results more difficult to monitor and interpret. Lipopolysaccharide (LPS) treatment of RAW 264.7 cells is reported to enhance the production of TNF, although at high dose rates (>10ng/ml), this compound inhibits cell growth (Raschke et al., 1978).

The initial treatment regimen of the RAW cells used in the first stage of the summer project is reflected in figure 1.

Figure 1 - RAW Cell Treatment

RFR treated	Sham treated	Incubated only
temp. variation by RFR	temp. variation by heated air	temp. constant at 37°C
LPS 1.0-> 0.1 ng	LPS 1.0-> 0.1 ng	LPS 1.0-> 0.1 ng
3-AT	3-AT	3-AT

RFR treated RAW cells were exposed at 2450 MegaHz at a power level of 100 watts per kilogram over a 30 minute period with the temperature maintained at a constant value. One ml of RAW cells, at 5 X 10^7 cells per ml, was suspended in a test tube during exposure.

The shammed cells were placed in a similar test tube within the same apparatus as the RFR treated cells and then heat applied with warmed air to mimic the heat rise associated with RFR treated cells. The incubated cells remained in the cell culture incubator under the usual incubation protocol (37°C with 5% CO_2). These incubated, but chemically treated, cells were never placed in the RFR apparatus.

The initial experiment was performed with variable amounts of lipopolysaccharide (LPS) and a constant value of 3-amino-L-tyrosine hydrochloride (3-AT) added. These two chemicals were used to influence the cells under RFR. Previous experiments had suggested the dose rates of LPS and 3-AT used in the experiment (personal communication, Johnathan Kiel, USAF/SAM RZP).

After finding viral particles in the first group of samples, the experimental treatment was broken into parts to determine what element might be affecting the presence of viral particles. Below, figure 2 lists the various samples and their treatment prior to preparation for electron microscopy.

Figure 2 - Samples Prepared for EM and Their Pre-treatment

Sample designation	LPS	3-AT	RFR	comment
A	-	-	-	poor sample prep.
B	1 ng/ml	+	40°C	S and I
C	0.5 ng/ml	+	40°C	S and I
D	0.1 ng/ml	+	40°C	S and I
E	-	-	-	control
F	1 ng/ml	-	37°C	S and I
G	0.5 ng/ml	-	37°C	S and I

H	0.1 ng/ml	-	37°C	S and I
I	-	+	37°C	S and I: problems with 3-AT dose
J	-	-	37°C	S and I

Ten different samples (variables) were prepared for EM. The first sample (A) was of poor quality, reflecting problems with starting up a new preparation lab. Under the comment column, "S and I" refers to Sham and Incubated only. All cell samples were investigated with "B," "D," "E," and "F" receiving the greatest attention. The time and supplies allotted permitted only a qualitative evaluation of the cell's fine structure.

The methodology selected to prepare the cells for transmission electron microscopy was relatively direct. A standard fix/post-fix scheme of glutaraldehyde followed by osmium tetroxide was employed. Additional contrast was provided by en-bloc exposure to uranyl acetate and post-section staining with lead citrate. An initial fixation osmolarity range of about 500 milliosmolal (mos) at physiological pH was selected.

It was decided to examine the RAW cells as in situ monolayers. By looking at monolayers the cells could be seen in their association with one another and provide an orderly way to observe the cells. After exposure and manipulation, cells were seeded into the eight-well culture slides (Lab-Tek #4838, 8 chamber, fixed gasket, tissue culture chamber/slide - Miles Scientific). Each well provides approximately 80 mm^2 of surface area and 0.4 ml of volume. The initial seed level was approximately 500 ± 200 cells per well or about 1000 cells per ml. The incubation medium consisted of RPMI 1640 with 10% fetal bovine serum added. Sodium bicarbonate and pen/strep was added. The cells were incubated for four to six days at 37°C and at 5% CO_2 after treatment and seeding. At one day into incubation the cells usually would turn the growth media pH indicator from pink to yellow

indicating viable metabolism. The cells were examined under the light microscope for gross morphology and then prepared for electron microscopy by the procedure outlined in figure 3.

Figure 3 - EM Preparation Scheme

Fixation:	3% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.4, ambient temp., 1 hour
Buffer Wash:	0.1M sodium phosphate buffer, pH 7.4, ambient temp.
Post-Fix:	1% osmium tetroxide in 0.05 M sodium phosphate buffer pH 7.4, ambient temp., 1 hour
Buffer Wash:	As above followed by distilled water wash
En-Bloc:	0.5% uranyl acetate in water 4°C, 18 hours
Wash:	distilled water
Dehydrate:	graded ethanol series (25, 50, 75, 95, 100, 100%)
Imbed:	Spurr plastic, medium grade polymerized at 60°C for 24 hours

In situ cell monolayers were removed from the culture slide base by a simple technique. Plastic filled Beam capsules were inverted over the adhered cells. After heat polymerization the inverted capsules were snapped off the glass slide which had been chilled to 0°C. The monolayer released from the slide and was contained in the upper surface layer of the resultant plastic block. The block face was trimmed down and gold sections were taken with glass knives. The gridded sections were examined in the electron beam and photographed.

Light Microscopy

Examination of the wells under the light microscope revealed that cells grew best near the edges of the well and least near the center. This observation was especially true for cells placed through the RFR and sham protocols. Light microscopy also revealed three general cell types. The dominant type was the tight, round "typical" cell. There were "fibroblast" looking cells which, when present, usually had clear areas around them (that is, little adjacent cell growth). And finally a "giant" cell which was multinucleate, usually with four nuclei. The "typical" cells would grow up to the borders of the giant cell.

The "fibroblast" cell has not be examined with EM yet. The other two cell types have. Light microscopy also revealed foci or places where the tumorogenic cells would pile up on the monolayer. No attempt was made to determine the frequency and density of these foci. EM has not been knowingly brought to bear on these foci as of yet.

Electron Microscopy

At the EM level, the "typical" cells had a "fried egg" organization with preferential location of various organelles in the in situ state. The nucleus had little cytoplasm over it on the side away from the glass slide. (See figure 4.) The cytoplasm was richest nearest the glass.

Those cells examined to date were rich in Golgi, as would be expected of an actively secreting cell. (See figure 5.) Probable receptor mediated endocytic vesicles were visible. (See figure 6.) Coated vesicles were a regular feature of the cells. (See figure 7.) Endoplasmic reticulum expectedly and richly occupied cytoplasmic prominence. Mitochondria were pleomorphic with some forms rich in osmiophilic bodies. (See figure 8.) Untreated cells frequently showed dense bodies in the cytoplasm. (See figure 9.) With only 20 beam hours to date spread over ten sample preparations representing 23

variables, it would be hazardous to make additional structural comments. No quantitative work or image analysis was performed in the time available.

IV: PHASE TWO: THE VIRUS

Finding viral particles in the RAW 264.7 cells changed considerably the direction of the initial fine structure analysis of the project. Figure 10 shows viral particles outside of the cells, in the act of being shed, and internal in the cytoplasm. Viral shedding seemed to demonstrate preferential locations, which means there are locations on the cell that have a greater probability of showing shed virus or budding virus. The untreated cells showed occasional particles of the type seen in figure 11.

Raschke et al. reported in September of 1978 the creation of a functional macrophage cell line termed RAW 264.7. The initial cell line "was established from the ascites of a tumor induced in a male BAB/14 mouse by the intraperitoneal injection of A-MuLV." These RAW macrophage cells were of interest to this RFR study for their ability to produce Tumor Necrosis Factor [TNF] (also called cachectin). TNF is a major protein produced by these cells and is an excellent marker of protein production. TNF is a trimeric molecule with a primary monomer length of about 155 residues (Beutler and Cerami, 1988). It begins as a prohormone of about 75 additional amino acid residues. Its structure would indicate considerable cytoplasmic processing before secretion. The prohormone monomer would have to be clipped and assembled into the functional trimeric form. TNF manufacture would provide a good EM target.

Tumor Necrosis Factor exerts a broad range of effects including participation in inflammatory responses and in cachexia (profound wasting). This cytokine generally is carefully produced and is used in a local, tissue -specific way. Macrophages can be

stimulated to produce large quantities of TNF by exposure to LPS (lipopolysaccharide). Raschke et al. in their original report (1978) recorded that RAW 264.7 was very sensitive to the effects of LPS. Exposure to as little as 0.1 μg LPS per ml inhibited cell growth completely and 0.001μg/ml produced a four-fold reduction in cell doubling rates when compared to control although some growth did occur at this exposure level. Apparently, growth is sacrificed in deference to the production of TNF.

Lipopolysaccharide (LPS) is a component of the Gram negative bacterial cell wall. The ability of bacterial wall extracts to evoke a response from some cells has been known for almost a century (Beutler and Cerami, 1988). And for over ten years LPS has been recognized as being able to activate macrophages into the production of TNF (Beutler and Cerami, 1988). The original design of this experiment employed three dose ranges of LPS: 1.0, 0.5, and 0.1 ng per ml of culture fluid. These levels were used to stimulate TNF production without being too detrimental to cell growth. The LPS used in this study was derived from phenol extracted E. coli 0111:B4, Sigma catalog L2630. Raschke et al.'s original study used Difco S. typhosa WO901 LPS to probe the RAW responses to the endotoxin.

The relationship of LPS and TNF represented a major factor in the selection of RAW cells for the RFR portion of this study. Another reason for using RAW cells is that Raschke et al. (1978) reported that virtually no virus were produced by RAW 264.7 cells. Their production values were less than 10 ffu and pfu per 10^6 cells. This is very important information because the original cell source for the RAW cell line was transformed by exposure to Abelson murine leukemia virus (A-MuLV).

Abelson murine leukemia virus (A-MuLV) is an RNA retrovirus that can cause transformation of certain cells (Abelson and Rabstein, 1970). The A-MuLV RNA is 5,600

bases long and forms the familiar nucleoid associated with C-type virus morphology (Aaronson and Stephenson, 1976). In other words, the Abelson murine leukemia virus is a C-type RNA retrovirus. Therefore, it is highly likely that the particles seen in the first EM of the LPS, 3-AT treated RAW cells is of A-MuLV origin. However, the original RAW citation (Raschke et al., 1978) stated that "The other macrophage line, RAW 264, fails to secrete detectable virus particles and is negative in the XC plaque formation assay, as well as the fibroblast transformation assay for Abelson virus but becomes positive for Abelson virus production after rescue by Moloney leukemia virus." An examination of the literature since the introduction of RAW 264 cells revealed no mention of shed C-type viral particles unless a Moloney rescue was performed. In fact, the only published electron micrographs of RAW 264 cells were in a 1983 cytochemical study by Petty and McConnell. These photomicrographs focus on cytochemical markers and not cell structure. No careful ultrastructural study of RAW 264.7 cells has been reported; nor has C-type viral shedding been reported for this cell line without rescue.

V. RECOMMENDATIONS:

If LPS proves to be inducing the expression of a viral genome, why should it do this? The literature provides some contact points for speculation. Macrophages in general have 40 or more known different membrane receptor activities (Petty and Berg, 1988). Where and how does LPS bind? Hampton et al., 1988 provide some insights. LPS is a complex oligosaccharide which is covalently bound to a moiety called lipid A. Lipid A is a glucosamine-derived phospholipid which is responsible for the LPS endotoxin response. For example, cells challenged with lipid A will produce TNF just as if they had been exposed to LPS. Hampton et al. using a radiolabelled lipid A identified a membrane protein of 95,000 molecular weight that binds with the LPS analog. The protein may be a member of a cell surface group called integrins. They also found another protein of 31,000

molecular weight that bound to the lipid A. This second protein was speculated to be histone H1. For someone looking for a way to express a viral genome, this is very exciting news. Their report went on to say that they were not able to get their labelled lipid A to bind to intact nuclear histone H1.

Ding et al. (1989) offered another point of consideration. Macrophages, including RAW 264.7, have membrane receptors for TNF. They determined that RAW cells have 1100±200 binding sites per cell for TNF. Further, they speculate that many of these receptor sites may be normally bound to the very TNF they produce. The bound sites may have something to do with the regulation of TNF manufacture and function. The most surprising aspect of their study was that RAW 264.7 cells internalize their TNF receptors when exposed to LPS. 15 minutes after LPS exposure the TNF receptors were pulled off the membrane. Why did this down-regulation occur? They had no conclusive information. Of greater interest to me is their speculation that LPS may increase the rate of fluid-phase pinocytosis. Could the possibility of a fluidity factor increase bring LPS into the cell and proximity to the nuclear histone, thereby expressing the viral genome?

Verifying that LPS can influence the RAW 264.7 genome in terms of expressing Abelson murine leukemia virus would be a highly useful piece of information. Such a finding could focus these latter two investigations of Hampton et al. and Ding et al. Also, assuming this viral observation is correct, a system would be in hand that could allow a chemical control over the induction of a viral genome. Such a system would be interesting to explore within an RFR protocol.

Confirmation of this initial finding is very necessary. Several sources of RAW cells should be compared to insure that something unique has not happened to the cell source used in this study. The threshold of LPS response should be sought. And, of course, replicates of

cell preparations should be compared. Shed virus should be collected and checked for retention of tumorgenicity or a specific probe for Abelson murine leukemia virus should be applied to the LPS RAW genome to see if it is being expressed at the molecular level.

I am quite excited by the initial results of this Air Force sponsored work. To bring the work to a logical conclusion, I will prepare a minigrant proposal. Can lipopolysaccharide really induce the expression of an Abelson muring leukemia virus in the RAW 264.7 genome?

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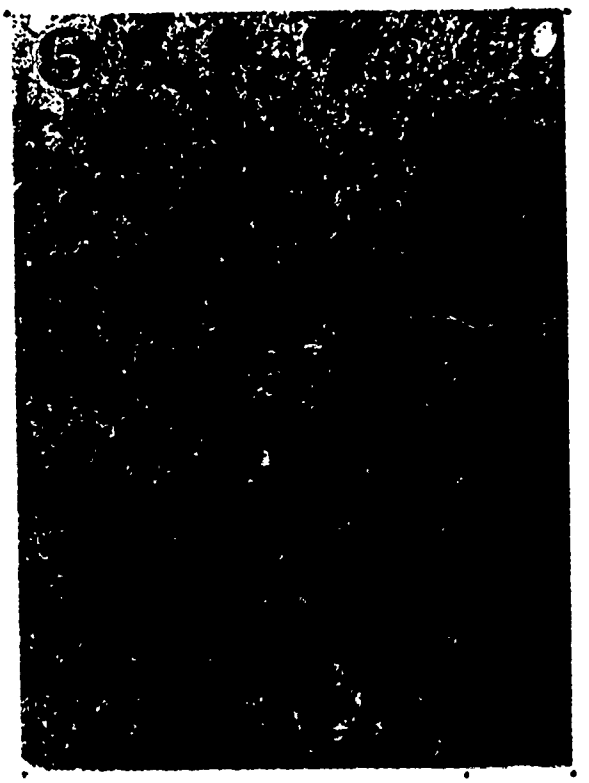




Figure 4. B series, sham. 3,800X. View of "typical" RAW cells.

Figure 5. B series, RFR treated. 11,500X. Arrows indicate area of Golgi.

Figure 6. I series, control. 15,000X. Arrow indicates possible forming endocytic vesicle.

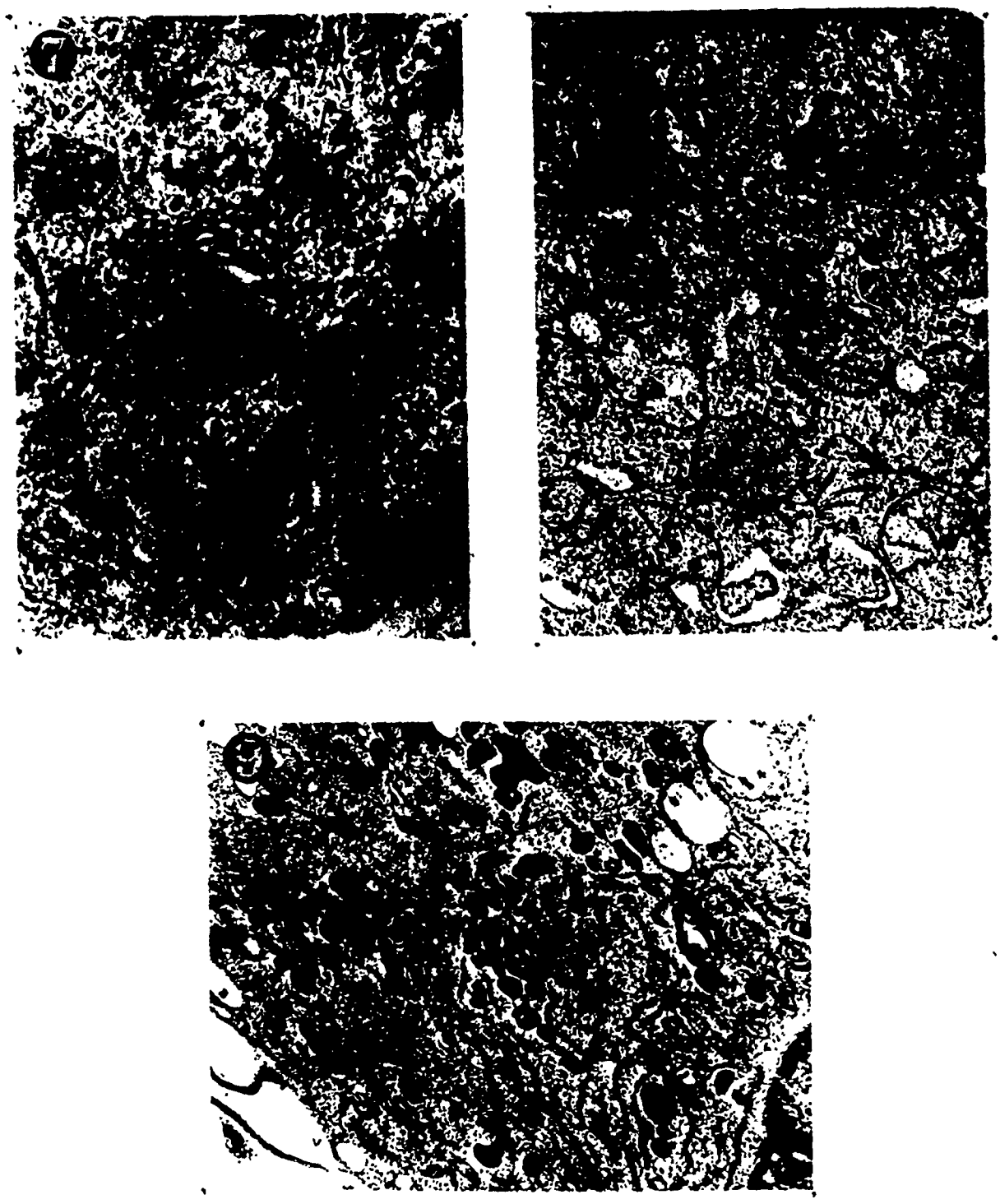


Figure 7. C series, RFR treated. 36,000X. Closed arrow indicates coated vesicle. Open arrow indicates Golgi vesicles.

Figure 8. E series. 26,000X. Note osmiophilic granules in mitochondria.

Figure 9. E series. 10,000X. Control cells frequently contain dense bodies.

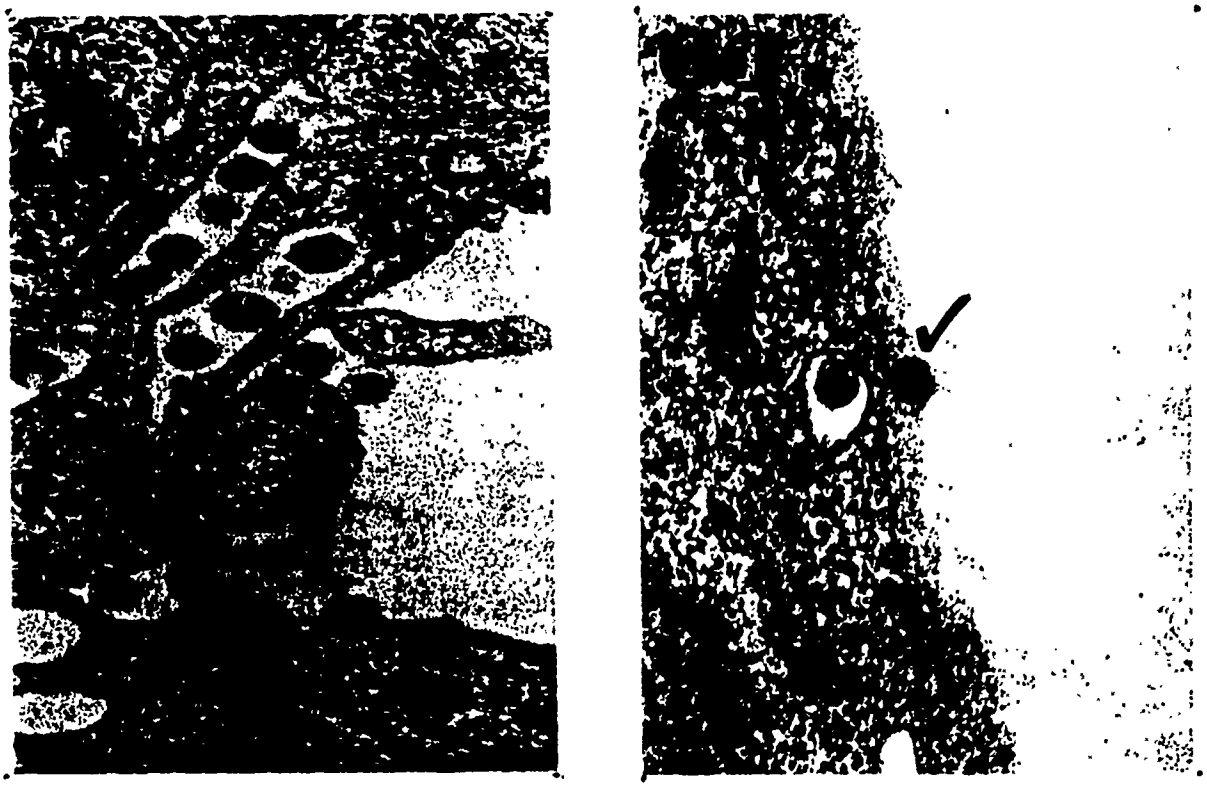


Figure 10. C series, sham. 47,000X. Virus at cell edge.

Figure 11. E series. 43,000X. Occasional "defective" virus in Control.

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FINAL REPORT

Effects of Microwave Radiation on Cultured Cells

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Date:	11 August 1989
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Effects of Microwave Radiation on Cultured Cells

ABSTRACT

The potential hazardous nature of radiofrequency radiation (RFR) has been of great concern for several years. This study was conducted to: (1) further elucidate the effects of low field intensity microwave radiation on cultured cells, (2) to determine if there would be elevated levels of the mRNA of certain oncogenes, and (3) understand more about the mechanisms of radiofrequency radiation on living cells and to characterize these interactions at a molecular level. DNA/RNA hybridizations were performed on cells which had been treated with the mitogen lipopolysaccharide (LPS) alone, or in combination with the metabolic inhibitor 3-amino-L-tyrosine (3AT). Results from such experiments can contribute to or establish guidelines for medical, industrial or military personnel who may be exposed to radiation in the course of their occupation.

INTRODUCTION

The human population has been increasingly exposed in modern times to microwave radiation, which is one of the most common forms of nonionizing radiation. Microwave devices are used widely in telecommunications, cooking, industries and medical technology. There has been great concern regarding the hazardous nature of microwave radiation and the safety of personnel has been the subject of great interest for many years.

A diversity of pathophysiological effects from exposure to microwave radiation have been reported. Some investigators have been able to correlate different biological effects with microwave exposure and others have failed to confirm such positive correlations. Dwivedi et al. (1989) presented evidence that distinct ultrastructural alterations occurred in Chang liver cells after exposure to 2450 MHz microwave of a field intensity ranging from 5-20 mW/cm^2 for different periods up to 2 hours. The effect is related to the intensity of the incident radiation because 5 mW/cm^2 for a period of 120 min will not cause complete degeneration of cell structure, but 20 mW/cm^2 for 30 min produced drastic damage. These results strongly suggest that the membrane may be the main target for microwave damage at the ultrastructural level.

Studies also suggest that the immune system may be influenced by extremely low frequency electric fields. Rao (1983) demonstrated that exposure of hamsters to 2.45-GHz microwaves (25 mW/cm^2) resulted in activation of macrophages which were viricidal to vaccinia virus. In vitro heating of the

macrophages to 40°C was not as effective as the microwave exposure in viricidal activation. Balcer-Kubiczek and Harrison (1985) reported an enhancement of transformation frequency of 10T 1/2 cells exposed to 2.45-GHz microwaves for 24h at an SAR of 4.5 w/kg with or without post treatment with the tumor promoter tetradecanoyl-phorbol-13-acetate.

Microwave radiation has not been reported to cause any noticeable changes in DNA, RNA or proteins, but a slight stimulation of B lymphocytes is known to occur (Wiktor-Jedrzeczac et al., 1980). Reese et al. (1988) were not able to detect any damage to DNA as measured by single-strand breaks (ssb) in Chinese hamster ovary cells (CHO) exposed to 60-Hz electric field as high as 38 v/m or a magnetic field of 2mT for 1 hr. Meltz et al. (1987) investigated the effect of continuous-wave (CW) and pulsed-wave (PW) radiofrequecy radiation and reported that DNA repair synthesis in MRC-5 normal human diploid fibroblast was not affected during up to 3 h of RFR exposure at 37°C at an average power density of 10 mW/cm^2. Cells which had been thermally stressed (39°C) and exposed to RFR at frequencies of 350 and 850 MHz did not show any effects on DNA repair synthesis from CW or PW.

Parker et al. (1988) using four different rodent lines exposed to 2450 MHz microwave radiation at specific absorption rate of 103.5 $\pm$ 4.5 w/kg for 37, 40, 42, and 45° C observed no significant difference in mRNA of certain oncogenes. Since RFR alone did not appear to change the expression of certain oncogenes, we decided to investigate the effects of microwave radiation in the presence of a metabolic stimulant LPS alone or

in combination with a metabolic inhibitor 3AT.

The biological effect of microwave radiation have been reported to be mainly thermal in nature, however; there are some effects that cannot be explained on a thermal level, particularly when low intensity radiations (below $10mW/cm^2$) are involved. Robinson et al. (1981) exposed mammalian cells (CHO) and bacteria (Serratia marcescens) to hyperthermal temperatures (43, 44, 45^oC) for CHO and (48, 49, and 50^oC) for the bacteria with or without microwave irradiation. From their data they concluded that the effects of microwave alone on the cell survival was not clinically significant, but suggested a simple thermal origin for the difference. Since microwaves are used in human cancer therapy, it is important to understand specific biological effects at elevated temperatures.

OBJECTIVES

This study was design to determine if there would be an increase in the expression of certain oncogenes after cells are exposed to microwave radiation in the presence of the metabolic stimulatant lipopolysaccharide (LPS) or the metabolic inhibitor 3-amino-L-tyrosine (3AT).

MATERIALS AND METHODS

Cell Culture, RNA Isolation and Electrophoresis

Raw 264.7 (macrophage, Abelson leukemia virus transformed BALB/c) was used in this study. This cell line was chosen because these cells produce tumor necrosis factor (TNF) which is sensitive to LPS. Approximately 1×10^8 . RAW 264.7 cells had been previously exposed to 100 w/kg RFR in the circular waveguide

apparatus as reported by Parker et al. 1989. LPS at final concentrations of 1, 0.5, and 0.1ng/ml was added alone or in combination with 0.2mM 3-amino-L-tyrosine. A sham-exposed control heated in the waveguide apparatus but not exposed to the microwave, and a control left at 37°C in the incubator was also included in the study. After exposure, the cells were washed in phosphate buffered saline (PBS) and the RNA extracted. The concentration of the RNA was determined spectrophotometrically. Most of the RNA samples had been previously extracted by Dr. Parker, according to the methods of Favaloro et al. (1980) and Manniatis et al. (1982), and stored at -70°C. The RNA samples were subjected to electrophoresis on a 1.0% agarose-formaldehyde gel according to modified methods of Thomas (1980). The gel was run at 60 volts (80 mAmp) for 6 hr in buffer containing 40 mM MOPS, 10mM sodium acetate and 1 mM EDTA. 25 ug of total RNA or 5ug of mRNA was dissolved in three volumes of 50 % formamide, 2.2 M formaldehyde and MOPS and heated for 10 min at 60°C to denature the RNA. Prior to electrophoresis, 5 % glycerol containing 0.025 % bromphenol blue and xylene cyanol was added.

Northern Blotting

After agarose gel electrophoresis, the gel was turned over on top of two pieces of Whatman 3mm filter paper the size of the gel. Nitrocellulose or nylon membranes wet with 2x SSC (0.3M sodium chloride, 0.03M sodium citrate) and soaked in 20X SSC (3M sodium chloride, 3M sodium chloride, was placed on top of the gel, followed by two more pieces of Whatman slightly smaller than the gel which had been soaked in 20x SSC. A stack of paper towels

and weight was placed on top of the gel and the surrounding tray filled with 20x SSC. Transfer was allowed to proceed for approximately 16h at room temperature. The filters were removed and immediately immersed in 2x SSC to remove any traces of the gel, air dried and baked in a vacuum oven at 80°C.

Source of probes

Strain MC1061 containing the plasmid pfos1 with the v-fos probe isolated from FBJ murine osteosarcoma virus was obtained from American Type Culture Collection. The plasmid was isolated by Dr. Parker using a large scale preparation according to Manniatis et al. (1982). The plasmid was digested with PstI and two v-fos specific fragments of 1.0 kbp and 0.3 kbp resulted. The 1.0 kbp fragment was used as the v-fos probe. This probe was chosen because it is smaller than the c-fos (7.8 kbp) which has been reported to be a Master Switch in the regulation of the cell cycle. This gene should also show changes with mitogenic stimulation. Previous studies demonstrated that both the c-fos and v-fos probes gave identical hybridization patterns with the RNA.

The 3611-MSV was isolated from integrated proviral DNA from FRF-3A Fisher rat cells. The E. coli host strain LE392 containing plasmid pF4 was also obtained from American Type Culture Collection. This plasmid contains 7.6 kbp fragment from the virus and 1.3 kbp fragment from the host inserted into the Eco_{RI} site of pBR322.

The v-abl probe was isolated from Abelson murine leukemia virus genomic clone AM-1. A 1.6 kbp fragment which is specific

for the p102 coding region of v-alb gene and a 0.6 kpb fragment which is specific for the p18 coding region of the v-abl were mixed together and used in hybridization studies. The E. coli strain C-600, with the v-alb inserts on plasmid, was also obtained from American Type Culture Collection.

The plasmids containing the probes were isolated by Dr. Parker, digested with the appropriate endonuclease to release the insert, separated on a 1% agarose gel in TAE buffer. The fragment of interest was cut out and electroeluted into a dialysis tubing, concentrated with butanol, phenol extracted, and ethanol precipitated.

Hybridization Of The Labeled-Probe To RNA On The Filter

The baked filter was wet with 2x SSC and put into a heat-sealable plastic bag. Prehybridization was carried out for 3h at 42°C in 50% formamide, 5x Denhardt's solution, 2.5 ug/ml denatured Salmon sperm DNA and 5x SSPE (1mM EDTA, 50 mM NaP and 0.9 M Nacl). The hybridization solution was the same as the prehybridization except the denatured labeled probe was added. The sealed bags were incubated at 42°C for 20h. The filters were removed from the bag and washed twice in 2X SSC + .1% SDS for 15 min at 52°C, followed by two washes in .2x SSC + .1% SDS. The filters were drained by placing them on Whatman paper and wrapped in plastic wrap, autoradiographed and exposed to Kodak XAR film for up to seven days.

Labeling of Probes

Probes were labeled by using the biotinylated non-isotopic

system for DNA which utilizes the nick-translation procedure of the biotinylated nucleoside triphosphates analog biotin-7-dATP and development with the streptavidin/alkaline phosphatase conjugate and a standard dye system as described by BRL or Oncor system which was a complete reagent set (Rigby, et al., 1977).

Probes were also labeled by using the oligo-nucleotide primer procedure described by Feinberg and Vogelstein (1983). The labeled DNA was separated from unincorporated dinucleotides by column chromatography through a Sephadex G-50 column. Sixteen 100 ul fractions were collected and counted for radioactivity in a scintillation counter. The fractions containing the peak activity were pooled and used as probes, or dot blotted onto nitrocellulose for determination of the Biotinylated probes. After labeling the probes were denatured by treatment with 1/10 volume 1N NaOH for 10 min at 37^{o}C, followed by neutralization with 1/10 volume of 1N acetic acid. Biotinylated probes were boiled for 10 min and cooled rapidly before using.

Removal Of Probes From Filter

Probes were removed from the filter by placing them in a boiling solution of 0.1% SSC + 0.1% SDS for 15 mim. This was repeated until all the probe was removed. Filters were then prehybridized and hybridized with another probe.

Analysis of Data

The intensity of spots from the autoradiographs will be determined by using a Hoefer Scientific Gs 300 Transmittance

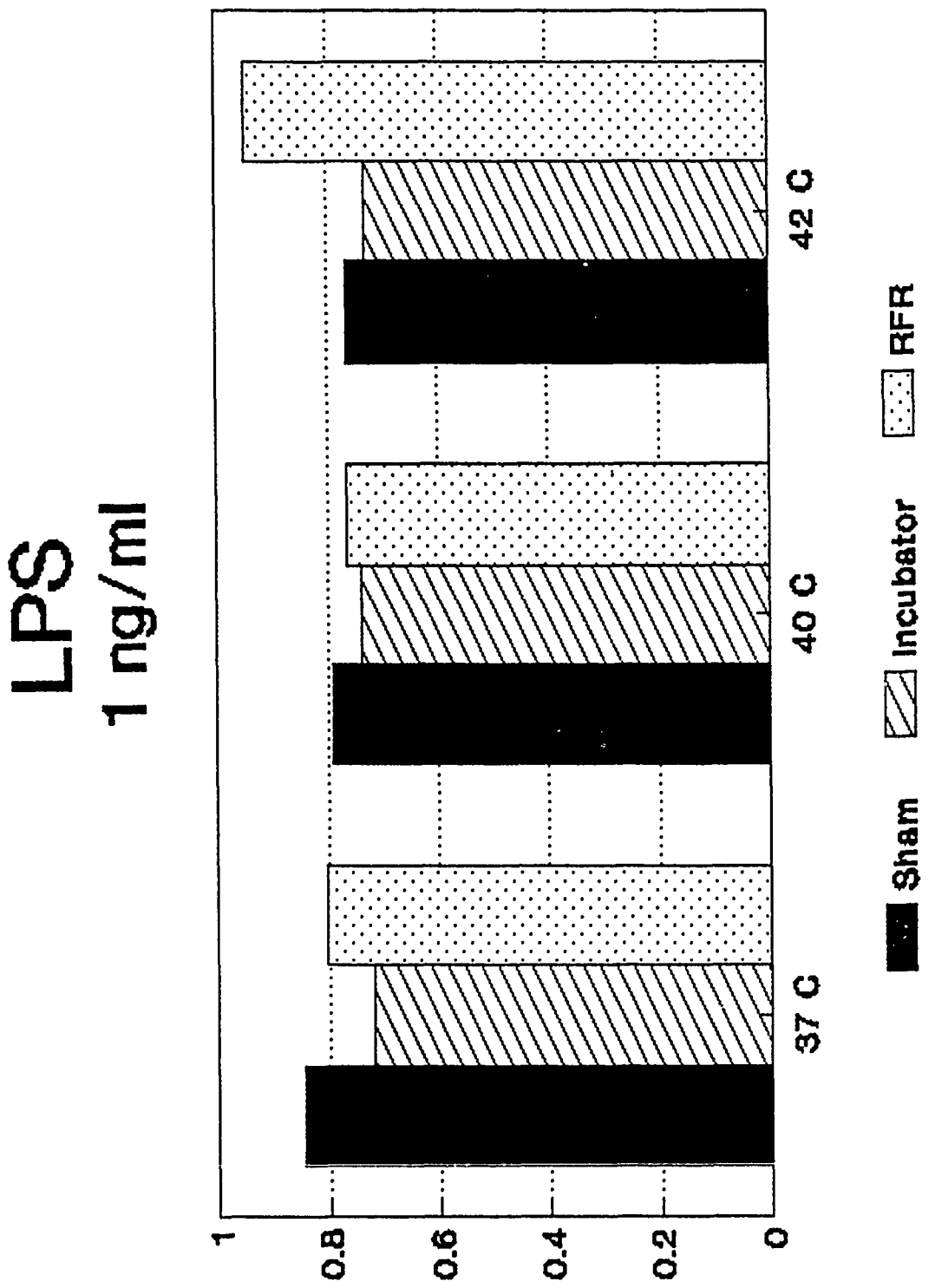
Reflectance Scanning Densitometer. To confirm that changes in intensity were due to expression and not to differences in the amounts of RNA applied to the gel, all samples were compared to the signal of the MSV probes, which from previous studies were shown not to be affected by exposure to microwaves.

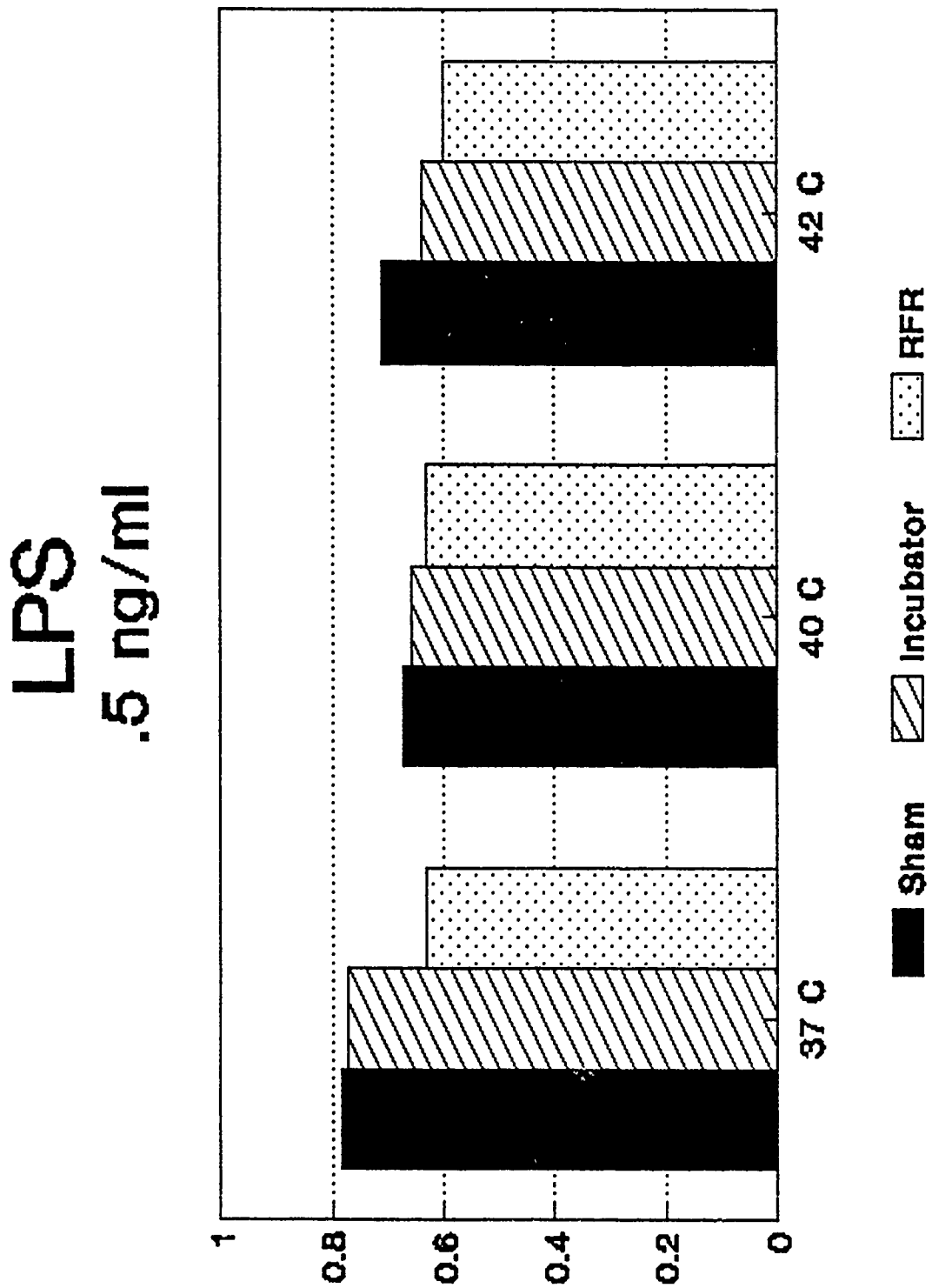
RESULTS

Hybridization with the non-radioactive biotinylated probes was not sensitive enough to detect the RNA. Filters were probed first with v-fos, which had been labeled by the oligo-nucleotide primer procedure. The results for 37, 40 and 42°C in the presence of LPS concentrations of 0.1 ng/ml, 0.5 ng/ml and 1 ng/ml are shown on the next three pages. There was no significant difference observed as a result of microwave exposure using the v-fos probe. The v-fos probe was removed from the filters as described in the previous section and filters were prehybridized and hybridized with the v-alb probe. There was no detectable hybridization of the RNA using this probe. The filters were again used in hybridization studies with the MSV probe. Results from this study are incomplete and will be continued by the lab. Other probes will also be used before any final conclusions are reported.

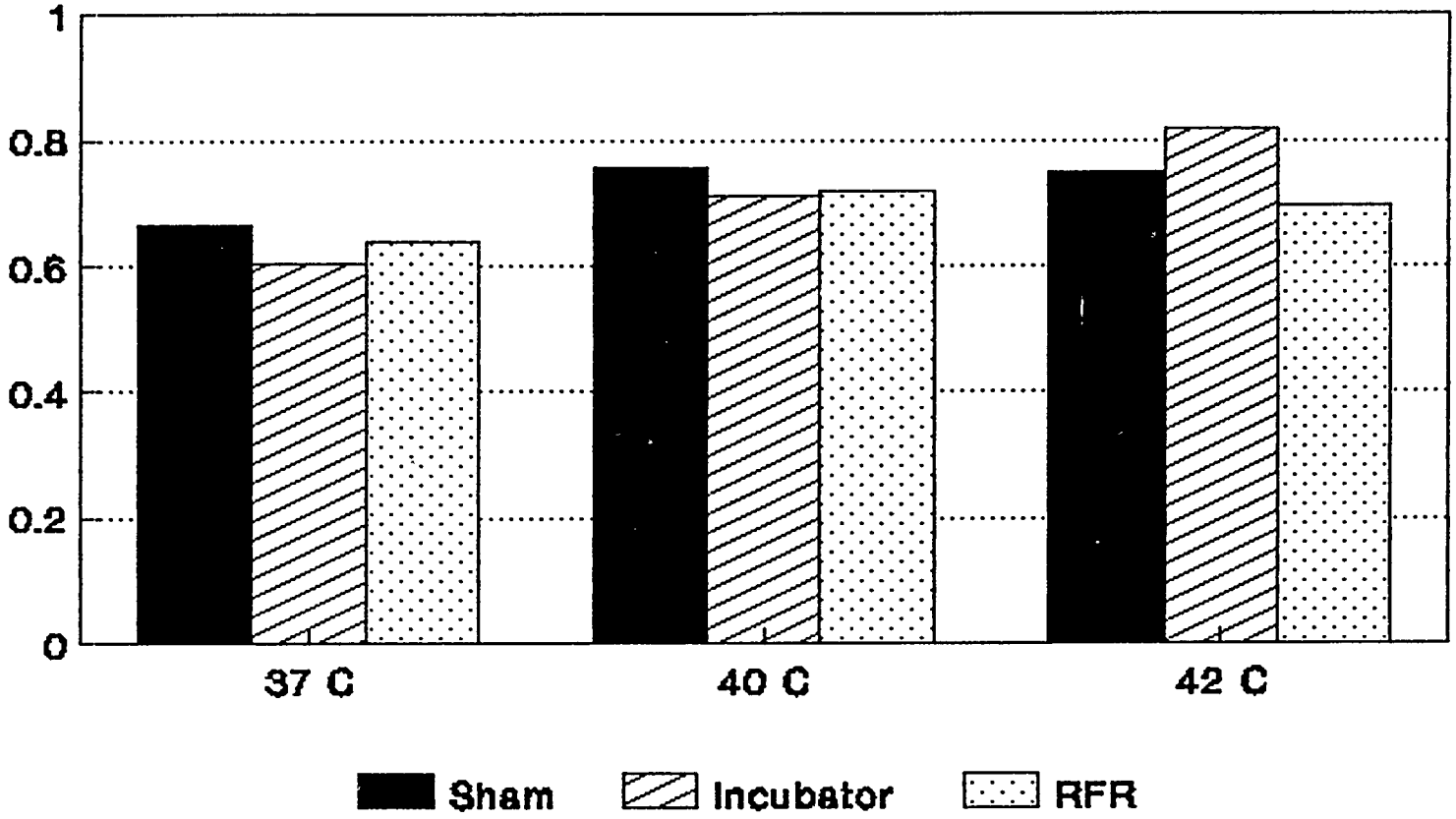
RECOMMENDATIONS

Cells in tissue culture are very sensitive to changes in environmental conditions including temperature. Care must be taken during microwave exposure to maintain cells at the proper temperature. Probes that have not been sufficiently purified can result in poor incorporation of the label. Since very small


LPS
1 ng/ml
1
0.8
0.6
0.4
0.2
0
37 C
40 C
42 C
Sham
Incubator
RFR


LPS
.5 ng/ml
1
0.8
0.6
0.4
0.2
0
37 C
40 C
42 C
Sham
Incubator
RFR

LPS
.1 ng/ml



volumes are used, care must be taken to be as accurate as possible.

It is important to determine the amount of Tumor Necrosis Factor (TNF) that is released in the supernatant by the RAW 264.7 cells and what effect the LPS concentrations will have on the TNF. It will also be interesting to determine what other factors or proteins may be found in the supernatant of the RAW 264.7 cells. Since no hybridization was detected with v-abl probe, it will be essential to do DNA/DNA hybridizations studies to determine if the Abelson murine leukemia virus is integrated into the genome. Some of these studies will hopefully be continued at Tennessee State University.

ACKNOWLEDGMENTS

I gratefully acknowledge the Air Force Systems Command and the Air Force Office of Scientific Research for the sponsorship of this research; and Universal Energy Systems and Dr. Russell Burton for administration of the program.

A special thanks is extended to Dr. Johnathan Kiel and Dr. Jill Parker for allowing me the opportunity to work with them, also for directing the project and providing encouragement and an enjoyable working environment. The technical help of Tsgt. David Simmons and Ssgt. Johns Alls was greatly appreciated.

Thanks to all of the staff of RZP who contributed to a very productive and rewarding summer.

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FINAL REPORT

The Effects of Three Reputed Carboxyesterase Inhibitors Upon Rat Serum Esterase Activity Using Paranitrophenyl and Naphthyl Esters as Substrates

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Date Prepared	September 25, 1989
Contract No:	F49620-88-C-0053

THE EFFECTS OF THREE REPUTED CARBOXYESTERASE INHIBITORS UPON RAT SERUM ESTERASE ACTIVITY USING PARANITROPHENYL AND NAPHTHYL ESTERS AS SUBSTRATES

by

James P. Chambers, Ph.D.

ABSTRACT

Using rat serum as source of esterase activity, a quick and efficient method of assay was developed using two paranitrophenyl esters taking advantage of the water solubility of the paranitrophenoxide anion. Esterase activity was terminated by addition of chloroform and released paranitrophenol converted to the 400 nm absorbing paranitrophenoxide anion by addition of 0.2 M phosphate buffer, pH 9.0. Paranitrophenoxide is quantitatively partitioned into the aqueous phase while leaving unhydrolyzed substrate in the organic (chloroform) phase. These conditions avoid nonenzymatic base catalyzed, hydrolysis of chloroform soluble, residual substrate. Using the above assay and Ecobichon's method for assessment of hydrolysis of naphthylacetate [12], the effects of three reputed carboxylesterase inhibitors (2-o-cresyl-4H-1:2:3-bensodioxaphosphorin-2-oxide, CBDP; bis-p-nitrophenyl-phosphate, BNPP and tetraisopropyl pyrophosphoramine, Iso-OMPA) were determined. Data presented here indicate the presence of an esterase activity in rat serum that when assayed with naphthylacetate is extremely sensitive to the inhibitor CBDP.

I. INTRODUCTION

Soman (pinacolyl methylphosphonofluoridate) is an extremely toxic organophosphorous poison. There is little doubt that the primary toxic manifestations and lethality of soman are due to inhibition of the enzyme acetylcholinesterase (EC 3.1.1.7) which inactivates the neurotransmitter acetylcholine. However, binding of soman to other tissue sites such as pseudocholinesterase (EC 3.1.1.8), aliesterase (non-specific carboxylesterase; EC 3.1.1.1) [1], chymotrypsin (EC 3.4.4.5)[2], trypsin (EC 3.4.4.4)[3] and probably a variety of other serine-containing hydrolase enzymes reduces the concentration of free soman in vivo. The USAF is very interested in the binding of soman at other sites which, in the short term, are not life threatening but may serve as a means of detoxification and/or "protection" in vivo against low dosages of this nerve agent.

It has been demonstrated that aliesterase is an important detoxification route for organophosphates in vivo [4]. Recovery of plasma aliesterase activity was suggested as the major factor in the tolerance of soman [5]. "The ethoxyethyl esters of both malathion and malaxon have been shown to be hydrolyzed by carboxylesterase enzymes" [6]; however, it is doubtful that carboxylesterase hydrolyses soman. If soman binding sites on aliesterase were already occupied, the toxicity of soman was potentiated.

CBDP(2-/O-cresyl/4H:3:2-benzodioxaphosphorin-2-oxide), a metabolite of tri-o-cresylphosphate [7] which is an irreversible inhibitor of aliesterase [8], potentiates the toxicity of soman almost 20 fold in rats [9] and 15 fold in mice [10]. Similarly, tri-o-cresylphosphate (TOCP) or CBDP potentiates the toxicity of sarin (isopropyl methylphosphonofluoridate) [9], tabun (ethyl N-dimethylphosphoramidocyanidate) [9], malathion

[7] and to a minor degree VX (O-ethyl S-[2-(diisopropyylamino)ethyl]methylphosphonothioate) [9].

Although our research interests are primarily enzymological in origin, my laboratory at The University of Texas at San Antonio has been involved in development of a receptor based biosensor using the [Ca^{2+} + Mg^{2+}]-dependent ATPase as its biosensing element for the US Army Chemical Research Development and Engineering Command (CRDEC). The work described here is a natural extension of our biosensor program due to the potential usefulness of these "protective" esterase(s) as "sensing" elements.

II. OBJECTIVES OF THE RESEARCH EFFORT

The paranitrophenyl and naphthyl esters are widely used esterase substrates. To date, no detailed analyses of effects of the reputed esterase inhibitors 2-(o-Cresyl)-4H-1:3:2-bensodioxaphosphorin-2-oxide (CBDP), bis-p-nitrophenyl-phosphate (BNPP) and tetraisopropyl pyrophosphoramine (Iso-OMPA) with regard to substrate specificity have been conducted. Inhibition with regard to substrate specificity is an important issue because an inhibitor may have a potent effect upon a particular esterase component but due to the substrate used to monitor esterase activity, may go undetected.

The goals of this study were to 1) develop a method of assay reflecting maximum hydrolysis of 2 paranitrophenyl esters, 2) determine the ex vivo effects of three reputed aliesterase inhibitors upon hydrolysis by rat serum of esters of paranitrophenol and naphthol, and 3) initiate in vivo work involving administration of these inhibitors to animals in order to correlate ex vivo observations.

III. EXPERIMENTAL APPROACH

a. Materials-Paranitrophenylacetate, paranitrophenylbutyrate, paranitrophenol, ^{14}C-paranitrophenol, sodium chloride, BNPP, Iso-OMPA and sodium phosphate were obtained from Sigma Chemical Company, St. Louis, Missouri. CBDP was obtained from the US Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, Aberdeen, Maryland. Chloroform was obtained from Fisher Chemical Company, Houston, Texas. Male, Sprague Dawley rats were purchased from Charles River Laboratories, Wilmington, Massachusettes. Liquiscint scintillation fluid was purchased from National Diagnostics, Manville, New Jersey.

b. Esterase Assays-Esters of paranitrophenol are widely used chromogenic substrates due to their being relatively stable and great sensitivity in detecting esterase activity. However, because paranitrophenol has a pKa value of 7.2, the protonated form predominates at neutrality. A potential problem that arises in quantitating degree of hydrolysis of these esters is that absorbance maxima for the yellow, chromophoric product, paranitrophenoxide and its protonated, colorless form, paranitrophenol, are significantly different [11]. Any attempt to convert to the paranitropehnoxide anion by reducing hydrogen ion concentration without first removing unhydrolyzed substrate may result in 1) significant enzymatic hydrolysis if the pH optimum of the esterase of interest is broad and/or 2) nonenzymatic, alkaline hydrolysis of unhydrolyzed substrate. In either case, the accuracy and sensitivity of the assay is compromised. Thus, a more sensitive method of assay using paranitrophenyl esters as substrate had to be developed.

Assays contained in a final volume of 100 ul, 20 ul 0.1M phosphate buffer, pH 7.0, 45 ul distilled water, 10 ul substrate (25 mM) dissolved in acetone and 10 ul of

serum. Reaction mixtures were kept in an ice slurry during addition of components. Prior to reaction initiation by addition of substrate, reaction mixtures were incubated at 35°C for 30 seconds. Reactions were terminated after 1 minute by addition of 1.0 ml cold, chloroform and shaken vigorously. Unless indicated otherwise, 1.5 ml 0.2 M phosphate buffer, pH 9.0 was added following extraction to facilitate partitioning of paranitrophenoxide anions. Reaction mixtures were vigorously shakened and centrifuged at 2000 rpm for 5 minutes at 5°C. A 1.0 ml aliquot of the upper, aqueous phase was removed and partitioned paranitrophenoxide anion quantitated spectrophotometrically at 400 nm. Paranitrophenol served as standard and was treated in identical fashion as experimentals. Blanks consisted of incubation mixtures to which serum was added after termination of the reaction with chloroform. Each experimental and corresponding blank was carried out in triplicate.

Hydrolysis of paranitrophenylacetate and paranitrophenylbutyrate was carried out at optimal hydrogen ion concentration (8.0) and determined by the method described above. Assays using naphthylacetate as substrate were carried out at pH 7.4 according to the method of Ecobichon [12].

c. Inhibition Studies-The effects of inhibitors were determined by mixing respective inhibitors and substrate together in acetone (2.5 %, V/V final reaction volume) at the indicated reaction molarities.

d. Preparation of Serum-Rats were decapitated and trunk blood collected. Blood was allowed to clot on ice for 30 minutes and serum carefully removed by aspiration.

IV. RESULTS

a. Development of Assay-As shown in Figure 1, increasing the ratio of conjugate base (paranitrophenoxide) to acid (paranitrophenol) increases significantly, absorbance at 400 nm. When the ratio of paranitrophenoxide anion to paranitrophenol ratio is 1.0, the midpoint of the curve should correspond approximately to an optical density of 0.5. We observed an optical density of approximately 0.35. In our calculations, we have used the pKa value of 7.2, but pKa values vary significantly with ionic strength. However, it is apparent that conversion of released paranitrophenol to paranitrophenoxide gives rise to a much more sensitive assay. Partitioning at pH 9.0 not only favors formation of conjugate base (paranitrophenoxide) greater than 60 to 1 but equally important, results in no more than 3% nonenzymatic hydrolysis. Extraction of paranitrophenol and paranitrophenoxide into the organic and aqueous phase is quantitative as evidenced by greater than 95% ecovery of carbon-14 labelled paranitrophenol under acid (pH 5.0) and alkaline (pH 9.0) partitioning conditions (Table I), respectively.

Some investigators choose to use the Molar Extinction Coefficient to quantitate released paranitrophenol. However, most physiologic assays are conducted between pH 6.5-7.5 and unless carried out under identical conditions used to derive Molar Extinction coefficients, considerable error arises in the determination of extent of hydrolysis. In this pH range, a significant quantity of released paranitrophenol is protonated resulting in reduced sensitivity at 400 nm (Table II).

Quenching the reaction with chloroform achieved two goals: 1) esterase activity ceases to occur and 2) unhydrolyzed substrate is extracted into the organic phase while released paranitrophenol is converted to the water soluble, paranitrophenoxide

anion following aqueous, alkaline partitioning. Thus, this method of extraction allowed quantitation of paranitrophenoxide either by direct comparison of identically treated paranitrophenol standard and/or utilization of appropriate Molar Extinction coefficients independent of assay pH.

b. Effects of Inhibitors-Shown in Figures 2 and 3 are effects of CBDP, BNPP and Iso-OMPA on the hydrolysis of acetate and butyrate esters of paranitrophenol, respectively. As indicated in Figure 2, addition of BNPP and Iso-OMPA to reaction mixtures resulted in a small decrease (20-25 %) in esterase activity using the substrate paranitrophenylacetate. Addition of CBDP to reaction mixtures resulted in approximately 35% reduction of esterase activity. We were interested in determining the lowest concentration of inhibitor required for maximum inhibition. Shown in Figure 2B, is titration of the reaction mixture with very low concentrations (0.25-1.25 uM) of CBDP indicating that maximum inhibition (approximately 30%) was achieved at 0.50 uM inhibitor. In contrast to CBDP, much higher concentrations (mM) of BNPP and Iso-OMPA were required to give similar levels of inhibition (Figure 2A). Shown in Figure 3 is an identical inhibition pattern using paranitrophenylbutyrate as substrate. In contrast to data presented in Figure 2, 50-60 % inhibition was observed in the presence of 0.50-0.75 micromolar CBDP (Figure 3B). As previously described for paranitrophenylacetate, inhibition of hydrolysis of paranitrophenylbutyrate by BNPP and Iso-OMPA, required much higher concentrations (mM).

Shown in Figure 4 are effects of inhibitors upon hydrolysis of the acetate ester of naphthol. The effects of CBDP are dramatic at very low concentrations (100 %

inhibition at 0.7 micromolar). In contrast to paranitrophenyl esters, hydrolysis of naphthylacetate was not inhibited to any appreciable extent by high concentrations (mM) of BNPP and Iso-OMPA. These data indicate that the esterase inhibitor CBDP, specifically inhibits at very low concentration, hydrolysis of naphthylacetate. Thus, naphthylacetate appears to be the preferred substrate of a serum esterase activity that is extremely sensitive to the inhibitor CBDP.

V. RECOMMENDATIONS:

a. Application of Research Findings-These data support the contention that esterase activity of rat serum is a heterogenous mixture of activities and that if specific parameters such as substrate and inhibitor specificity are known, they can be used effectively to biochemically define an animal model designed to ascertain effects of low dose neurotoxic agents. Not presented in this study are supportive data derived using serum from animals previously treated with these inhibitors. Injection of CBDP (1mg/kg) resulted in reducing naphthylacetate hydrolysis greater than 90 % with no serious effect upon brain acetylcholinesterase activity (data not shown, manuscript in preparation). Furthermore, exposure of animals previously treated with CBDP (1 mg/kg) to Soman resulted in potentiation of this nerve agent at low dosage.

b. Suggestions for Follow-on Research-A complete assessment of the possible protective effects of one substrate (e.g. paranitrophenylbutyrate) upon that of naphthylacetate hydrolysis should be carried out. Of great importance is the tissue source of these various esterases and determination of their respective kinetic properties using substrates (esters of naphthol) of varying chain length and degree of saturation. Finally, a complete

in vivo testing of effects of pretreatment of animals with CBDP, BNPP and Iso-OMPA upon potentiation of agents such as Soman should be conducted.

Although, the rat is unusual in that serum contains high levels of a "protective" esterase activity that hydrolyzes naphthylacetate and is very sensitive to CBDP, a complete survey of substrates and effects of these inhibitors using serum from primates should be conducted. It is quite possible that natural, protective "scavenger" molecules reside in the plasma compartment of primates. If so, could their synthesis and/or effectivness as protective agents against threat agents such as soman not be increased by diet? drug administration?

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Lt. Col. Stanley Hartgraves and Drs. Russell Burton and Michael Murphy for the opportunity to participate in the USAF Summer Faculty Research Program. Additionally, special thanks to Miss Stephanie Miller and Mr. Robert Noonan for their diligence and hard work in the laboratory.

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FIGURE LEGENDS

Figure 1: A Comparison of Various Conjugate Acid-Base Ratios on Absorbance at 400 nm. Assay mixtures contained 0.2 M buffer at indicated hydrogen ion concentrations and 25 nmoles of paranitrophenol (closed circles) or paranitrophenylacetate (open circles) in a final volume of 100 ul. Partitioning following chloroform extraction was achieved by adding 1.5 ml of 0.2 M buffers (pH 5.2, 6.2, 7.2, 8.2, 9.2 and 10.2). One milliliter aliquots were removed and paranitrophenoside monitored spectrophotometrically at 400 nm. Blanks consisted of assay mixtures containing no paranitrophenol or paranitrophenyl-acetate.

Figure 2A and B: The Effects of Reputed Carboxylesterase Inhibitors Upon Hydrolysis of Paranitrophenylacetate by Rat Serum. Assays were carried out as previously described under "Esterase Assay". The effects of CBDP (closed circles), BNPP (open circles) and Iso-OMPA (closed triangles) were determined at the final concentrations indicated.

Figure 3A and B: The Effects of Reputed Carboxylesterase Inhibitors Upon Hydrolysis of Paranitrophenylbutyrate by Rat Serum. Assays were carried out as previously described under "Esterase Assay". The effects of CBDP (closed circles), BNPP (open circles) and Iso-OMPA (closed triangles) were determined at the final concentrations indicated.

Figure 4A and B: The Effects of Reputed Carboxylesterase Inhibitors Upon Hydrolysis of Naphthylacetate by Rat Serum. Assays were carried out according to the procedure of Ecobichon [12]. The effects of CBDP (closed circles), BNPP (open circles) and Iso-OMPA (closed triangles) were determined at the final concentrations indicated.

Table I

Efficiency of Chloroform-Buffer Partitioning

	Organic	Aqueous
pH 5.0	960,650(96)	23,300 (2.3)
pH 9.0	22,300 (2.2)	962,330 (96)

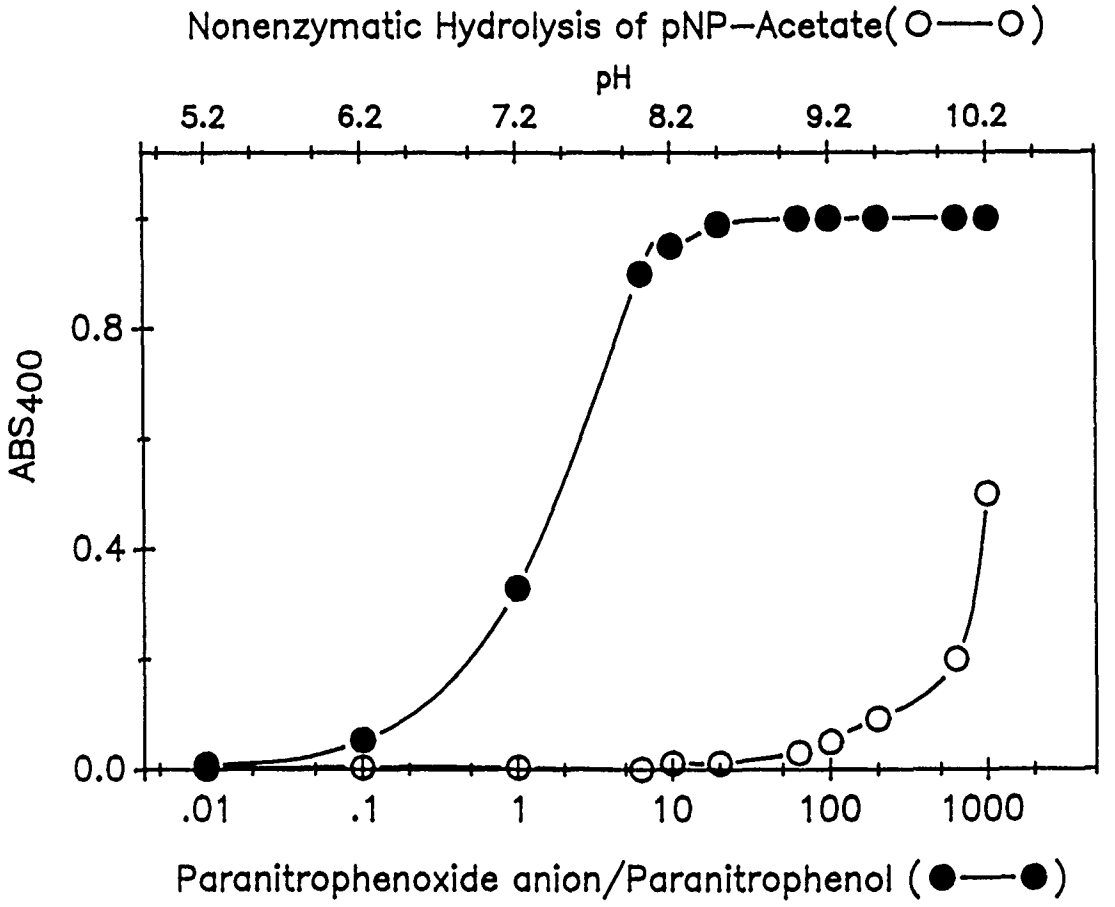
Legend: Reaction mixtures containing 1.0×10^6 cpm carbon-14 labelled paranitrophenol were extracted with chloroform and partitioned with pH 5.0 and 9.0 phosphate buffer as previously described. Aliquots (100 ul) of both organic and aqueous phases were removed and radioactivity determined. Values shown in parentheses represent the percentage of radioactive paranitrophenol recovered in the organic and aqueous phase of each assay mixture.

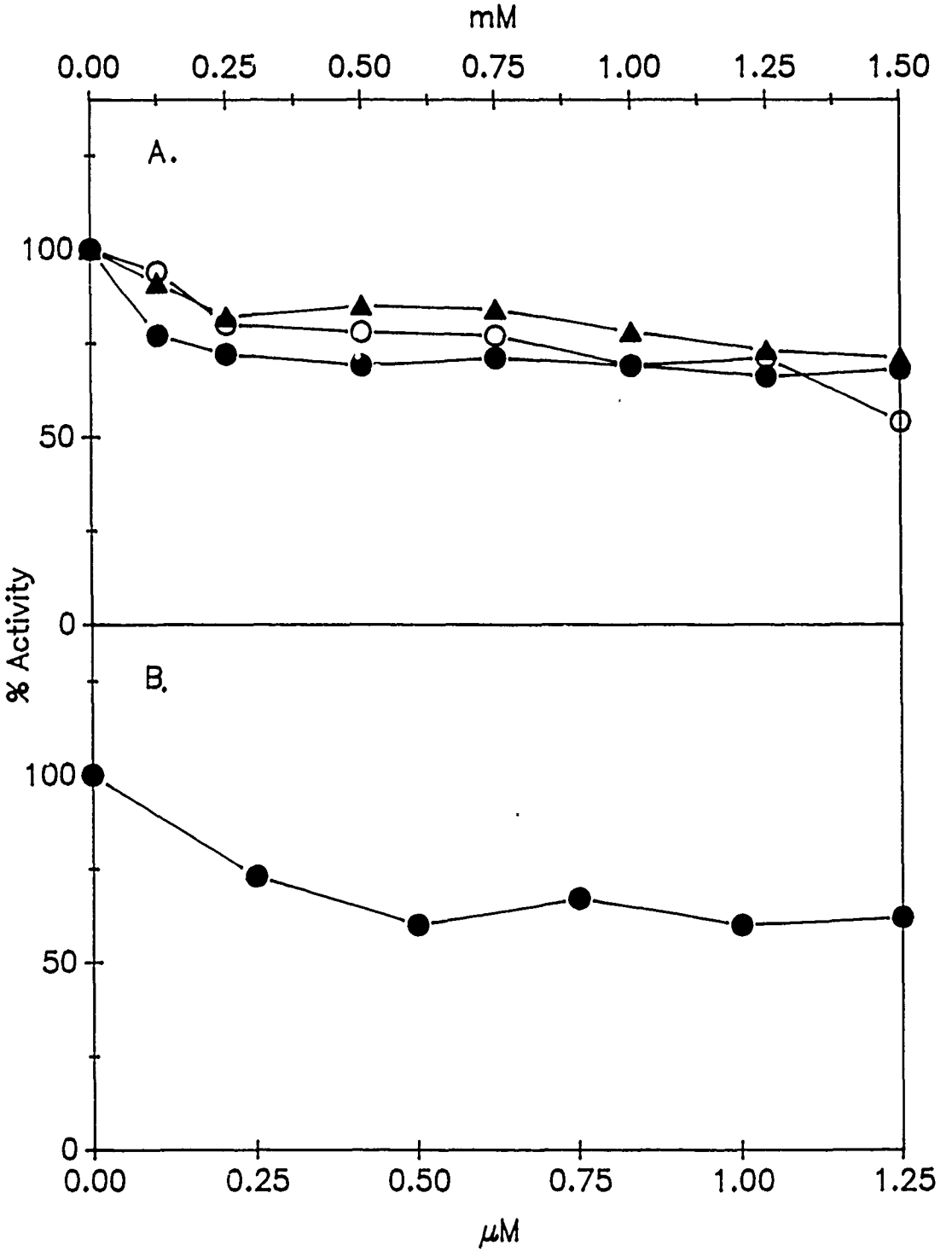
Table II

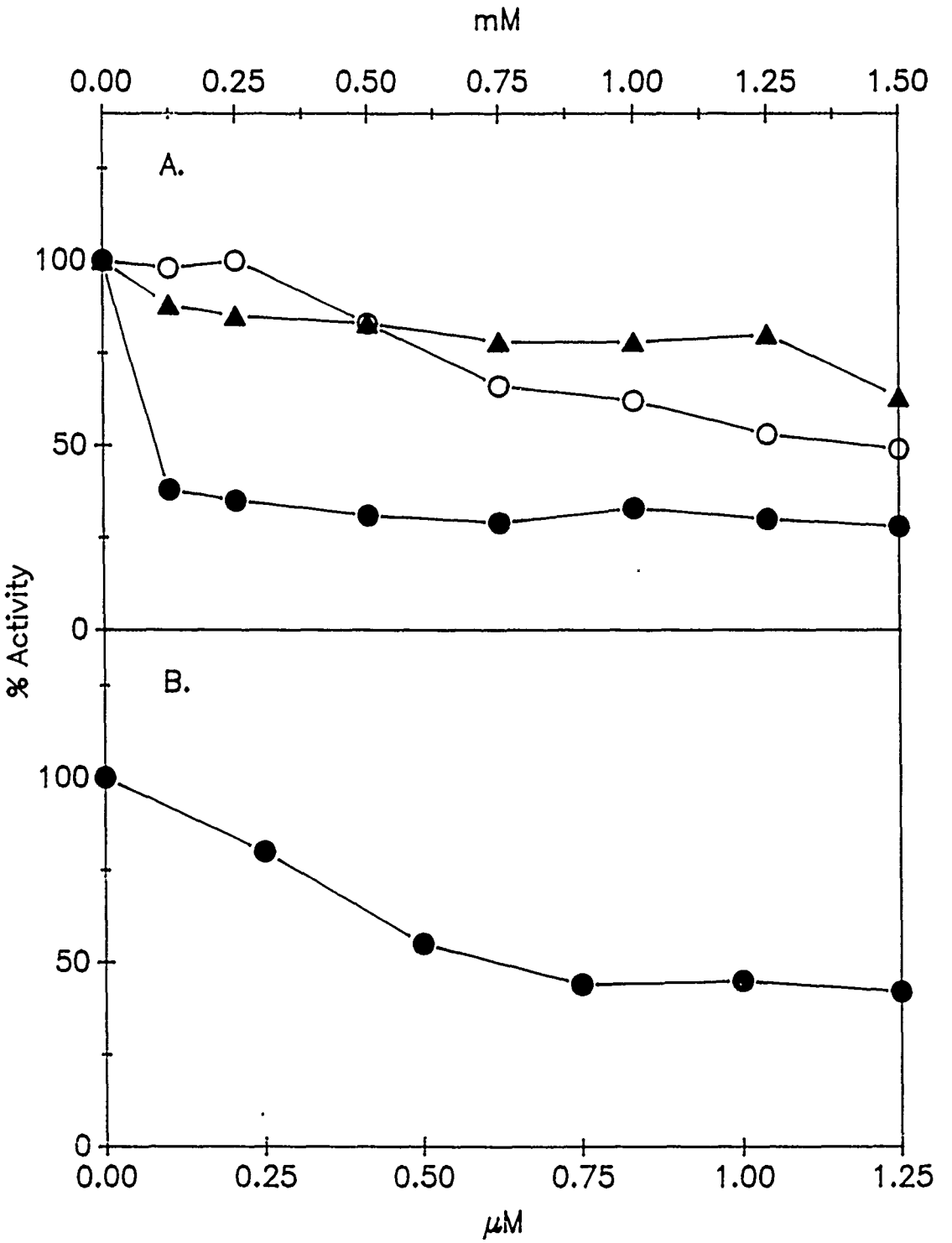
Comparison of Absorbance, Conjugate Acid-Base Ratios and Serum Esterase Activity Obtained by Partitioning at Various Hydrogen Ion Concentrations

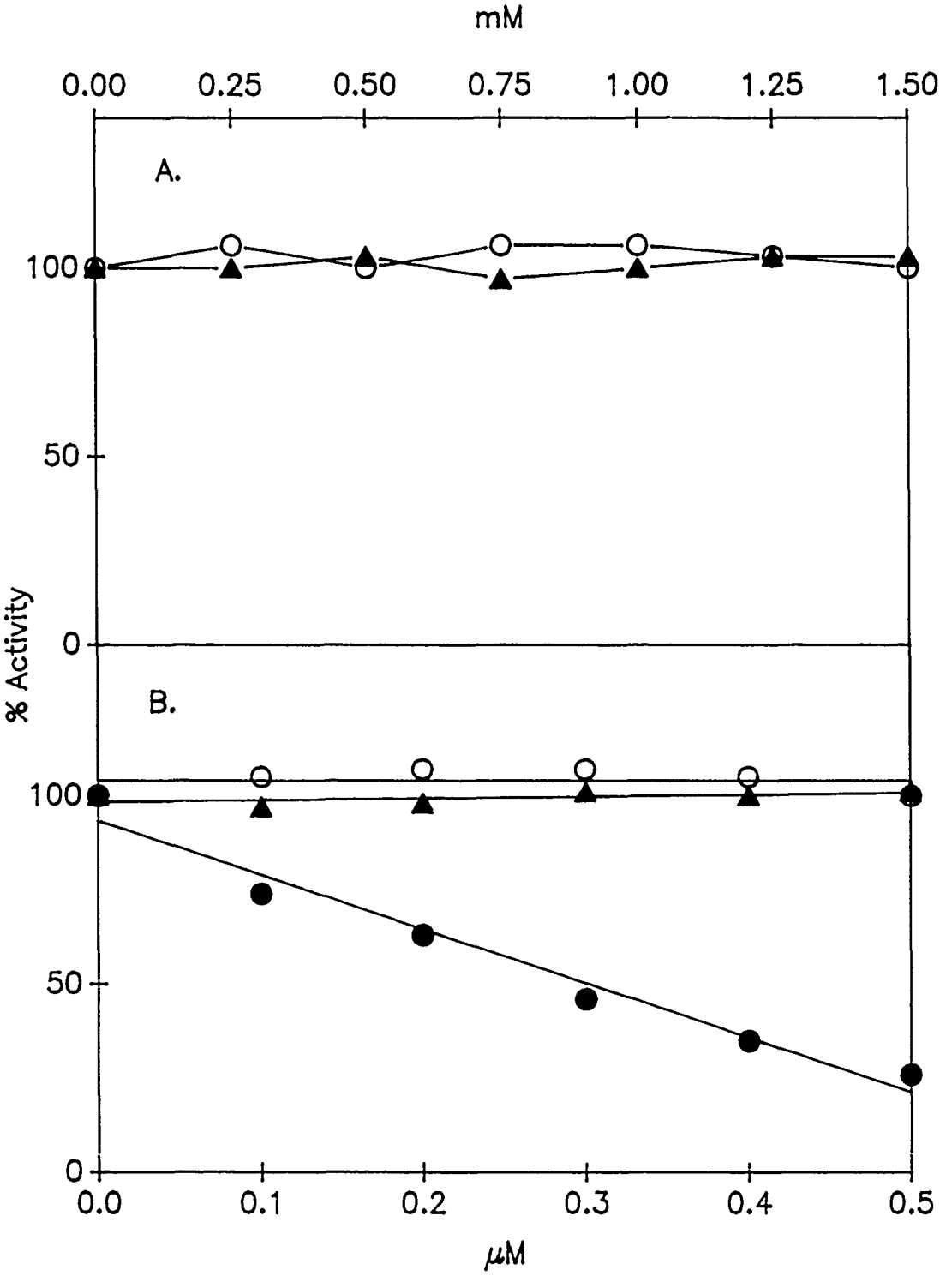
Assay	A_{400}	A^-/HA	Sp. Activity
1	0.047	0.63/1	1.4
2	0.149	6.3/1	4.5
3	0.198	63.0/1	6.0
4	0.198	630/1	6.0

Legend: Enzyme assays were carried out at pH 7.0 and quenched by addition of chloroform. Partitioning was accomplished by addition of 0.2 M buffer adjusted to pH 7.0 (Assay 1), pH 8.0 (Assay 2), pH 9.0 (Assay 3) and pH 10 (Assay 4). Specific Activity (nmoles paranitrophenol released/min/ml serum) was obtained by direct comparison to a standard paranitrophenol curve, chloroform extracted and partitioned with 0.2M phosphate buffer, pH 9.0.


Nonenzymatic Hydrolysis of pNP–Acetate(O——O)
pH
5.2
6.2
7.2
8.2
9.2
10.2
ABS400
0.8
0.4
0.0
.01
.1
1
10
100
1000
Paranitrophenoxide anion/Paranitrophenol (●——●)


mM
0.00
0.25
0.50
0.75
1.00
1.25
1.50
A.
100
50
0
% Activity
B.
100
50
0
0.00
0.25
0.50
0.75
1.00
1.25
μM


mM
0.00
0.25
0.50
0.75
1.00
1.25
1.50
A.
100
50
0
% Activity
B.
100
50
0
0.00
0.25
0.50
0.75
1.00
1.25
μM


mM
0.00
0.25
0.50
0.75
1.00
1.25
1.50
A.
100
50
0
% Activity
B.
100
50
0
0.0
0.1
0.2
0.3
0.4
0.5
μM

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FINAL REPORT

Anti-G Suit Inflation Influence on Lower Extremity Muscle Performance during Sustained $+G_z$ Acceleration

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Anti-G Suit Inflation Influence on Lower Extremity Muscle Performance during Sustained $+G_z$ Acceleration

by

Mark W. Cornwall

ABSTRACT

The purpose of this project was to investigate the influence of the Anti-G suit on muscle performance during exposure to sustained $+G_z$ acceleration force. The amplitude and frequency of the myoelectric signals from the erector spinae, abdominal, hamstring, quadricep and gastrocnemius/soleus muscles were recorded from 3 subjects during sustained gravitational stress of 6 $+G_z$. Each subject was tested under two separate conditions: 1) with the Anti-G suit and 2) without the Anti-G suit. Because of the low sample size, the results are certainly not conclusive. Within that limitation, they do seem to indicate that 1) subjects contract their muscles less while wearing the G-suit compared to when they are not wearing the G-suit, 2) individuals with longer duration times at sustained $+G_z$ are maintain a higher level of muscle activity during the exposure compared to those with low duration times and 3) only the abdominal muscle showed signs of fatiguing as a result of the $+G_z$ stress.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsoring this research effort.

I would also like to express my appreciation to Dr. Larry Krock for his willingness and enthusiasm in accepting me for this summer research effort. He was always willing to drop what he was doing to help me solve the many problems that I encountered as well as those that I created. I have gained a great deal from my time spent at USAFSAM/VNB and he was no little part.

I. INTRODUCTION:

Pilots of high performance aircraft are often required to perform in a high-G environment. It is imperative in these situations that the pilot maintain complete control of the aircraft by being able to tolerate the resulting physical and physiological demands. Among the current methods employed to assist pilots sustain the high $+G_z$ acceleration forces are the Anti-G suit and the Anti-G Straining Maneuver (AGSM). The Anti-G suit is designed to provide pneumatic pressure over the abdomen, thighs and calves during positive acceleration stress. The AGSM, on the other hand, requires the individual to perform "near-maximal" activation of numerous muscle groups in response to the acceleration stress. Both methods have been found to contribute significantly to the tolerance of sustained $+G_z$ stress [1,2].

Although both methods have been shown to be effective, the AGSM is frequently accompanied by contractile inefficiency and rapid muscular fatigue. This is particularly true in the untrained individual. Previous research into ways to improve a person's tolerance to $+G_z$ stress has, in part, dealt with the AGSM. The literature has shown that a generalized resistance (strength) training program can improve a person's tolerance to acceleration stress [3,4]. In contrast, however, selective training programs have not been successful in significantly improving G-tolerance [5,6,7]. Current research is now being focused upon

the formulation of individually tailored resistive training programs which will be more efficient and effective in their strain mechanics.

My research interests and expertise has been in the area of motor skill and strength acquisition and particularly the use of electromyography (EMG). In addition, I am a licensed Physical Therapist and have worked extensively in the area of muscle strength assessment and exercise. As such, my background fit well into the future goals and direction of $+G_z$ stress tolerance research.

II. OBJECTIVES OF THE RESEARCH EFFORT:

a. Central to the development of these specific training programs is the question of the Anti-G suit's influence on muscle contraction during the AGSM. It is not yet known whether the Anti-G suit enhances or hinders the performance of the straining maneuver. The first objective of my summer research was, therefore, to establish whether the Anti-G suit influenced muscle contraction while performing the AGSM.

b. Inherent in the development of an individual exercise program for $+G_z$ tolerance is the understanding of which muscles are most active during the AGSM and to what extent are they active. This, therefore became a secondary goal of the research project.

c. Finally, it was of interest to understand the level of muscular endurance required by individuals during

$+G_z$ acceleration. This then became our final question. What is the magnitude and rate at which muscles fatigue during $+G_z$ acceleration?

III. G-SUIT INFLUENCE

a. **Approach.**

Three normal, health, subjects were tested under two different conditions while being exposed to high, sustained accelerative ($6+G_z$) force. The conditions were: 1) while wearing the standard (CSU-13) Anti-G suit and 2) while not wearing the Anti-G suit. Subjects performed a maximal AGSM during each exposure to prevent loss of consciousness. The duration of each acceleration exposure was to the tolerance of the individual (ie. 100% peripheral light loss and 50% central light loss). Surface electrode EMG was recorded from the erector spinae, abdominal, hamstring, quadriceps and gastrocnemius/soleus muscle groups of the dominant limb of each subject during all rides. The normalized amplitude of electrical activity in each muscle was then compared to determine if a statistically significant difference existed between the two conditions.

b. **Results.**

Our results indicates that each of the five muscles, except the erector spinae showed more activity during the no G-suit condition. Assuming that EMG

signal amplitude is representative of muscle tension, this would indicate that the G-suit does, in fact, hinder activation of muscles in the lower extremity. Although this is a possibility, it is also likely that the lower activity during the G-suit condition is more of a reflection of subjects knowing that the suit is present and will provide a certain amount of $+G_z$ protection. Knowing this, they may have subconsciously lowered the level of muscle contraction.

IV. INDIVIDUAL MUSCLE CONTRIBUTION

a. **Approach.**

The same methods as outlined above were used to collect data for this objective as well. The test of the hypothesis was made by comparison of the relative magnitude of activity between each of the five muscle groups. In this way the relative contribution of each muscle while performing the AGSM could be assessed.

b. **Results.**

The lack of sufficient numbers of subjects makes interpretation of the data with respect to this goal very difficult. Making that statement at the start, it appears that no differences could be seen with respect to which muscles are used preferentially by those classified as "good" strainers (duration times of greater than 20 seconds) and those who are "poor" strainers (duration times of less than 20 seconds).

There is, however, a definite ability for the "good" strainers to maintain the level of muscle activity throughout the $+G_z$ exposure. The "poor" strainer showed a very rapid decrease in his muscle activity during the $+G_z$ exposure.

V. $+G_z$ RELATED MUSCLE FATIGUE

a. **Approach.**

Again, the same testing protocol was used for this objective. The method of data analysis consisted of looking at the spectral density function of the EMG signal during the course of the AGSM. It has been shown that the median frequency of the EMG signal decreases with the onset of fatigue[8]. This decrease is linear for isometric contractions. The magnitude of decrease in the EMG spectral density function as well as its rate of decrease will provide an indication of the muscular endurance requirements of the AGSM.

b. **Results.**

Using the shift in the spectral density function of the EMG signal as an indicator of localized muscle fatigue, only the abdominal muscles became fatigued as a result of the exposure to $+G_z$ force. The frequency of the abdominal muscle decreased from 50.31 to 30.76 Hz in the no G-suit condition, and from 49.34 to 33.74 Hz in the G-suit condition. The erector spinae, quadriceps and gastrocnemius, on the other hand, showed

increases in their median frequency. This increase in frequency could be the result of increased frequency of motor unit firing, especially when considering that the amplitude for each of these same muscles decreased during the same time. The decrease in amplitude, without a concomitant decrease in the frequency suggests that the muscles used less motor units during the $+G_z$ exposure. This is certainly contrary to what we originally theorized. It is not clear at this point why there is this apparent decrease in motor unit recruitment, and simultaneous increase in motor unit rate coding. Further research, with a larger sample size is certainly needed to shed more light on this phenomenon.

VI. RECOMMENDATIONS:

a. **Application.**

Even though it appears from our data that the Anti G-suit is not accompanied by the same level of muscle contraction compared to the no G-suit condition, it is certainly not our recommendation that the G-suit be discarded. It is obvious from our study that subjects had significantly greater duration times when the G-suit was worn compared to when they did not have the G-suit. Because of the small sample size, any recommendation concerning the application of these results would be suspect. It does seem to indicate

that our thinking about muscle fatigue as a primary mechanism responsible for determining $+G_z$ duration times needs to be re-thought.

b. **Follow-up Research.**

Research is already planned to look at the fatigue characteristics during the Simulated Aerial Combat Maneuver (SACM). Other areas to be investigated in the future include, 1) upper extremity muscle activity during $+G_z$ exposure, 2) upper and lower extremity muscle activity during the SACM, 3) relationship between muscle strength and endurance to $+G_z$ tolerance, 4) comparison of muscle activity patterns of trained and untrained centrifuge subjects and 5) measurement of the amount of force produced by the muscles under conditions of $+G_z$.

c. **Other Suggestions.**

Because of the small sample size in the present study, it is difficult to make specific suggestions. Certainly a major suggestion would be to continue with research into this area so that our understanding of the AGSM can be improved.

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FINAL REPORT

PCR Analysis and in situ Detection of Ureaplasma urealyticum and Mycoplasma hominis.

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USAF Researcher:	Ferne K. McCleskey
Date:	11 August 1989
Contract No:	F49620-88-C-0053

PCR-Analysis and in situ Detection of Ureaplasma urealyticum and Mycoplasma hominis

by

Paul Calvo and
Vito G. Del Vecchio
and Raymond Wolfe

ABSTRACT

Three DNA probes which were specific for either U. urealyticum or M. hominis were used as models for PCR analysis. The sequence and composition of these target sequences are in the process of being determined in order to profile their restriction endonuclease sites and to synthesize oligonucleotide primers. These primers will define and limit in vitro amplification of only target sequences.

In situ DNA hybridization using biotinylated probes and an FITC-streptavidin signal was proven to be specific and offer the ultimate sensitivity for elucidating the presence of these organisms in artificially infected tissue cultures as well as clinical samples. The probe for Ureaplasma DNA was specific; however, that for Mycoplasma displayed some cross reactivity with Ureaplasma DNA. Investigation of stringency may render the Mycoplasma probe more specific.

Acknowledgements

We wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems must be mentioned for their concern and help to me in all administrative and directional aspects of this program.

Our experience was rewarding and enriching because of many different influences. Ferne K. McCleskey provided support, encouragement, and a truly enjoyable working atmosphere. The help of Cliff Miller and Martha Barrera was invaluable. Dr Jerome Schmidt's, Dr Vee Davison's and Dr Russ Burton's interest in every phase of this project truly served as a source of stimulation. The encouragement and help of Dr Louis Blouse clearly added to every aspect of this research project.

I. INTRODUCTION

Mycoplasma hominis and Ureaplasma urealyticum have been associated with infertility, pregnancy wastage, pelvic inflammatory disease, pneumonia of neonates, and non-specific urethritis. Currently, there is no satisfactory method for the direct identification of these organisms in clinical samples since culture assays involve complex media, lengthy incubations, frequent growth failure and contamination; therefore, their total involvement in the disease process has never been elucidated. With this in mind we have established DNA libraries of these organisms in E. coli. These libraries have afforded a probe collection of varying specificity and sensitivity as defined by hybridization and visualization by a color change using biotinylated probes, streptavidin-alkaline phosphate signal instead of the more expensive and hazardous radioactive technique which is used in some DNA hybridization techniques. Although, some of these probes are specific for as little as 270pg of DNA, the DNA hybridization assay is labor intensive and involves relatively long periods of time (3 days).

II. OBJECTIVES

a. Recently, a new technique called Polymerase Chain Reaction (PCR) has become available to researchers and clinicians. The application of this technique is expected to revolutionize diagnostic medicine for it offers the most sensitive, rapid, and least labor intensive means of detecting specific nucleic acid segments. Thus, we will apply this technique specifically to detect the cloned Ureaplasma and Mycoplasma sequences present in clinical samples.

The PCR technique is based upon the natural DNA replication process since the number of DNA molecules doubles after each cycle. Target DNA is first denatured, or converted from the double-to-single-strandard state, at a high temperature (95^{o}-100^{o}C). The denatured single-stranded DNA will then anneal to a complementary primer once the temperature of the reaction is lowered. Primers are single-stranded oligonucleotides which limit and define a target DNA segment of gene or organism. Primers are added in molar excess so that they may easily anneal to dissociated target single-stranded DNA. Once annealing has occurred, the enzyme DNA polymerase calatyzes the synthesis of new strands of target DNA segments. The synthesis occurs by the DNA polymerase adding nucleotides which are complimentary to the unpaired DNA strands onto the annealed primers 3'OH. After 30 cycles of denaturation, linker annealing and primer extension, a single target DNA can be amplified or duplicated up to 1,000,000 copies in the space of 3 hours. The amplified DNA can then be identified via agarose gel electrophoresis or hybridization techniques.

b. In addition to PCR analysis, it was decided to reassess the in situ detection of these two organisms. This method enables one to detect, via nucleic acid hybridization using our probe library, the presence of specific target DNA sequences. Visualization is accomplished by biotin-streptavidin/FITC complexing. The in situ system should provide a positive signal in clinical specimens suspected of harboring mycoplasma. One disadvantage of the technique it is extremely labor intensive. Since retention of host cell morphology is not a consideration in the diagnosis of mycoplasma infections, we attempted to shorten and streamline the methodology so it can easily be applied in a clinical setting.

III. DEVELOPMENT OF EXTENSION PRIMERS

PCR amplifies specific DNA sequences one million times in a few hours. The specificity of this technique resides in the structure and composition of oligonucleotide extension primers which are complementary to areas flanking the sequence of interest. Extension primers are single stranded and anneal to denatured target DNA regions in opposite and overlapping directions. DNA synthesis proceeds in the 5' to 3' direction with Taq polymerase and the addition of deoxyribonucleolide triphosphates (dNTPs).

Three cloned segments, which had determining hybridization specifics were selected to focus on portions of the mycoplasma genome. These probes were: pUP18 which had absolute specificity for Ureaplasma urealyticum and pMD7 and pMD37 which had high stringency specificity for Mycoplasma hominis. These were subcloned into M13 bacteriophage to determine the nucleic acid sequence of these clones (and thus target portions of the mycoplasma genomes) after the method of Messing, 1981.

Each insert was isolated from its pBR322 vector via hydrolysis with PstI, agarose gel electrophoresis, and electroelution. These were then ligated into the PstI-sites of M13 RF18 and 19 DNA. This insertion causes inactivation of the gene for B galactosidase. Plaques which contained specific inserts were selected by hybridization with either pUP18, pMD7, or pMD37. Orientation of the inserts was determined by isolation of the M13 bacteriophage, release of its ssDNA, and C-testing of various ssDNA to determine complementation. The ssDNA was then sequenced by the dideoxy chain termination method of Messing (1981) and Sanger (1977).

After sequencing the first halves of pMD37 and pMD7 which have a composition of 1000 and 1500 base pairs respectively, it was determined that they share the same origin of insertion into pBP322 and the 1000 bp of pMD37 is found in the first 2/3rds of pMD7.

Although we were not able to perform specific probe/PCR analyses on the mycoplasma DNA, we did determine all of the PCR standards for these organisms using extension primers for the 677bp fragments of the 16S-rDNA.

IV. IN SITU DETECTION OF MYCOPLASMA HOMINIS AND UREAPLASMA UREALYTICUM

Sample Preparation: Clinical samples obtained were broken into smaller fragments using a razor blade. The cells were then centrifuged at 400 x g for 5 minutes. The pellet formed was resuspended in trypsin and placed at 37°C for 15 minutes, centrifuged at 400 x g for 5 minutes, and then washed and resuspended in 1 X PBS. The cells were cytocentrifuged at 1,000 rpm's for 5 minutes onto poly-lysine (ONCOR# S1311) or chitosan-coated microscope slides using a Cyto-Tek centrifuge.

Tissue culture (MRC-5) cells were also used in the in situ analysis. Cultures of MRC-5 were inoculated with either a drop of control Mycoplasma hominis or Ureaplasma urealyticum. The cultures were placed at 37°C for a minimum of 2 days to facilitate absorption of mycoplasma onto the plasma membrance of the MRC-5 cells. Half of the growth medium was discarded and the MRC-5 cells were scraped off the side of the test tube using a sterile wooden applicator stick. The suspensions were then cytocentrifuged onto the treated microscope slides.

Mycoplasma smeared onto microscope slides served as the ultimate control for this experiment. A drop of either Mycoplasma hominis or Ureaplasma urealyticum culture was placed on treated slides and air dried in a laminar flow hood. All of the above stated specimens were fixed onto the slides with 3:1 ethanol/acetic acid for 15 minutes and 95% absolute ethanol for 15 minutes at room temperature. The slides were air dried and stored at +4°C.

Probes. Probe DNA molecules (pUP-18 and pMD-37), obtained from our libraries, were nick-translated with biotin-dUTP (ONCOR) according to the instructions of the supplier. They were then treated with 50 ul of 7.5 M ammonium acetate, and 300 ul of 100% ethanol and allowed to precipitate overnight at -20°C. The probes were centrifuged at 14,000 rpm's for 10 minutes, washed with ice cold 70% ethanol, centrifuged, and the precipitates were resuspended in 50 ul of sterile distilled water.

In situ hybridization. Slides were treated with 50 ul of a stock solution of Proteinase K (20 mg/ml in 10 ml of 0.5 Tris-HCl, 5 mM EDTA pH7.6) and incubated at 37°C for 30 minutes. They were then washed twice for 3 minutes in 1X PBS, post-fixed in 3:1 ethanol/acetic acid for 10 minutes and 95% ethanol for 5 minutes at room temperature and then air dried. Twenty ul of the probe hybridization solution was placed on each slide, covered with a coverslip, and sealed with rubber cement. Denatured probe solution was prepared by suspending 50 ul of probe DNA in 0.5 ml of hybridization solution and boiling for 10 minutes. The slides were then incubated in 42°C water bath overnight in a moist chamber saturated with hybridization solution.

Cytochemical Detection. The coverslips were removed and the slides were washed 3X 5 minutes in 2X SSC, washed in Reagent 1 (100mM Na_2HPO_4) for 5 minutes and in Reagent 2 (Reagent 1 + 10 mg/ml Bovine Serum Albumin) for 10 minutes. The streptavidin-FITC was diluted 1:1250 with Reagent 1 and 70 ul of this dilution was applied to each slide. The slides were covered with a coverslip, incubated for 1 hour at 37°C in a moist chamber, washed 3X for 5 minutes in 2X SSC at room temperatures, and then visualized under UV microscopy.

pUP18 probe was confirmed to be specific for Ureaplasma urealyticum and not for Mycoplasma hominis under the streamlined procedure of fixation and hybridization described. pMD37 was not specific for it was to react with Ureaplasma DNA although it was expected that it would have absolute specificity for Mycoplasma hominis.

The streamlined procedure did result in less morphological detail of the host cells but no decrease in signal was seen for the target organisms. Thus, a more realistic procedure is evolving for clinical application.

The use of the Cyto-Tek centrifuge greatly improved the deposition of host cells. Chitosan treatment of microscope slides appeared to retain the cells sufficiently so that subsequent procedure would not result in loss of cells from the slide surface.

V. RECOMMENDATIONS

It may be necessary to search for different oligonucleotide primers which vary in their specificity for Ureaplasma and Mycoplasma species. This will only be needed if the PCR-products do not contain sufficient restriction endonuclease sites for subsequent electrophoresis analysis and/or Southern blotting. Once the sequence of the probe DNA molecules is known, restriction endonuclease site analysis can be carried out on the

GENIUS DNA analysis computer software system. It may also be necessary to use several primers on PCR analysis, form larger PCR-products, and to clone larger mycoplasma segments into vectors which can accommodate longer genomic segments.

Specificity of the PCR and *in situ* system should be determined by testing other mycoplasma species and other organisms which may be present in clinical samples. Different biotypes and serotypes should also be characterized by this technique.

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FINAL REPORT

Ribosomal DNA Fingerprinting of Microorganisms of Epidemiological Significance

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Ribosomal DNA Fingerprinting of Microorganisms of Epidemiological Significance

by

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ABSTRACT

Complementary DNA which was reverse transcribed from E. coli rRNA was used as a probe for the fingerprinting of E. coli, Citrobacter freundii, Mycoplasma hominis, and Ureaplasma urealyticum. Southern blots of EcoRI-cut genomic DNA from these organisms demonstrated that the rDNA probe was of sufficient sensitivity to detect genomic differences in these microorganisms.

PCR analysis was also initiated using extension primers which focused in upon a 677 bp segment of the rDNA which encodes for the 16S-rRNA. These primers were demonstrated to initiate DNA amplification in E. coli as well as the other microorganisms listed. The PCR-produced DNA was electrophoresed and also hydrolyzed with selected restriction endonucleases.

I. INTRODUCTION

Current procedures for determining the passage or origins of epidemic bacteria in a community of patients are often lacking in sensitivity or cannot be applied to certain species of organisms. These methods include serotyping, phage typing, and plasmid profiling. Recently investigators (Owens, 1988; Pitcher, 1987; and Wilkenson, 1986) have demonstrated that restriction fragment length polymorphisms (RFLP) of rDNA can provide a sensitive means of distinguishing between microorganisms derived from clinical isolates. rDNA is the segment of the genome which encodes for the 16S- and 23S-rRNA.

The Microbiology Section of the Epidemiology Division of the USAF School of Aerospace Medicine at Brooks Air Force Base is involved in the identification of microorganisms in clinical samples dispatched from medical facilities worldwide. The rapid identification of nosocomial infectious agents and the determination of the common source of infectious outbreaks in clinical communities are of critical importance for patient care and outbreak control.

Our research interests are in the realm of recombinant DNA technology with concentration on formulating DNA probes engineered to specifically detect Mycoplasma in clinical specimens. Our expertise in the area of genetic engineering contributed to our assignment to the Epidemiology Division.

II. OBJECTIVES OF THE RESEARCH EFFORT

RFLP of rDNA is extremely helpful in fingerprinting a variety of bacteria which are not easily typed by conventional method. Application of this technique to a wide range of bacterial species would result in a more

precise means of determining the common source of an infectious outbreak. Thus, several approaches were taken to refine, streamline, and increase the general application of this potentially useful technique.

III. SYNTHESIS OF rDNA PROBES

16S- and 23S-rRNA of E. coli MRE600 (purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN 46250) were reverse transcribed to form its complimentary rDNA molecules by the method outlined by Gubler, 1983. The rDNA was biotinylated using the nick translation method of the Oncor Non-Isotopic System, Oncor, Inc., Gaithersburg, M.D. 20877, which was first described by Rigby, 1977.

The two rDNA (i.e., those reverse transcribed from the 16S- and the 23S-rRNA templates) were also cloned into pUC18 by the following strategy. The mixture of rDNA was placed in solution with SmaI-cut pUC 18 plasmid. SmaI will hydrolyze the recognition sequence 5'-CCC GGG-3' and as such produces blunt ends. T_4DNA ligase was used to attach either the 16S- or 23S- derived rDNA molecules onto the pUC18 plasmid. According to the method of Sgaramella, 1972. Since the SmaI recognition sequence is located in the gene which encodes for galactosidase, recombinant-DNA containing plasmids cannot express the enzyme. The Ap+ (ampicillin-resistant) gene is found on a segment of pUC18 which is unaffected by SmaI hydrolyses. The circular pUC18+rDNA insert was used to transform E. coli strain JM83. Transformants were differentiated from non-transformed JM83 by inoculation on Luria agar plates containing ampicillin since only Ap+ containing pUC18 plasmids are a isolated on Luria agar+Ap+Xgal+IPTG plates for they will be white due to the insertion inactivation of the galactosidase gene.

JM83 cells which contain only pUC18 will be blue in color because the B-galactosidase gene is undisturbed.

A segment of the 16S-rDNA of Mycoplasma strain PG50 was chosen to serve as the target for the polymerase chain reaction analysis (PCR). This technique will be discussed below. PCR allows the in vitro synthesis of a 677 base pairs sequence of rDNA from gram negative bacteria. This synthetic PCR-generated DNA was cloned by insertion into pUC18 and cloning into JM83 by the cloning strategy outlined above.

IV. ISOLATION, SEPARATION, AND DETECTION OF DNA SEQUENCES

Bacteria derived from clinical samples were grown overnight, the cells were lysed, and the genomic DNA was isolated after the method of Ausubel, 1987. The DNA was then hydrolyzed with an appropriate restriction endonuclease according to the directions of the manufacturer, and electrophoresed in agarose gels containing TAE buffer (40mM Tris Base, 20mM Citric Acid, 1mM EDTA, pH 7.8). Blotting onto nylon paper was accomplished using the LKB 2016 Vacugene blotting unit, and hybridization of target DNA with biotinylated DNA probes as well as signal development with streptavidin-alkaline phosphatase were performed by the methods outlined by the Oncor Non-Isotopic System as originated by Southern, 1975 and Leary, 1983.

V. POLYMERASE CHAIN REACTION OF 677 bp 16S-rDNA SEGMENT

DNA was amplified using the Gene-Amp DNA Amplification Kit, Perkin Elmer Cetus, Norwalk, CT 06859. Extension primers were formulated using the published 16-SrDNA sequence of Frydenberg, 1985. Twenty one bp primers which had

at least a 50% G.C. content were chosen. These primers were:

P-I CAT TGG GAC TGA GAT ACG GCC > 3'

P-II 3'< GGA ATG GTC CCG AAC TGT AGG

P-I spanned bp 306 to 336 whereas P-II extended from 963 to 983. These will achieve a polymerization of approximately a 677 bp segment of target DNA.

Thirty cycles of duplication were accomplished in an Eppendorf MicroCycler with the following duplication cycle:

Denaturation	95°C	1 min.
Renaturation	44°C	2 min.
Taq Polymerization	72°C	1.5 min.

Following amplification, DNA was either directly analyzed on 4% agarose gels or samples requiring further enzymatic treatment were purified by adding 1 volume of a 2M ammonium acetate and 3 vol 95% ethanol followed by precipitation and washing with 70% ethanol.

VI. RIBOSOMAL DNA, which was reverse transcribed from a mixture of E. coli 16S- and 23S-rRNA, was tested for its ability to serve as a general probe for fingerprinting microorganisms. This cDNA was biotinylated and utilized directly on Southern blots of EcoRI-cut genomic DNA isolated from clinical specimens of E. coli and Citrobacter freundii. Citrobacter freundii clinical isolates were tested since this organism was found in contaminated water used for food preparation during an investigation of an outbreak of an intestinal infection at the USAF Academy in Colorado Springs.

Although Owens (1988) and Wilkenson (1986) recommended overnight digestion, we have found that EcoRI will completely digest 250 to 1000 ng quantities of genomic DNA. No appreciable difference was apparent in fingerprints which were generated by 1, 16, or 23 hour digestions.

The quality of the fingerprints obtained was excellent. Clinical isolates contained anywhere from 12 to 18 distinct bands ranging in size from 1,425 to 18,000 bp. All of the isolates contained 5 common bands which had base pair compositions of 1500, 1635, 2300, 2500, and 2800. All other bands were characteristic for each individual isolate. Thus, rDNA fingerprinting is sensitive and specific enough to distinguish strains of Citrobacter derived from patients.

Two of the strains, C-9 and C-10, were isolated from the sewage-contaminated water source of the Air Force Academy. Raw sewage contamination would give rise to a myriad of different bacteria. This explains the different fingerprint obtained for these two strains. All other fingerprints were of Citrobacter strains isolated from patients at the Academy. In most cases, each patient had a specific rDNA profile. the heterogenity of the fingerprints indicated that Citrobacter freundii was not the causal agent for the clinical outbreak otherwise similar fingerprints would have been obtained.

The strategy used for cloning the cDNA mixture as well as the 677 bp fragment was not successful. This could have been due to incomplete linearization of pUC18 DNA or the fact that the JM83 host is not restrictionless.

The method of DNA extraction for Southern blot analysis also permitted excellent restriction endonuclease digestion and intense ethidium bromide staining.

VII. THE SYNTHETIC PRIMERS for the 677bp segment of the 16S-rDNA were of general use for PCR analysis of Mycoplasma, Ureaplasma, E. coli, and Citrobacter. A renaturation temperature of 44°C was ideal since it was suspected that the primers did not possess complete homology with portion of the DNA which matched the primers. Higher reannealing temperatures did not always result in a PCR product.

Restriction endonuclease digestion of the PCR products with DraI and TaqI did not generate fragments. This was most likely due to the fact that there are no recognition sites for these enzymes present on the 677 bp target sequence.

VIII.RECOMMENDATIONS

Cloning of DNA of E. coli origin could use the pUC 18-JM83 system since this restriction positive system will not affect this molecule. However, cloning of Citrobacter and mycoplasma DNA should utilize the restrictionless pUC19-JM109 system. Post ligation analysis of the recombinant DNA should be carried out to ensure that the insert and vector are combined. These recommendations should result in successful cloning of the rDNA and thus provide a wide range of probes.

An ultra rapid method of DNA isolation should be developed in order to allow fast PCR analysis. A method patterned after Kogan, 1989 should be investigated. This would include harvesting of bacterial cells by centrifugation in an Eppendorf microcentrifuge, suspension of the precipitate in 50 ul of 0.1MNaOH, 2MNaCl, and 0.5% SDS, followed by vortexing, boiling for 2 minutes, and centrifugation for 10 minutes to remove cellular debris.

In addition to probing for the 16S-rDNA, the investigations should be extended to an analysis of the 23S-rDNA via PCR. Such an analysis will give use to a much larger PCR product (as much as 2500 bp), and thus permit a possible RFLP study of this rDNA fragment. Such a system would greatly reduce the time and labor presently associated with Southern blot analysis of the RFLP system.

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FINAL REPORT

The Influence of Broad Spectrum Illumination on Circadian Neuroendocrine Responses and Performance

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The Influence of Broad Spectrum Illumination on Circadian Neuroendocrine Responses and Performance

by

Patrick Roy Hannon

ABSTRACT

Plasma levels of the pineal hormone melatonin are greatest during the sleep phase of the vertebrate circadian cycle. Orally administered melatonin has been associated with sleepiness and increased measures of fatigue. Recently, melatonin levels have been shown to be acutely suppressed by bright, white light in mammals including man. If increased light intensity can be used to control levels of circulating melatonin, then it may phase shift the sleep stage of the circadian cycle and attenuate fatigue degraded performance. The objective of this research effort was to assess the effects produced by wide spectrum, bright illumination on plasma melatonin and to determine if this treatment can reduce fatigue and enhance performance during and/or immediately following stimulus presentation. This summer's research effort entailed exposing subjects to a dim light condition or one of two bright light treatments from 1800 hrs to 0800 hrs the following day. Bright light conditions consisted of one of two presentations: (a) overhead and (b) facing presentation. Data collection included behavioral, physiological, and blood chemistry measurements on 9 subjects in a repeated measures research format.

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I. INTRODUCTION:

Human circadian rhythms have been hypothesized to result from social factors and/or light entrainment. Although much is known about human physiological responses during the 24 hour solar day, the underlying variables which are responsible for the entrainment to the circadian cycle and subsequent performance variability are still unclear. The Crew Performance Section of the USAF School of Aerospace Medicine is interested in how sustained work performance will be affected by the circadian cycle. Further, it is possible that a light stimulus may be sufficiently strong to entrain the circadian cycle and therefore enable human performance as well as human neuroendocrine responses to be manipulated by differing levels of ambient illumination.

This investigator's background is in exercise science and his research work has focused on animal and human motor performance. His research experience during the past 8 years at Northern Arizona University includes interdisciplinary work with colleagues in biology, engineering, physical therapy, and psychology. His interest in illumination and the light-dark cycle in humans is relatively recent with initial funding resulting from a proposal he submitted to the DURIP United States Dept. of Defense program in June of 1988.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The overall objective of this summer's research effort was to evaluate the effect of "level of illumination" upon a wide range of human performance, physiological, and neuroendocrine measures. Some of these measures are well established markers of the circadian cycle. These markers included oral temperature, plasma melatonin, salivary melatonin, and plasma cortisol measures. The setting for this study was a sustained cognitive work operation for a 26 hour time epoch beginning at 1000 hours and finishing at 1200 hours the following day.

This overall objective was only possible through sub-objectives which included doing a review of literature, writing the research proposal/protocol, collecting the data, reducing the data, performing the statistical analysis, and finally reporting the results and preparing manuscripts for publication. The present summer's 10 week time frame dictates that some of these objectives must remain in progress with reduction and statistical analysis of all data expected to be complete by December, 1989. Manuscripts are expected to follow.

III. REVIEW OF LITERATURE OBJECTIVE

An extensive review of literature was accomplished early during this investigator's research effort. A summary of the relevant literature is presented in this section.

Several studies have shown that cognitive and complex

motor tasks demand arousal (alertness) and selective attention on the part of human performers (Sage, G, 1984; Schmidt, 1982). It appears that the activation of arousal and attention functions which affect human performance are in large part mediated through the brainstem reticular formation (i.e. the reticular core). The reticular formation is composed of net-like structures of small nuclei and transmitting fibers which are able to stimulate the cerebral cortex, thalamus, hypothalamus, cerebellum and spinal cord. Reticular formation nuclei receive direct neural inputs from the cerebral cortex and spinal cord (reciprocal connections) and may be affected by endocrine secretions from a variety of sources (Sage, 1984).

Melatonin is a hormone which appears to have a depressant effect upon arousal, attention and motor activity in animals that is ultimately mediated through the brainstem reticular formation (Anton-Tay, et al., 1971; Lieberman, et al., 1985; Weaver, 1985). Many studies suggest that melatonin acts as a naturally occurring sleep enhancing compound. For example, human subjects given relatively low doses (2 mg) of melatonin for three weeks experienced increased fatigue (Arendt et al., 1984). Similarly, Lieberman, et al. (1985) using an acute oral dose of 240 mg of melatonin administered mid-afternoon found subjects experienced reduced vigor, elevated fatigue, increased confusion and slowed reaction time. Orally

administered melatonin has also been found to alleviate transcontinental disruption of circadian sleep wake cycles (Petrie, et al., 1989; Reiter, R, 1989).

Melatonin is derived principally from the pineal body and retina by N-acetylation of the neurotransmitter serotonin. Plasma levels of melatonin follow a 24-26 hour cycle and are approximately 10 times greater at night than during the day. Further, melatonin has a high affinity for receptor sites in the suprachiasmatic nucleus (SCN) of the hypothalamus where it is purported to trigger hormonal entrainment and regulate circadian and circannual rhythms (Reppert, et al., 1988; Brainard et al., 1985). Light acutely suppresses plasma melatonin levels in rodents (Benshoff, et al.,1987; Brainard et al., 1986), monkeys (Brainard et al.,1981) and man (Lewy et al., 1980; Brainard et al., 1985). Animal studies indicate that this entraining is mediated through the retina in response to photic stimuli via the retino-hypothalamic projection and the retino-geniculo-suprachiasmatic projection (Groos, et al.,1985). Finally, a pathway projects from the SCN to the pineal gland by way of the intermediolateral cell column and the superior cervical ganglia (Moore et al., 1974).

Two factors which influence light-induced pineal melatonin depression in animals and man are light irradiance and wavelength. First, light must meet specific illuminance/irradiance levels in order to suppress melatonin (Brainard, et al., 1983). Typical indoor

lighting (50-600 lux) does not meet this minimal level for humans (Abramov, 1985; Lewy et al.,1980 and Weaver et al., 1985). Second, Brainard has found that there are differential effects of light wavelength upon melatonin suppression in animals (Brainard, et al., 1983; Brainard et al., 1984; Brainard et al, 1986) and man (Brainard et al, 1985 and Brainard et al., 1988). Two additional factors affecting melatonin suppression include the duration of the light stimulus and the time of day in which the light stimulus is administered (Brainard et al., 1986). Recently, several studies have detailed the human sensitivity of melatonin release to environmental light and the uses that may be made of such control. For example, sleep onset can be delayed in subjects suffering from seasonal affective disorder as well as normal subjects when bright broad spectrum illumination (2000-3000 lux) is administered in the early evening (Lahmeyer, 1988; Czeisler, 1986 and Weaver, 1985). Lahmeyer (1988) reported this effect as cumulative over a seven day period with a "pleasant activation" being reported by subjects after the evening illumination treatment. The administration of 3000 lux illumination for a period of 7 hours (early evening through early morning) delayed the onset of 6-sulphatoxy melatonin (a major urinary metabolite) the following evening in 6 male subjects (Kennaway et al., 1987). Unfortunately, performance measures were not assessed. Still unaddressed is this relationship between illuminance

induced melatonin suppression and sustained human performance.

IV. COLLECTION OF DATA OBJECTIVE

This research effort addressed the effectiveness of the intensity of illumination in suppressing melatonin, perturbating selected physiological measures, and attenuating fatigue impaired performance. The study included data collected on 9 male subjects ages 23-38 years, throughout a complete circadian cycle from 1000 hrs to 1200 hours the following day. Data were collected on the weekends of 7/22, 7/29, 8/5, and 8/12 during 1989. Two weeks between the first and second experimental sessions allowed proper re-entrainment of the circadian cycle.

Subjects were evaluated at each of 12 treatment sessions and each subject was assigned to a separate sound attenuating testing booth that contained the Vita-lite illumination source and a PC work station. The light source was mounted on a wooden frame over the work station and suspended from an adjustable height to provide directed illuminance within either the dim or the bright treatment ranges. Specifically, the wide spectrum Vita-Lite fluorescent lamp (Duro-test Corp., Fairfield, N.J., 07007 Part # 1157030) was used for the experimental levels of intensity and therefore wavelength was held constant across all conditions. Bright light conditions were administered as an a) overhead and as a b) anterior presentation of the light stimulus to subjects. In the bright light

conditions, subjects were exposed to 3500 lux for a 14 hour period while measures were collected. In the overhead bright condition, a fixture adjustment was made to achieve the 3500 lux with the Tektronics J6511 illuminance probe facing up at the subject's eye level. It was noted by the investigators during the first weekend of bright illumination condition testing that the light actually entering the subjects' eyes was at a much lower level (X = 1550 lux). The investigators were concerned that photic entrainment threshold may not have been met with this overhead bright illumination condition. Therefore, the next weekend, the bright condition administered to subjects was achieved by configuring the light fixture in front of the subject. Using this same probe, adjustment of the fixture at the beginning of this condition achieved a 3500 lux level perpendicular to the subject's face, thereby ensuring that more illuminance reached the subjects' retinas. In the dim light condition, the fixture was always mounted overhead (probe facing up measurements) and the lighting condition did not change from the 100 lux level. The duration of this control treatment condition also extended over 14 hours. In all conditions, the first 3 trials were under dim light condition and allowed behavioral performance measures to stabilize. Seven treatment trials under a dim or a bright light condition began at 1745 hours and were followed by 2 trials beginning at 0800 hours under the dim light condition. The dim light

condition presented an illumination level which is typical in some military video monitor tasks (Thorington, 1985). The subjects were required to stay in the booth throughout the study with the exception of short (<10 minute rest room breaks). Social interaction was kept to a minimum between subjects by the experimenter and by the demands of the testing schedule.

The behavioral measures consisted of tests from the Complex Cognitive Assessment Battery (CCAB), the Walter Reed Performance Assessment Battery (WRPAB), Navel Medical Research Institute (NMRI) and the PED. Further, self rating scales included the mood, sleepiness, fatigue, and subjective symptom measures. Electrophysiological monitors evaluated electroencephalograms, electrocardiograms and electrooculograms. Oral temperature was also monitored during each testing time session. Additionally, the work stations presented computer tasks such as a air traffic controller role playing task also served as a quantitative measure of human performance.

IV. REDUCTION OF THE DATA OBJECTIVE

The cognitive/motor performance data were reduced automatically by the software programs used in the data collection process. Variables were organized in preparation for the statistical analysis through word processing and spreadsheet software.

V. STATISTICAL ANALYSIS OBJECTIVE

This research effort used a repeated measures ANOVA

design to evaluate the main effects of illumination conditions upon neuroendocrine, physiological, and performance responses. Subjects were exposed to the dim and bright lighting conditions in a counterbalanced design to evaluate the order of presentation effects. This design measured the main effects (illumination condition) and the order of presentation effects across the 7 treatment and 2 post-treatment sessions for each weekend of data collection.

VI. REPORTING OF RESULTS OBJECTIVE

Results for one of the WRPAB tests are presented below.

Manikin is a test of a subject's ability to perform rotations and related transformations of a mental image. It has been termed a spatial orientation test. The repeated measures ANOVA found a main effects (illumination condition) favoring the bright light conditions, X =.913 errors vs the dim condition, X = 1.362 errors for the "number of errors" variable, F = 11.66, p = .0112. The two bright light conditions were counter-balanced across order of presentation. Order of presentation was not statistically significant. Further, when comparing the bright anterior presentation (3500 lux, entry through the eyes) with the overhead dim condition (100 lux), several dependent measures were statistically significant. Again a main effect was found for this bright illumination condition where the bright condition subjects averaged

.9167 errors over the 12 testing sessions and averaged 1.7143 errors over this same time period in the dim condition, $F = 11.54$, $p = .0426$. Further, in comparing these same two conditions, significant interaction effects were found between illumination and session time period for the remaining 4 dependent variables for Manikin (Table 1). Values for mean overall response time, response time for correct responses, slowest response time, and total task duration time were all superior under this bright illumination condition vs the dim illumination condition at selected time periods. Interestingly, a reversal occurred for mean response time, response time for correct responses and total task duration time. All reversals occurred at 0800 hours which began 30 minutes after the end of this bright illumination treatment. In other words, this bright light condition produced significantly poorer results (after treatment) at 0800 hours when compared to same subjects' performance under the dim illumination condition.

Oral temperature was also plotted across the circadian cycle for the dim and bright (overhead and anterior) conditions (Fig.1). It should be noted that during treatment presentation from 2330 to 0530, the 9 subjects under the bright conditions maintained a 0.2 to 0.6° F temperature elevation compared to their oral temperature under the dim illumination condition, $X = .44°$ F.

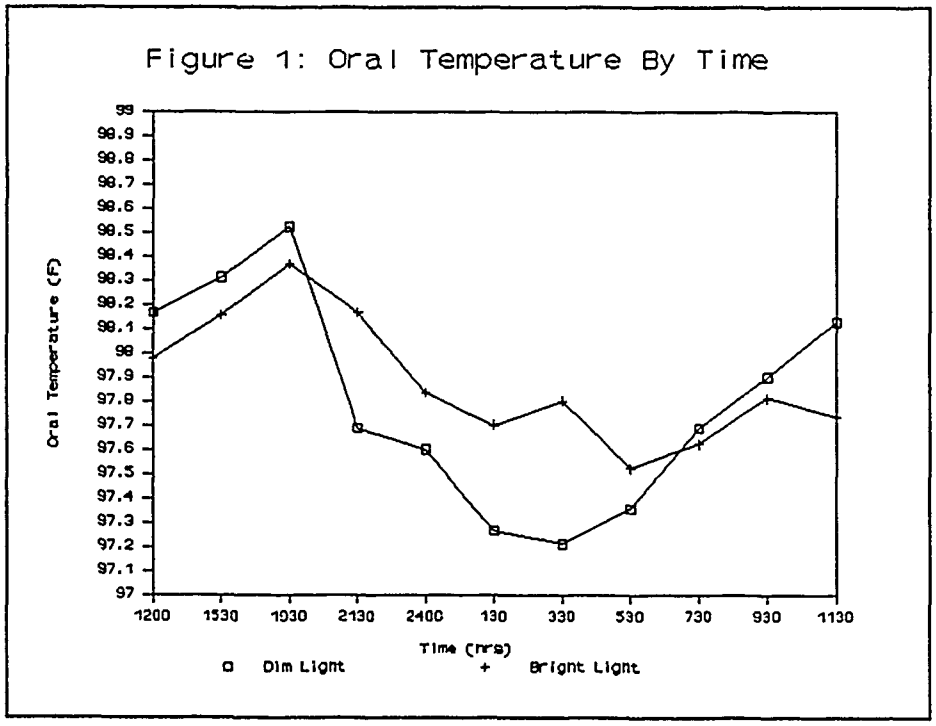


TABLE 1: MANIKIN DATA FOR BRIGHT FACING CONDITION VS DIM CONDITION

SESSION TIME (HRS)	VARIABLE	BRIGHT MEAN	DIM MEAN	p value
2000	Task Duration	70.5s	79.0s	.0499
2200	Response Times: for Correct Responses	1.59s	2.13s	.0342
0400	for Overall responses	1.99s	2.81s	.0042
	for Correct responses	1.95s	2.85s	.0010
	for Slowest response	3.55s	6.67s	.0089
	Task Duration	76.5s	89.75s	.0037
0600	Slowest Response Time	6.70s	9.93s	.0190
0800	Response Time for Overall responses	3.46s	2.63s	.0039
	for Correct responses	3.50s	2.62s	.0012
	Task Duration	100.25s	86.75s	.0032

VII. RECOMMENDATIONS

Recommendations must be guarded at this particular time. The investigators have examined one measure of spatial orientation-cognitive performance and oral temperature across time. If other tests and measures show similar results to these measures, then it would seem that the light intensity manipulation with anterior presentation may be significant and judging from the means, an appreciable factor in enhancing cognitive performance. Further, if a similar experimental protocol and design can be performed with a larger number of subjects, and significant findings result; then broad implications will follow. These implications would include making recommendations as to the appropriate level of illumination in a variety of work environments. It should be appreciated however that other factors (eg., visual glare and contrast) are also important concerns and must be factored into any illumination level recommendation. Clearly, this is beyond the scope of the present research problem.

At present, the investigators are beginning the data analysis for the remaining subjective feeling and cognitive/motor performance measures. These data analyses will be completed by October 1, 1989. It is this investigator's intent to submit a RIP grant proposal as a follow-up to this summer's research effort. Problems of significant interest to this investigator are the time

dependency of the light stimulus and the possible differential human response to wavelength manipulations. Further, this investigator has and will be able to test each subject individually over a sustained test/work operation at his home institution. This isolation of the subject during treatment-testing will help to eliminate the social interaction variable and any possible effects of social circadian entrainment. Specific research questions to be addressed would best wait until analysis of the data is more complete.

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FINAL REPORT

The Relationship Between locus of control, performance on cognitive tasks, and affective states after the consumption of antihistamines in hi-and low-workload conditions among aircrew personnel

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The relationship between locus of control, performance on cognitive tasks, and affective states after the consumption of antihistamines in hi-and low-workload conditions among aircrew personnel

by

Cynthia Ford

ABSTRACT

Three hypotheses will be tested in this study. They are: (1) There is a significant relationship between LOC and cognitive performance. (2) There is a significant relationship between LOC and affective states and (3) Cognitive performance of internals will be superior to that of externals across all conditions. Cognitive performance of aircrew personnel in the three groups (benadryl, seldane and placebo groups) will be assessed in high- and low-workload conditions. The Rotter I-E Scale will be used to assess LOC and will be administered at the commencement of the study. The Performance Assessment Battery (PAB) which is a battery of cognitive tests will be used to assess cognitive performance. The Profiles of Mood States (POMS) will be used to assess affective states. Each team of subjects will be tested one week at a time. To date, only one team has been tested. Therefore, the attached is a progress report.

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The author would like to express gratitude and appreciation to the Air Force Office of Scientific Research and Universal Energy Systems for sponsorship of this research. I would also like to extend gratitude to Dr. Russell Burton for his administrative endeavors which made participation in the program both a productive and enjoyable experience.

As a researcher, surrounded by individuals with diverse research interests directed toward a common goal, I must say that my experiences in the School of Aerospace Medicine at Brooks Air Force Base were enriching and priceless. I would also like to express my deepest gratitude and appreciation to the staff in VNB. Special thanks are extended to Drs. Douglas Eddy, Tom Nesthus and Bill Storm for their being instrumental in facilitating the conduction of this research project.

I. INTRODUCTION

A lucid understanding of the present study requires that one is cognizant of the larger ongoing study at SAM/VNB Brooks Air Force Base from which the present study emanated. The paramount purpose of the larger study is two-fold. The initial objective is to ascertain the sensitivity of selected C^3 and synthetic performance measures to the effects of two antihistamine medications, Benadryl and Seldane. The second objective is to assess the magnitude of individual and team performance impairment engendered by antihistamines during high-and low-workload C^3 scenarios.

One of my research interests has been in the area of cognitive psychology. More specifically, this investigator has conducted research to ascertain the relationship between personality and cognitive performance (e.g., The relationship between Field-Dependence and Field-Independence and performance on intelligence tests).

II. OBJECTIVES OF THE RESEARCH EFFORT

As a participant in the 1989 Summer Faculty Research Program (SFRP), I collaborated with Drs. Douglas Eddy and Tom Nesthus, the two researchers who designed the larger study to which I referred earlier in this document. After discussions with both investigators, this researcher decided to investigate the relationship between locus of control (LOC), performance on cognitive tasks and affective states after the consumption of antihistamines in high- and low-workload conditions among aircrew personnel.

My primary objective was to investigate the aforementioned relationships by incorporating the variables of interest into the larger research project on which Drs. Eddy and Nesthus were working. A second objective was to conduct an extensive literature review which is an integral part of the research process. This review, without dubiety, aided in my learning as much as possible about the LOC-cognitive performance and LOC-affective states relationships. A third objective was

to identify and locate the instruments to assess the variables of interests. A fourth objective was to decide on the data collection procedures in relation to the previously designed study. A fifth objective was to decide on the ways in which the data were to be analyzed. A sixth objective was to begin data collection prior to the end of my participation in the summer faculty program.

III. ACCOMPLISHMENT OF OBJECTIVE 1-LITERATURE REVIEW

At the commencement of the research period, this researcher conducted an extensive literature review which culminated in the written summary of the research findings cited later in this document.

Currently, research which entails the scrutiny of the relationship between locus of control and cognitive performance and affective states is exiguous. The author was unable to locate research which investigated the relationship between LOC-cognitive performance and the effects of antihistamines.

For clarity to the reader who is unfamiliar with the terminology and research in this area, the author has provided background information which should facilitate the understanding of this research project.

Locus of control is a concept generated by Rotter (1966) and refers to the individual's proclivity to perceive reinforcements as contingent upon external forces or internal forces. In tandem, Rotter theorized that there were two types of individuals. They are referred to as internals and externals. Externals are those individuals who contend that events in their lives are contingent upon external factors of which they have no control. More explicitly, externals attribute eventualities to chance, luck, fate or powerful others. Contrariwise, internals contend that events in their lives are controlled by internal factors. Internals believe that events are contingent upon their own behaviors or relatively permanent characteristics such as ability or skill. A comparison of internals to externals is tantamount to a

comparison between an actor and a pawn of fate. Internals feel that they are determiners of their fate while externals feel that they are the object of manipulations by external forces.

The individual's view of perceived control should not be taken as a simple isolated variable but should be scrutinized as a pervasive and profound perception with diverse ramifications and implications for a host of behaviors. One's perception of control can profoundly influence one's transactions with the environment. Locus of Control profoundly determines one's judgement of his potential for survival (Lefcourt, 1976). Subsequently, a fatalistic perception as perceived by externals, without dubiety, rids one of the fecundity needed to be a productive and self-reliant individual.

Additionally, one who is beleaguered by an external locus of control is likely to expend less effort in attaining a goal than one who feels that (s)he is in control. This is only one of the many lucid distinctions in behavioral responses of internals and externals as this review will substantiate via scientific research.

An extensive review of the locus of control literature elucidates the large volume of empirical findings regarding the relationship between locus of control and a host of behaviors such as: response to aversive events (Glass et al., 1973; Bowers, 1968; Houston, 1972), response to social influence (Baron, Cowan, Ganz & McDonald, 1974; Baron & Ganz, 1972; Fitz, 1971; Pines & Julian, 1972), and academic performance (Crandall, Katkovsky, & Preston, 1962).

The relationship between cognitive performance and locus of control is one of the paramount foci of this investigation. Documentation of the LOC-cognitive performance relationship appears less frequently than that of the other aforementioned relationships. The frequency of occurrence is unequivocally unrelated to the salience of this relationship.

The implications of the LOC-cognitive activity relation are so major that an extensive review of the literature and profound scrutiny are merited.

Locus of Control and Cognitive Activity

Albeit, there is a paucity of research verifying the relationship between locus of control and cognitive activity, the vast majority of the research reported tends to corroborate the fact that there is a relation between LOC and cognitive activity.

The idea that there is a relationship between locus of control and cognitive performance is commensurate with logic. Internals should be more circumspect, calculating and meticulous about their choices as well as more sedulous and persistent in attaining goals than externals since they believe that they determine their consequences. Such self direction should entail more active cognitive processing of information relevant to the attainment of goals (Lefcourt, 1976).

Research has verified the veracity of the hypothesized relationship between LOC and cognitive performance. One of the first studies conducted to ascertain the relationship between LOC and cognitive activities was conducted by Seeman and Evans (1962). These researchers found that internals, moreso, than externals avail themselves of information. The provenance of this disparity is the fact that internals believe that they control their fate and therefore act in their own behalf. This mode of thinking dictates the fact that a large repertoire of knowledge is a sine qua non for internals. Such is quite the contrary for externals. For they accept dependency on more competent others and thus perceive information to be less requisite for them.

Congruent with this finding are the findings of Prociuk and Breen (1977). These researchers investigated the relationship between locus of control and information seeking in a college academic situation. Results indicated that internals

manifested a propensity to actively seek course-relevant information moreso than externals. A subsequent comparison of final grades of internals and externals who sought information was made. Internals had higher grades than their external counterparts. In view of this finding these authors then concluded that in addition to actively seeking more information than externals, internals also used information more effectively than externals.

In a similar investigation conducted to ascertain whether locus of control is a determinant of information seeking, Davis and Phares (1967), found that internals tend to actively seek more information when the outcome determination is contingent upon information seeking. Lefcourt (1976) noted that such information seeking should engender an increment in the probability that internals will succeed more often in skill demanding tasks than their external counterparts.
In a similar study, Pines and Julian (1969) found that when stimulus materials are presented successively, internals tend to seek more information initially followed by a subsequent decline in information seeking behaviors while information seeking for externals remained virtually at the same level.

One's control beliefs are also believed to be causally related to the amount of effort one exerts in attaining a goal. More specifically, internals tend to exert more effort than externals in attaining goals (Phares, 1976). Kahneman (1973) has identified effort as a salient dimension of cognitive performance. Tangentially, Hasher and Zacks (1979) (as cited in Skinner & Chapman, 1984) distinguish between effortful performance and automatic performance. The former refers to mental processes which interfere with other concurrent cognitive processes. Exemplars of effortful performance include imagery, rehearsal, organization, and mnemonic techniques. Automatic processes include the encoding of spatial location, time, frequency of occurrence, and word meaning. Accordingly, these investigators noted (as cited in

Skinner & Chapman, 1984):

"The foregoing analysis leads to the conclusion that control beliefs affect cognitive development in the aggregate by influencing the kind of sustained effortful performances which facilitate structural development. The personal control mobilizes more mental effort for repeated cognitive operations of a constructive nature which leads in turn to the formation of new cognitive structures." p.133

It is because of the aforementioned reasons that Skinner et al., (1984) believed that internals differ from externals in two ways (1) They are more adept at mobilizing mental efforts in performing a given task. (2) They develop a larger repertoire of operational structures and more access to cognitive resources. These differences cause the same performance to require less effort for internals than it did originally.

Similarly, Pines (1973) observed that when given memory tasks, internals tend to recall more information over time, whereas a significant increment in retention over time for externals did not exist. This finding corroborates Pines' hypothesis that internals tend to indulge in covert information processing such as subjective organization of the information to be recalled to facilitate memory. The aforementioned processing resulted in improved recall by internals throughout most of the testing while improved recall for externals was present only at the last testing. It appears that effortful performance as hypothesized by Hasher and Zacks (1979) may be similar to covert information processing as hypothesized by Pines (1973). Both appear to be characteristic of internals and aid in explaining, in part, the superior performance of internals on cognitive tasks.
The varied trends in covert information-processing behavior for internals as compared to externals parallel the trends in overt information seeking.

Handel (1975) explored the relationship between attitudinal orientations and cognitive performance. Attitudinal orientation measures were locus of control, self

concept, and educational aspirations. Multiple regression showed that 18%-31.6% of the total variance in cognitive performance was accounted for by attitudinal orientation measures. Locus of control proved to be the most potent attitudinal variable in predicting cognitive performance among subjects from the low socioeconomic stratum. Comparatively, a greater amount of the variance in cognitive functioning was determined by self-concept and aspirations in the two groups (middle class and upper class) of higher socioeconomic status.

In sum, internals have proven to be superior to their external counterparts in several areas of performance. Quite tersely, internals search more actively for information and they process, utilize and reproduce information better than their external counterparts.

Locus of Control and Affective States

A second focus of interest in this study is the LOC-affective states relationship. Lefcourt (1976) investigated the LOC-affective state association using the Profile of Mood States (POMS) measure. The POMS consists primarily of negative moods (tension, depression, anger, fatigue and confusion) and one positive mood (vigor). Both tension and depression proved to be more common attributes of externals than internals. Vigor was more a characteristic of internals. Lefcourt further noted that if tension and depression can be construed as debilitating and vigor as mood facilitating, then the aforementioned correlation indicates that internals are less likely to succumb to demanding circumstances and to remain active in confrontation with challenges. Other investigations of the LOC-mood association have resulted in similar findings (POMS-McNair, Lorr & Doppleman, 1971; Kilpatrick, Dubin and Marcotte, 1974).

Tangential to this finding, Cook, Navaco, and Sarason (1980) studied the attrition rate among medical students who were subjected to stressful training programs and found that the attrition rate was highest among externals. This finding

lends credence to the supposition noted previously by Lefcourt (1976) that internals are less likely to succumb when faced with challenges. A number of studies substantiate this fact by demonstrating that internals tend to cope better than externals under stressful conditions (Cromwell, Butterfield, Brayfield, and Curry, 1977; Anderson, 1977; Kilpatrick, Dubin, and Marcotte, 1974).

IV. ACCOMPLISHMENT OF OBJECTIVE 2-INCORPORATING VARIABLES IN PREVIOUSLY DESIGNED STUDY AND GENERATING HYPOTHESES

In view of the previous research and on the basis of the construct properties of the internal-external dimension, the present study was designed to investigate the following hypotheses:

1. There will be a significant negative correlation between locus of control and cognitive performance.

2. There will be a significant correlation between locus of control and affective states.

3. Cognitive performance of internals will be superior to that of externals across all conditions.

V. ACCOMPLISHMENT OF OBJECTIVE 3-LOCATING INSTRUMENTS TO ASSESS VARIABLES OF INTEREST

The following instruments were obtained and reviewed for validity and reliability to assess locus of control, cognitive performance and affective states.

1. Locus of Control. The Rotter I-E Scale will be used to assess Locus of Control. The Rotter consists of 23 question pairs, using forced-choice format, plus six filler questions.

2. Cognitive Performance. The Performance Assessment Battery (PAB) will be used to assess cognitive performance. This battery consists of various tests taken from other batteries. The PAB consists of the following tests: Dual STM Tracking Task, Logical Reasoning, Code Substitution, Pattern Comparison II, Tower Puzzle, Numbers and Words, Dichotic Listening, and Matching to Sample.

3. Affective States: The Profile of Moods States (POMS) will be used to assess affective states. The POMS consists of six affective states (anger, tension, depression, confusion, fatigue and vigor).

VI. ACCOMPLISHMENT OF OBJECTIVE 4-DECIDING ON DATA COLLECTION PROCEDURES IN RELATION TO THE PREVIOUSLY DESIGNED STUDY

Twelve teams of subjects will be tested. Each team will be tested in two 3.5 hours scenarios each day for 3 days. Subjects in the teams will be divided into three groups (Benadryl Group, Seldane Group, and the Placebo Group).

The Rotter Scale and the POMS will be administered the first day of testing. The cognitive tests will be administered each day between the C^3 scenarios.

VII. ACCOMPLISHMENT OF OBJECTIVE 5-COLLECTING DATA

One team (3 subjects) was tested for four days (July 10-13). All instruments requisite to this study were administered. Eleven more teams will be tested for 11 consecutive weeks starting July 31, 1989. Therefore, the next objective which is to analyze the data cannot be accomplished until all of the data are collected. However, a discussion of how the data will be analyzed is noted.

VIII. ACCOMPLISHMENT OF OBJECTIVE 6-MODES OF DATA ANALYSES

The relationship between group membership (whether one is internal or external) and cognitive performance will be analyzed by means of a zero-order correlation in high- and low-workload conditions after ingesting antihistamines. Correlations will also be used to determine the relationship between locus of control and scores on the POMS in high- and low-workload conditions after ingesting antihistamines. These data will serve as a baseline from which to view the results of multiple regression analyses insomuch as the analyses will be used to determine how well group membership predicts cognitive performance and affective state under the 3 foregoing conditions. Mean comparisons will be made between internals and externals across all conditions.

IX. RECOMMENDATIONS

Since locus of control is a personality variable which has many ramifications, scrutiny of the many such ramifications is merited. Locus of control has been found to be related to

variables (e.g., cognitive performance, ability to cope with stress, and affective states) which are so relevant in research in academia and the military. Completion of this study should further elucidate the role of locus of control in cognitive performance, stress and coping behaviors and affective states. If, in fact, internals do persist and perform superiorly to externals in the placebo group after ingesting antihistamines, researchers should, without dubiety, give the construct of locus of control greater scrutiny and investigate the many ramifications of this personality variable in military research. This variable could prove to serve as a puissant predictor of cognitive performance, affective states and coping behaviors and other variables which are relevant to research in the military. This author would like to further recommend that the Rotter I-E scale is administered to all potential aircrew personnel during the qualifying exams. This data would subsequently provide a means of predicting how well individuals will cope with stress, how well they will perform under stress, in addition to how well they will perform on cognitive tests. Please note that the author is not recommending that this measure is used as one of the criteria for admission but rather that it is used as a descriptive measure of the individual's characteristics which would predict performance on cognitive tests and performance under conditions of stress.

The author would like to continue work on this project as well as a second ongoing study at Brooks AFB. I have discussed this desire with the principal investigators, Drs. Jonathan French and Pat Hannon. The second ongoing project entails an investigation of the effects of wide spectrum, bright illumination on plasma melatonin. The researchers, are endeavoring to ascertain whether wide spectrum, bright illumination can engender a decrement in fatigue and enhance performance. This author would like to further determine if the personality variable, locus of control is related to how individuals perform on cognitive tests under focused and

diffused lighting conditions. She would also like to ascertain whether mood changes as measured by the POMS are different for internals as compared to externals during the 28 hours in which subjects are involved in the experiment.

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FINAL REPORT

Aspects of the Diffusion of Inert Gases in Biological Systems

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Date:	August 25, 1989
Contract No.	F49620-88-C-0053

Aspects of the Diffusion of Inert Gases in Biological Systems

by

Jeffrey F. Himm

ABSTRACT

Recent experimental work by Novotny et. al. (1989) on the uptake and elimination of radioactive xenon in dogs has revealed some interesting trends, as noted by Ingle (1989). First, the processes are not symmetric, with the elimination of xenon from the dog occurring at a different rate than the uptake of the gas. And second, both uptake and elimination can be modeled as hyperbolic functions of time. Various possible explanations are explored.

ACKNOWLEDGEMENTS

I would like to thank the Air Force Systems Command and the Air Force Office of Scientific Research for the opportunity to perform these investigations. I am also thankful to the Hyperbaric Medicine Division of the School of Aerospace Medicine for providing the facilities for carrying out my studies this summer.

Col Robert Ingle was of invaluable assistance; helping to ease my vast ignorance in the areas of decompression sickness and in hyperbaric medicine, providing fruitful discussions on the research topic, and introducing me to La Calesa and to Reversi.

I also want to thank Col John Touhey and the rest of the members of the HM Division for making me welcome and at home at Brooks AFB. Ann Potter, Raymond Sanchez, and the staff at Strughold Aeromedical Library were extremely helpful in my researches.

And finally, thanks to Dr. Russell Burton and his right arm Marilyn McConkley, and to any others associated with Universal Energy Systems, for their efforts in my behalf.

I. INTRODUCTION

Decompression sickness (DCS) has been an area of active research for the past 100 years. It is of interest to the Navy and the Air Force, since both divers and pilots are susceptible to the disease. The Hyperbaric Medicine Division of the School of Aerospace Medicine here at Brooks Air Force Base provides treatment for people suffering from DCS.

The causative mechanism of DCS seems to be that inert gases, which dissolve in biological tissues, often come out of solution at reduced pressures. My current research interests are in the area of solubility and diffusion of inert gases in liquids, a suitable background for exploring some of the aspects of DCS.

II. OBJECTIVES OF THE RESEARCH EFFORT:

Novotny et. al. (1989) have recently completed some experimental studies on the uptake and elimination of a radioactive inert gas, ^{133}Xe, in dogs, specifically in the calf of the hind leg. An analysis of the data by Ingle (1989) has shown that (a) the uptake and elimination of the gas is asymmetric in time, with off-gasing occuring at a different rate than the on-gasing, and (b) both uptake and elimination of the gas are hyperbolic, as opposed to exponential, functions of time. Traditionally, both processes are modeled using a sum of exponential terms.

The primary objective of my research this summer was to understand points (a) and (b) above. Secondary goals for this summer include the thermodynamics of bubble formation, and how these bubbles contribute to DCS. Unfortunately, time did not permit these secondary objectives to be addressed.

III.

In the experiments of Novotny et.al., uptake and elimination of xenon was measured in both hind legs of 8 different dogs. Shown in figure 1 is a plot of the data for the uptake of ^{133}Xe in a typical experiment. Also included in the plot is the best fitting hyperbolic curve, given by

$$f(t) = 5134[t/(t + 70.5)] \qquad (1)$$

The agreement is very good.

A first question which needs to be addressed is whether the hyperbolic time dependence is unique to this series of experiments. A review of the literature reveals a paucity of actual data. However, several authors have published theoretical fits to their data. For example, the data of Behnke on the washout of nitrogen from the human body was analyzed by Smith and Morales (1944), yielding

$$\Phi(t) = 800 - 257e^{-0.087t} - 357e^{-0.019t} - 185e^{0.0054t} \qquad (2)$$

The original data of Behnke is not available, but we can try

to fit a hyperbolic curve to equation (2). Such a fit is given by

$$f(t) = 839.9[t/(t + 30.84)] \qquad (3)$$

A plot of the two curves is shown in figure 2. On the scale of the plot, the two curves are virtually indistinguishable. It would seem reasonable to expect that the hyperbola also provides a good fit to the actual data, and consequently that it is worthwhile to search for an explanation for this hyperbolic behavior. The hyperbolic fit requires only two parameters, the asymptotes of the curve, whereas the exponential fit $\Phi(t)$ requires six parameters.

IV.

The Michaelis-Menten equation relates reaction rates in the presence of an enzyme to the concentration of the substrate. It too is a hyperbolic relation. The enzyme and the substrate must pair up before the chemical reaction can be catalyzed. Is it possible that the inert gas atoms are pairing up in solution? Is there some hydrophobic effect which leads to the hyperbolic time dependence of uptake and elimination?

The pair correlation function for multicomponent mixtures is represented by $g_{ij}(r)$. It describes the distribution of component j particles around a component i particle. For a two component mixture, we can define the second osmotic virial coefficient as (Watanabe and Andersen, 1986)

$$B_2 = (1/2)\int_0^\infty [1 - g_{22}(r)]4\pi r^2 \, dr \qquad (4)$$

where subscript 2 refers to the solute molecules, and 1 refers to the solvent. Values of $B_2 < 0$ indicate that the solute particles tend to cluster together when in solution, and $B_2 > 0$ indicates that solute particles tend to avoid each other in solution.

Watanabe and Andersen (1986) performed molecular dynamics calculations on the krypton/water system. They obtained a large positive value of B_2 for krypton in water, indicating that the krypton atoms tended to avoid each other when in aqueous solution, as opposed to clumping together due to the hydrophobic effect. Indications are that the second osmotic virial coefficient for xenon in water would be even larger. In contrast, nitrogen has a large negative value of B_2, which is comparable in magnitude to that of krypton. Since both nitrogen and xenon display a hyperbolic time dependence during elimination, the hydrophobic effect, the strength of which is indicated by the second osmotic virial coefficient, can probably be ruled out as a factor leading to the hyperbolic time dependence.

It is important to keep in mind that these values of B_2 have been determined only for the relatively simple, two component gas/water systems. No indications were found in the literature of any effects on B_2 due to the presence of other solutes. The intercellular fluid is a highly complex,

electrolyte solution which will undoubtedly affect inert gas solubility unpredictably. However, even if such complex mixtures lead to hydrophobic clumping of inert gases, it is difficult to see how this might translate into the hyperbolic time dependence seen for both xenon and nitrogen.

V.

Perhaps the best clue to understanding the data of Novotny et.al. is in the recent work of Leaist (1988) and of Kim et.al. (1988). Both groups are studyng diffusion coupled with adsorption. The two processes combined can produce some interesting effects, including asymmetries in uptake and elimination of the diffusant, and also non-exponential time dependence of both on-gasing and off-gasing.

How does this apply to the dog calf data? Cells in tissues are bathed in intercellular fluid. Diffusion of gas atoms or molecules through this liquid is relatively fast. If we assume that tissue is made up of cylindrical bundles of tissue around a central capillary, it is possible to solve the diffusion equation for this situation. The concentration as a function of radial position and of time is given by

$$c(r,t) = 1 + \sum_j f(r,a_j)g(a_j,q)h(a_j,t) \qquad (5)$$

where

$$f(r,s) = J_0(rs)Y_0(s) - Y_0(rs)J_0(s)$$

$$g(s,q) = \frac{(J_1(qs))^2}{(J_0(s))^2 - (J_1(qs))^2}$$

$$h(s,t) = \pi e^{-\left(\frac{Dts^2}{a_0^2}\right)}$$

and $q = b/a_0$, the ratio of the outer radius of the cylinder to the radius of the capillary. The a_j are roots of an equation given by Crank (1975), the J_i and Y_i are cylindrical Bessel functions, and D is the diffusion constant.

Figure 3 shows how the concentration varies as a function of position for 4 different times. The intercellular fluid is over 90% saturated within 2 seconds, assuming $D \cong 10^{-5} cm^2/s$, and that $b/a_0 = 10$. Tissue halftimes quoted by the various diving tables are of the order of 100 minutes. These long diffusion times must be due to diffusion from the intercellular fluid into the cells, since the fluid itself reaches equilibrium very quickly.

In order to enter a cell, the gas must first pass through the cell membrane. The interior of the membrane is non-polar, while the interior of the cell and its exterior envirnment are polar, aqueous solutions. The solubility of non-polar gases is typically one to two orders of magnitude

higher in non-polar liquids than in polar liquids. The membrane, along with any other deposits of fatty, non-polar material, may act as sinks, or adsorption sites, for the diffusing molecules. As noted above, diffusion combined with adsorption is a possible explanation for the hyperbolic time dependence seen by Novotny et.al.

VI. RECOMMENDATIONS:

Unfortunately, this last line of approach to the problem was not discovered until late in the 10 week research period. I feel it is definitely one that should be followed up. The model to be investigated should include a central capillary which acts as a constant source of the diffusing gas (or as a zero concentration sink for gas elimination). This central capillary should then be surrounded by a cylindrically symmetric distribution of non-polar materials to act as adsorption sites for the gas. A further possible refinement might include regions of significantly lower diffusion rates to represent the interiors of the actual cells. The results of such studies could shed some light on these experimental results.

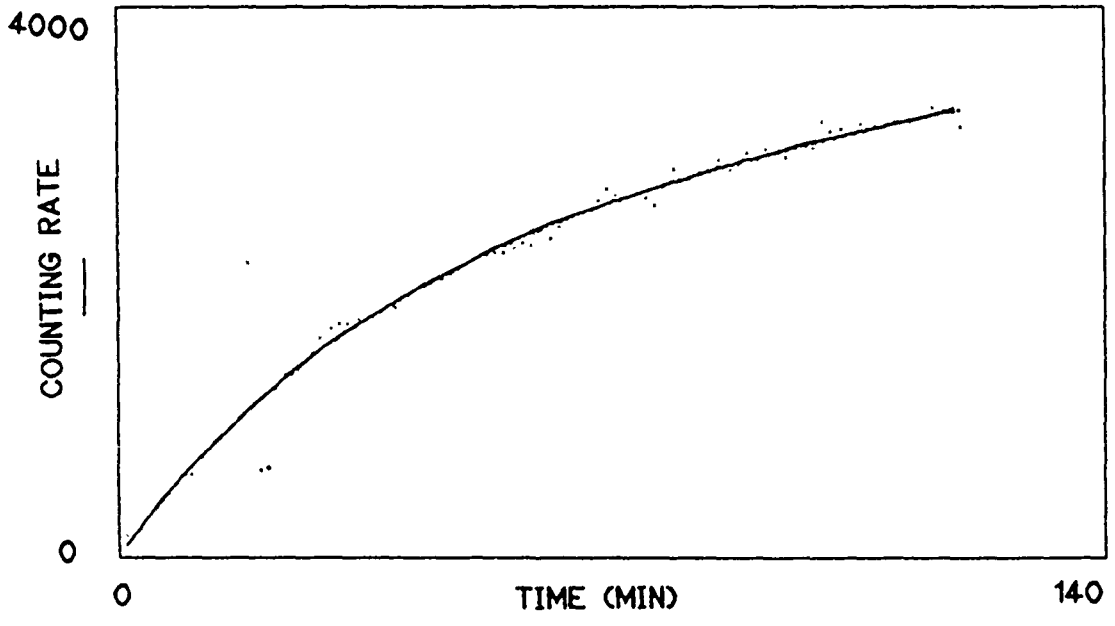


Figure 1. Graph of counting rate vs. time for the uptake of radioactive ^{133}Xe in dog calf. Dots are experimental data. Solid curve is equation (1).

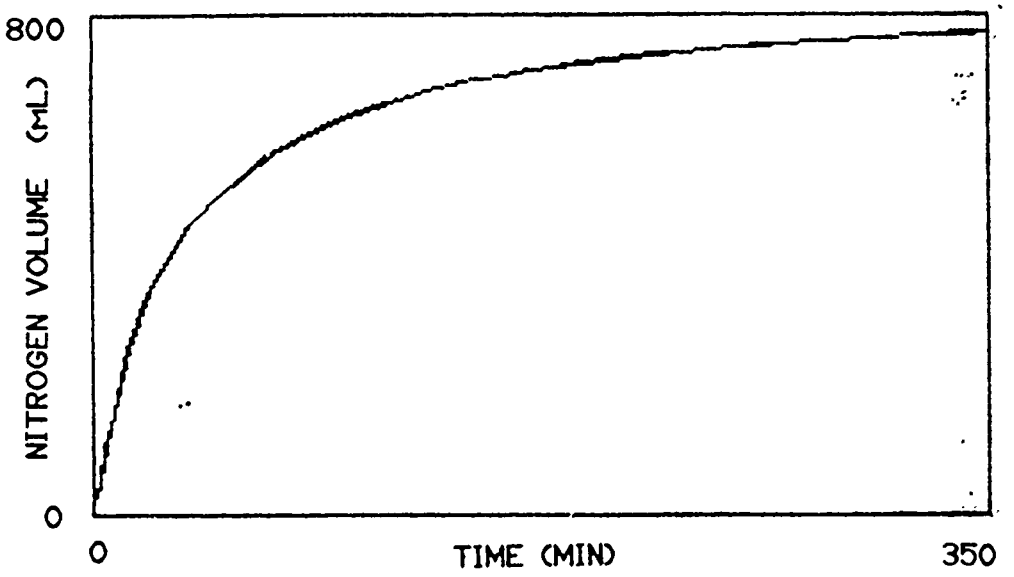


Figure 2. Plot of equations (2) and (3), describing the elimination of nitrogen from the human body. On the scale of the plot, the two curves are virtually indistinguishable.

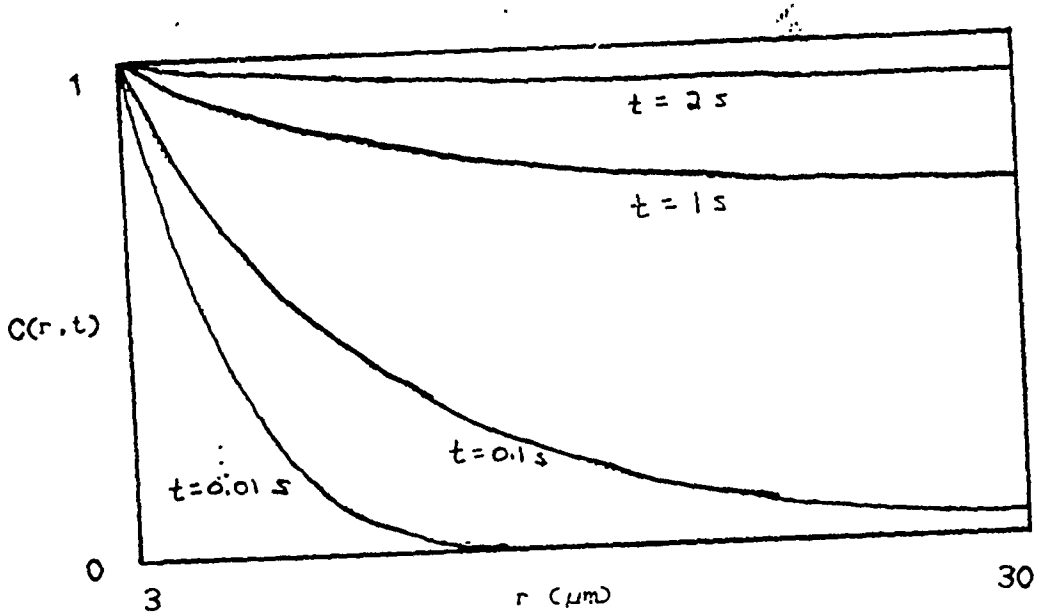


Figure 3. Plot of concentration vs. time (equation (5)) for radial diffusion from a central capillary of radius 3 μm into a cylindrical volume of radius 30 μm, at 4 different times t. ($D = 10^{-5}$ cm^2/s.)

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FINAL REPORT

STUDIES OF INTERACTIONS BETWEEN MICROWAVES, MELANIN AND MELANOCYTES

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Date: September 28, 1989

Contract No: F49620-88-C-0053

Studies of interactions between microwaves, melanin and melanocytes

by

Gwendolyn B. Howze

ABSTRACT

A synthetic form of the biopolyner melanin causes the reduction of cytochrome C. When oxidized cytochrome C and DOPA melanin were mixed, the absorption spectrum of cytochrome C changed to the profile characteristic of reduced cytochrome C. This reaction was studied in some detail. The reaction rate is concentration dependent. Microwave irradiation had no effect on the completed reduction reaction. Microwave (2450 MHz) irradiation seems to speed up the polymerization of L-β-3,4-dihydroxyphenylalanine (DOPA) to form melanin. Three types of evidence support this conclusion: increased absorption of irradiated melanin samples, decreased pH as compared with control samples, and a faster rate of reduction of cytochrome C as compared with controls. Preliminary studies of microwave effects on B16 melanocytes indicated no effect on growth.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. The excellent administrative appar s provided by Universal Energy Systems is also greatly appreciated.

Many people at Brooks AFB contributed to make this a productive and interesting research experience. Dr. Johnathan L. Kiel, my effort focal point, was supportive and readily available for suggestions and interesting discussions. Sgt. Hilrie M. Kemp provided essential technical assistance at a very critical juncture. Sgt. John Alls was a constant much appreciated source of information on tissue culture and irradiation procedure. I also wish to thank Sgt. David Simmons who was helpful in many roles during the summer. Many other people were helpful and hospitable beyond the call of duty.

I. INTRODUCTION

Because radio frequency radiations (RFR) are ubiquitous in the environment, there is much interest in determining the effects of these energy frequencies on living systems. Microwave frequencies are of particular interest and there have been studies describing the effects of microwaves on living systems. A partial list of such studies is given in ref. (2). Unfortunately, it is not always clear whether the effects may not have been due to hyperthermia rather than microwaves. The importance of designing experiments so as to distinguish between microwave and thermal effects has been shown in several papers (1,2,3).

It was thought that the melanocyte would be an especially interesting system for studying microwave interactions with living matter. The interest arises due to the biopolymer pigment, melanin. Melanocytes synthesize melanin in membrane bound cytoplasmic organelles called melanosomes. The melanin, which is a major component of the melanosome, is complexed with membranes which are composed of protein and lipid.

Melanin is of particular interest because it absorbs energy in a wide range of the electromagnetic energy spectrum, e.g., ultra violet, visible, infrared, ultrasound (4,5,7). It also responds to applied electrical fields. At specific combinations of hydration, temperature and applied electric field, synthetic and natural melanins exhibit conductivity changes compatible with biological semiconduction (6,7). Ultrasound absorption by melanin in the frequency range of 0.1 to 10 MHz has been described. Melanin samples absorbed ultrasound in an efficient resonant transfer mechanism. It was speculated that the melanosome might function in two different modes, as energy storage device and as an energy transducer(7).

Due to its versility as an absorber of electromagnetic wavelengths, it was speculated that melanin and melanin containing cells should be good systems for attempting to detect nonthermal interactions between microwaves and living systems.

Most of the experiments to be reported upon are studies of a modcl synthetic melanin. The melanin was originally synthesized with the aim of using it to produce a standard curve for a projected assay procedure to be used in determining melanin content of extracts from melanocytes cultures irradiated by microwaves. The chance observation that the melanin modified the absorption spectrum of cytochrome C led to most of the experiments which will be reported. Briefly, when melanin and oxidized cytochrome C (cytC) are combined, the absorption spectrum of cytC shifts from the oxidized profile to that characteristic of the reduced form of the molecule. The basis for interest in this particular reaction was the possibility that the reduction of cytC by melanin could be used as a target reaction for studying nonthermal microwave effects in living systems.

II. OBJECTIVES OF THE RESEARCH EFFORT

The objective of the research effort was to study the effects of microwaves on melanin and melanocytes under conditions where the temperature is controlled. This objective was compatable with one of the goals of the laboratory, which is to study the interactions between radiofrequency radiations (10 kHz to 100GHz) and living cells and tissues. The resultant data will have relevance to health and safety issues and quidance for Air Force personnel who will work with emitters of radiofrequency radiation.

Originally, the objective was to determine if it were possible to

demonstrate nonthermal microwave effects in melanocytes. It was felt that the melanocyte would be a likely candidate for detecting such an effect if it existed because these cells produce melanin. Melanin is a polymer pigment which absorbes throughout the electromagnetic spectrum. The first melanocyte cell line selected, Cloudman,Clone3, melanoma,did not activate from the frozen state into a thriving culture. During the period while trying to activate the Cloudman strain and subsequently while trying to activate a second melanoma strain, the B16 melanoma strain, it was decided to see if a nonthermal microwave effect on synthetic melanin could be detected.

The objective was therefore expanded. The objective was to determine if microwave irradiation, evokes a detectable nonthermal response in melanin or melanin producing cells. Because of time constraints this involved the use melanin synthesized on site from L-β-3,4-dihydroxyphenylalanine

III. EXPERIMENTAL

Research Plan

The following types of experiments were done: 1) synthesis of melanin from L-β-3,4-dihydroxyphenylalanine; 2) studies of the interaction of melanin with cytC; 3) studies of the effects of microwave irradiation on the melanin cytC reaction; 4) study of the effects of microwave irradiations on melanocytes.

Methods

1. Synthesis of DOPA Melanin

Melanin was synthesized by the method of Sealy (10). one gram of dihydrophenylalanine (DOPA) was added to 200 ml of distilled water, the pH was adjusted to 8.8 and the mixture was stirred with aeration for three

days. The DOPA was relatvely insoluble. The melanin was soluble at alkaline pH. Melanin was percipitated by adding enough HCl to give a 6N solution. The ppt. was concentrated and dried to produce powdered melanin. Unless indicated otherwise, powdered melanin was used to prepare the solutions for the experiments.

2. **Spectrophotometry**

Three ml samples were routinely used, containing 50 mM pH 7.5 phosphate buffer, 30 micro molar cytC, and melanin. Melanin concentrations are indicated in the results section. In moost experiments dry powdered melanin was used to prepare the solutions. In a few experiments diluted synthesis reaction mixture was used. These details are indicated in the results section.

3. **Cultivation of Cells**

The B16 melanoma cell strain was used. The cells grew well once they had recovered from the stress of freezing. A one/three split worked well for subculturing. Three commercially available media were compatible with good growth: RCMI, F10 and F12. It appeared that the main factor controlling good growth and pigment production was the amount of fetal calf serum. Visible pigment production usually began after the cultures were confluent. The stock cultures were grown in T-flask. The experiments were done in 12 or 24 well plates. The experiments on the cells are very preliminary because sufficient numbers of cells were not available until the last week. Therefore one large survey type of experiment was done. Trypsinization was essential for obtaining single cell suspensions. At least two washes with calcium/magnesium free salt solution were required. Gentle application and removal of the washes worked best.

RESULTS

Figures one and two are the absorption spectra of melanin and oxidized cytochrome C (cytC) respectively. Melanin absorbes throughout a range of 200 to 800 nanameters, without any maxima in the visible range, absorption increases substantially at wavelengths below 250 nm. The melanin spectrum corresponds to published reports (11). The spectrum for oxidized cytochrome C is also similar to published data (12).

When melanin and cytC are combined, the cytoC profile changes to the spectrum for reduced cytC. The two profiles are shown for comparison in figure three. The two new peaks at 521 and 550 are characteristic of reduced cytC. Aliquots of the soluble melanin from the reaction mixture were used for the experiment behind figures 1,2 and 3.

The reaction between cytoC and melanin has been studied in some detail. Figure four shows the effect of melanin concentrations on absorption at 550 nm. Each reading was taken one minute after adding tha melanin to a cuvette of buffered cytC The reaction is concentration dependent. Saturation conditions were not attained in the experiment. In this and other experiments the melanin stock solution was prepared from melanin powder, the concentration used was 0.2 mg/ml unless otherwise indicated. That concentration seems to be near saturation for the reaction, e.g.,reduction of cytC.

The time that it takes for the reaction to go to completion is shown in figure five. The graph describes two experiments. When the melanin concentration was 0.2 mg/ml, the reaction was over 90% complete within 5 min of adding the melanin to the vessel containing CytC.

The question was asked: does microwave irradiation influence the reaction between melanin and cytoC? When the reaction was allowed to go

to completion before irradiation at a constant temperature of 37 +0.2 degrees C., there was no effect on the absorption at 550 nm. see section A in Table 1.

Each section or group in Table 1 should be considered to be an independent experiment comparing the treatments listed. There were several design flaws in this group od experiments. Although the concentration of the melanin in the all of the tubes was identical, the exact concentration was not known in this experiment because aliquots from the synthesis reaction flask was used,rather than a known solutions of the powdered melanin. The interactions of concentration and time were not recognized at that juncture, and a saturating concentration was not employed. The concentration was probably less than 0.1 mg/3ml for comparison with figure four.

It is very likely that all of the samples in table 1 would have had a final value, after two hours, similar to the samples in group A. For example, after 30 minutes the readings for the samples in group B were 0.66 and 0.70 respectively. Therefore in each group the reading for a tube should have a value slightly less than that of the succeeding tube if there was no effect due to the treatment. There seems to be a trend, however, in that for most of the groups, the tubes containing irradiated melanin have lower O.D. values than the other tubes of that group. During the experimentation, it was easier to maintain a constant temperature during the irradiation, as compared with the sham irradiation. Is it conceivable that the melanin was modified in some way such that it increased the time required to reach completion of the reduction reaction? What would be the effect of irradiation on an experiment such as the one behind figure five?

The question was asked: does microwave irradiation effect the formation

of melanin by autooxidation/polymerization of DOPA? The melanin synthesis reaction was started and run for one hour. The stirring and aeration were stopped and three one ml samples were placed in opaque plastic tubes. Tube C was held at room temperature, tube B was sham irradiated at 37 degrees C and tube A was irradiated at 37 C. Absorption spectra of diluted samples from the three tubes are shown in figure six wherein the graphs correspond to tubes A,B,C respectively.There seems to be both a temperarure effect, which was expected, and also a microwave effect on the melanin absorption spectrum in the 350 to 800 nm range.

The three melanin samples were used to reduce cytC. As expected, (from figure 6 and due to interactions of concentration and time) the the cytC samples treated with the irradiated melanin had higher O.D. values at 550 nm. This is shown in figures 7, 8, and 9. Figures 7c, 8b and 9b are the tracings taken 45 min after adding the melanin samples (room temperature, sham irradiated and irradiated respectively).

Table 2 summarizes a cell growth experiment. Two inocula were used, four treatments were compared: RFR at 2450 MHz at 37 C sham irradiation at 37 C, incubation in the usual CO_2 incubator, 3-amino-L-tyrosine (3-AT). The duration of the treatments were for 30 minutes. No attempt was made to remove the 3-AT. Duplicate samples were used but no statistical treatment was attempted. There probably was no effect due to the treatments. The observed differences are probably due to the difficulty of obtaining single cell suspensions in preparing the inocula and also during the 24 hours count. The results do deem to indicate that the lower inoculum was better. The 12 wells plates were used for that experiment. A red neutral viability technique was also developed. The cells were treated, incubated for 48 hours, treated with the vital stain neutral red. Subsequently the

medium was decanted, the cells were washed, and the neutral red was extracted with 70% ethanol. The absorption of the extract was read at 460. All of the cultures absorbed equally. Live cells absorb the r- ral red, dead cells do not.

The melanocyte experiment had two additional components, pigment production and mitotic index. Time ran out and it was not possible to collect the data for these two components.

DISCUSSION

The studies with synthetic DOPA melanin support the belief that melanocytes will be very good systems for studying microwave effects. It will be necessary to determine the most useful parameters to study in the melanocyte system. Which aspect of pigmentation to study is not clear since in the melanocyte the melanin is packaged in the membrane bound melanosome.

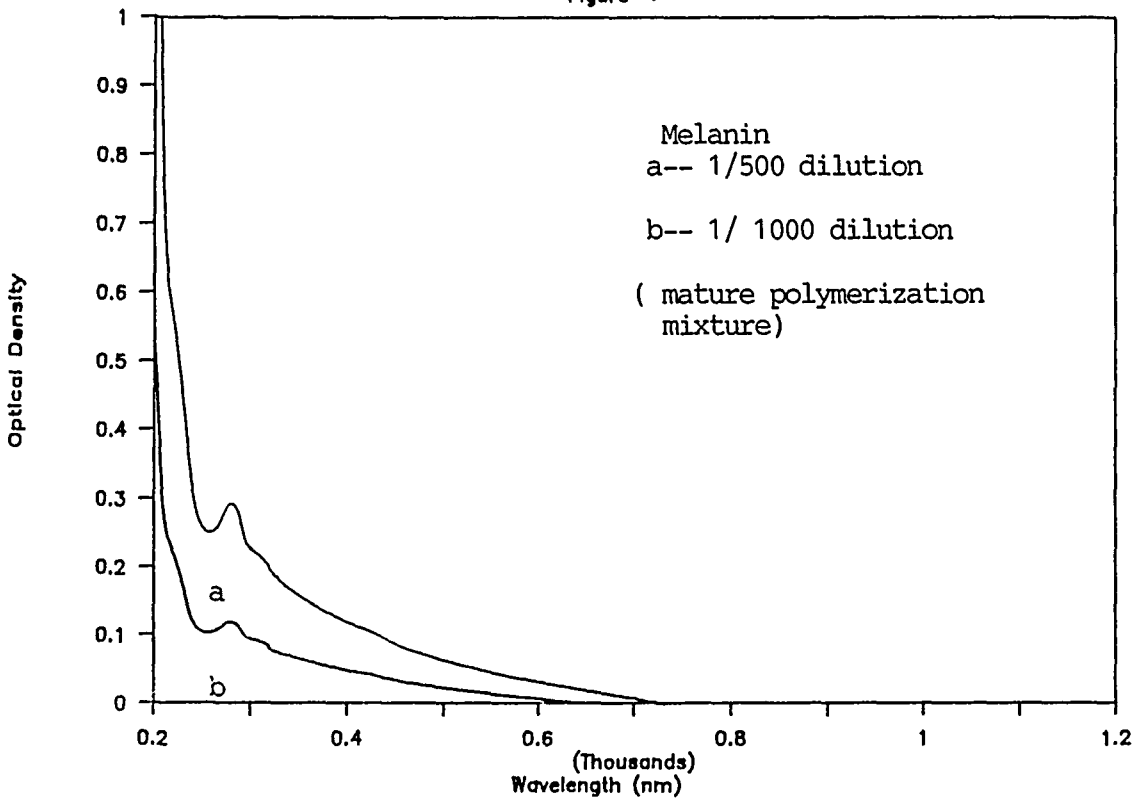
Melanin seems to exhibit both oxidative and reductive properties (11,13, 14). Melanin oxidizes reduced NAD (13). It is reported here, and also in the literature that melanin reduces cytC (14). Slawinska, Slawinska and Ciesla reported that melanin inhibits peroxyradical-induced chemiluminescence of luminol and cytC (14).They also reported that melanin protected cytochrome C from photo-degradation by UV-irradiation. Their data might also be explained as the UV light reversal of the melanin reduction of cytochrome C. This interpretation is more likely since melanin and cytC were mixed before exposure to UV-light (14). It has been suggested that melanin exerts its effects via superoxide (8,9,13,14). Superoxide dismutase, however did not protect cytC from reduction by melanin (these data were not included due to page constraints).

IV. RECOMMENDATIONS

Because of the time constraints, these studies should be considered preliminary. The effects of microwave irradiation on the apparent reduction of cytochrome C by melanin are intriguing. It is recommeded that they be repeated in a better designed context. Experiments on the effects of microwaves and microwave heating on melanocytes should prove interesting. Propably the most useful parameters to study are pigment production and cell injury as assayed by neutral red uptake or neotetrazolium reduction to formazan.

MELANIN vs CYTOCHROME C

Figure 1



MELANIN vs CYTOCHROME C

Figure 2

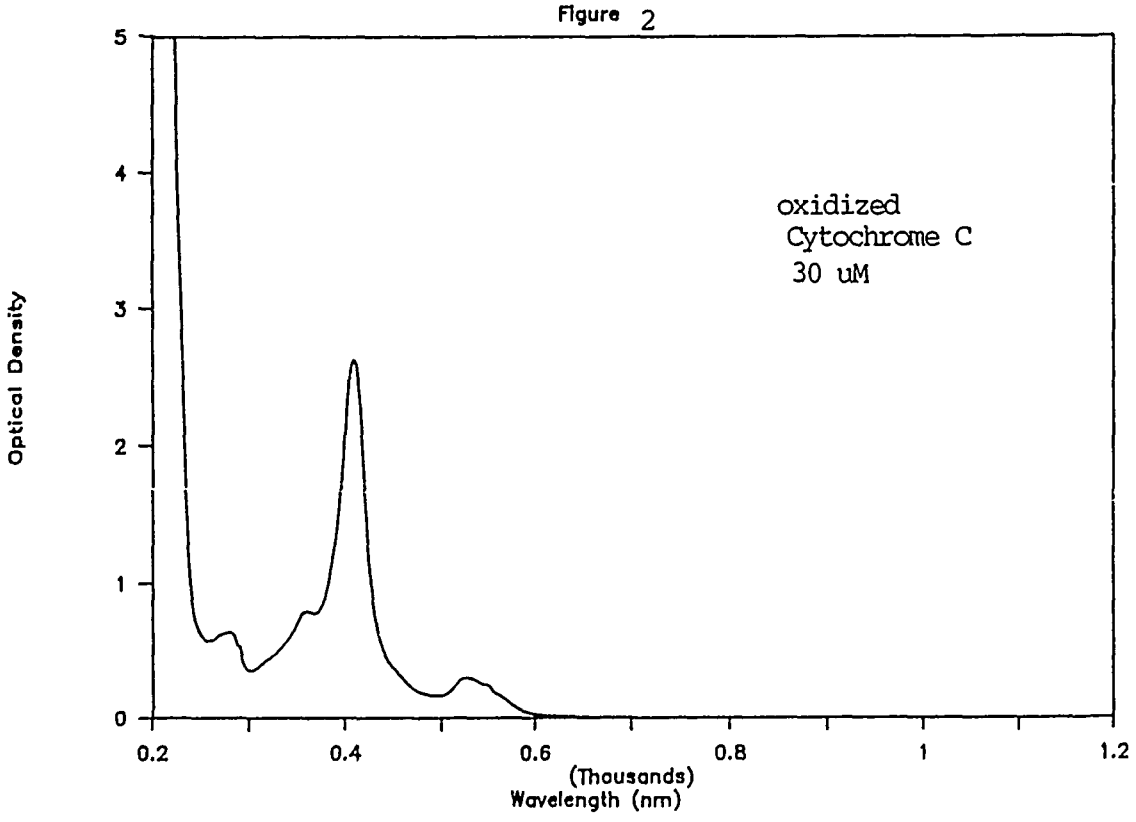


Figure 3

CYTOCHROME C

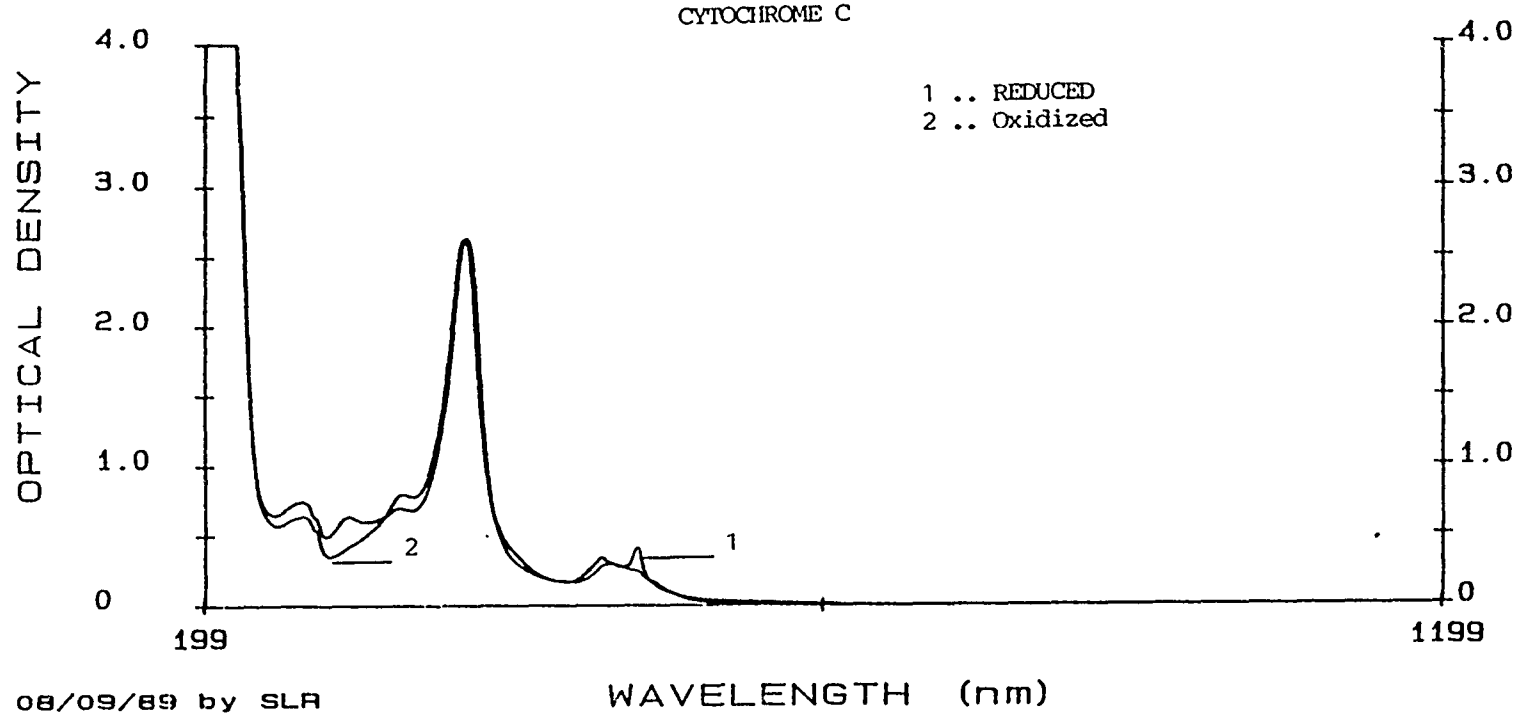


Figure 4

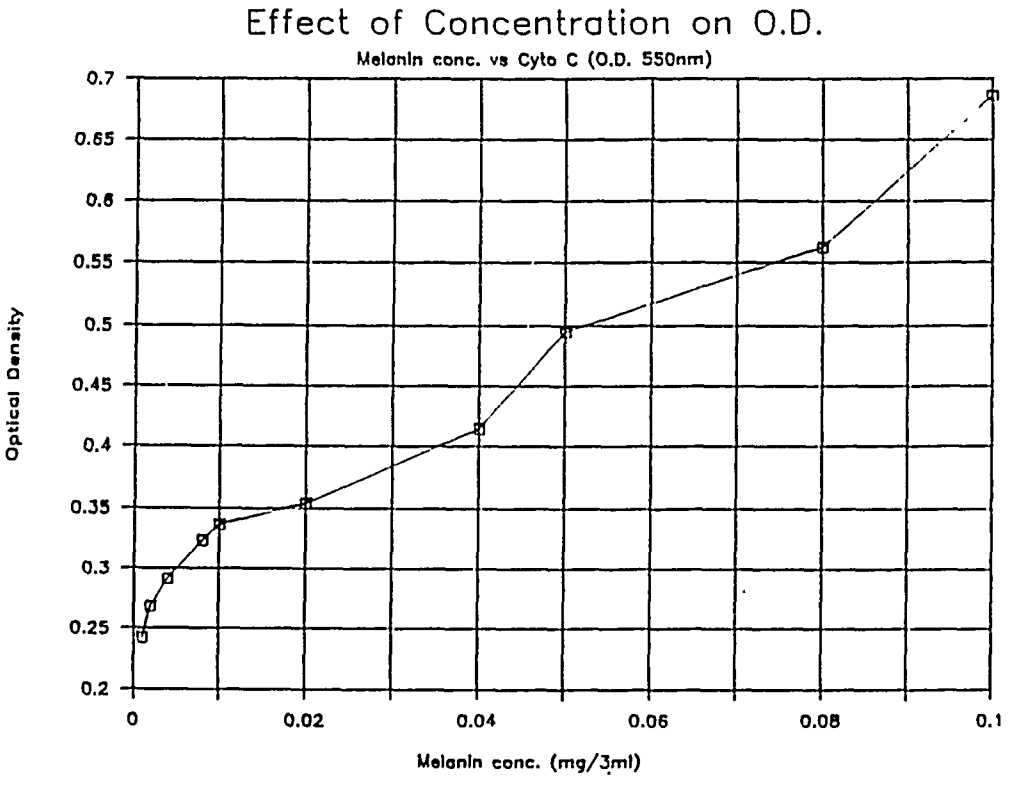


Figure 5

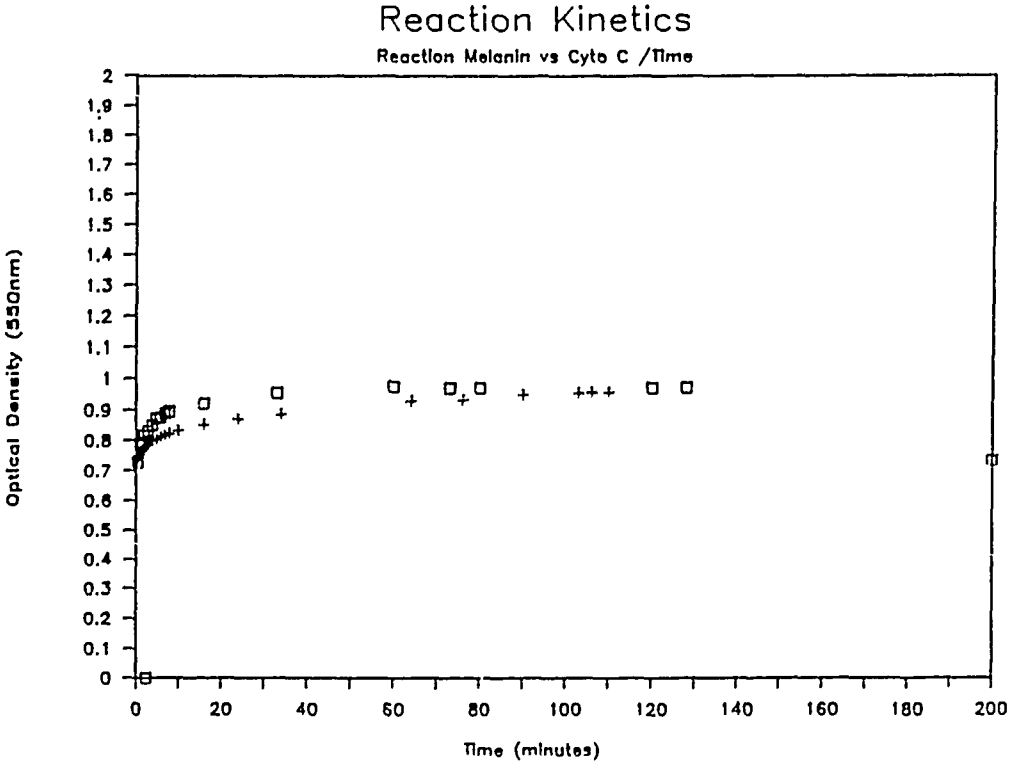
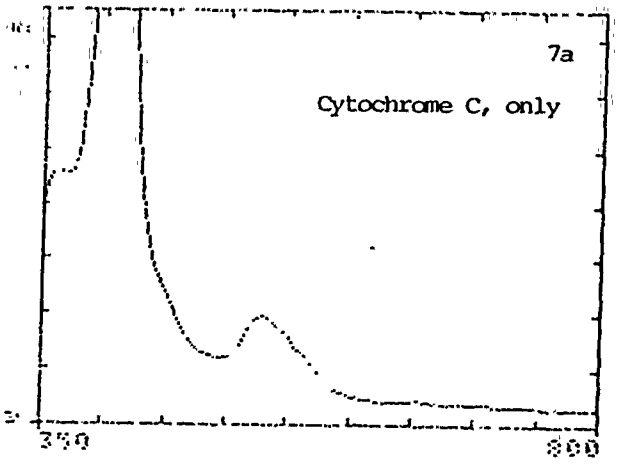
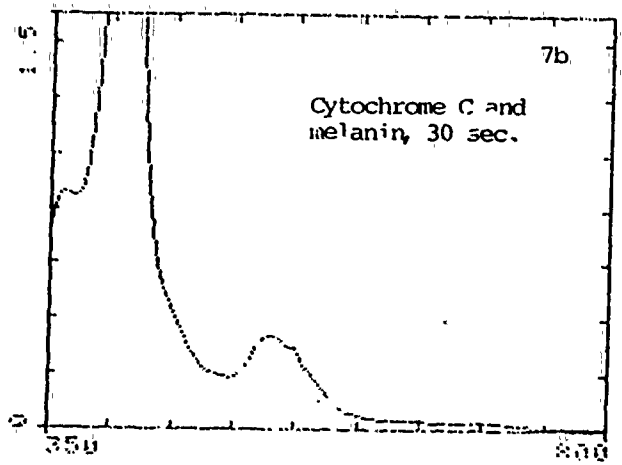


Figure 6
Treatment of
Polymerization
Mixture





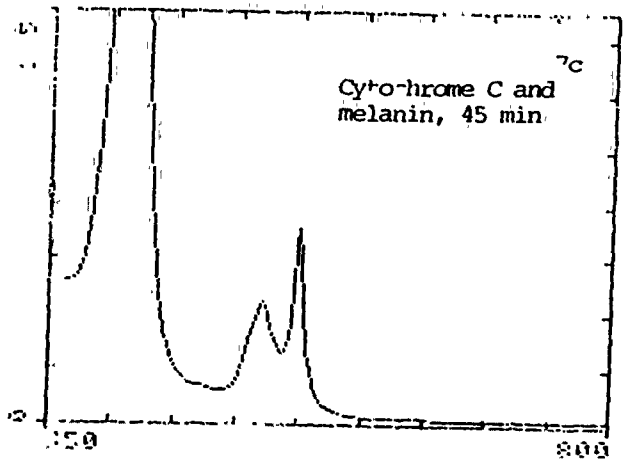
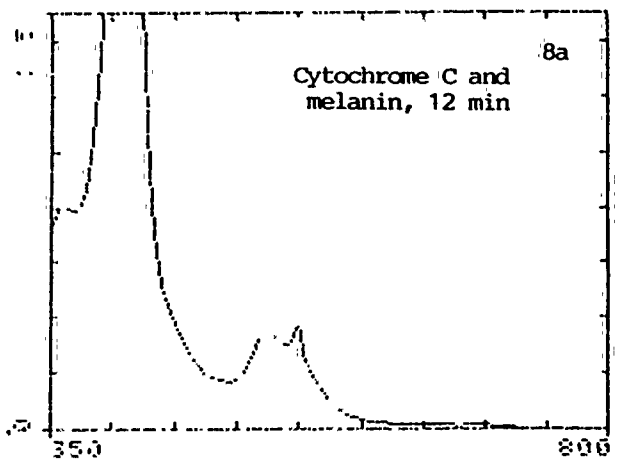
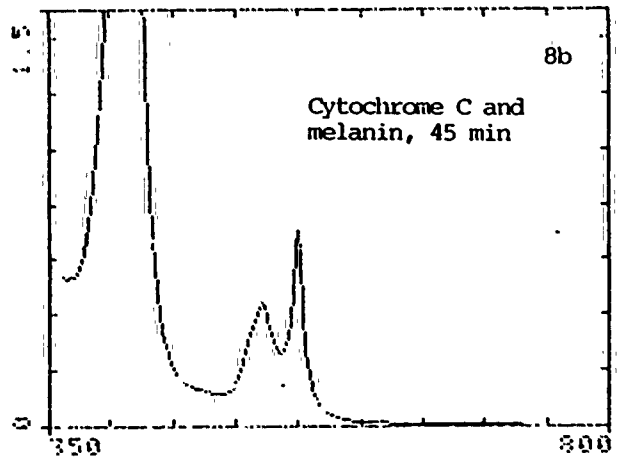


Figure 7
Sample held at room temperature during the during polymerization





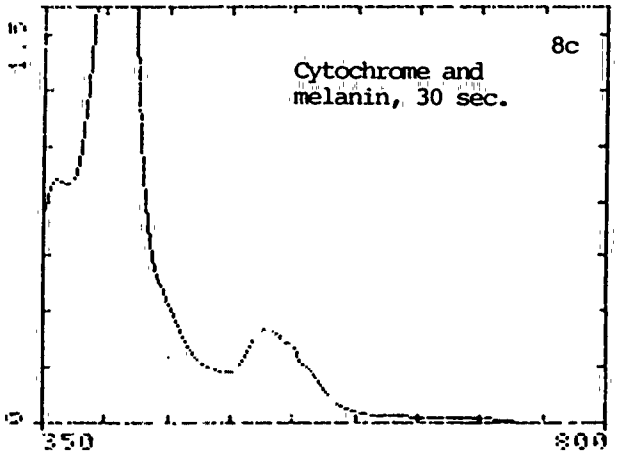


Figure 8
Sham treated sample of polymerization mixture, mixed with Cytochrome C for the time period indicated

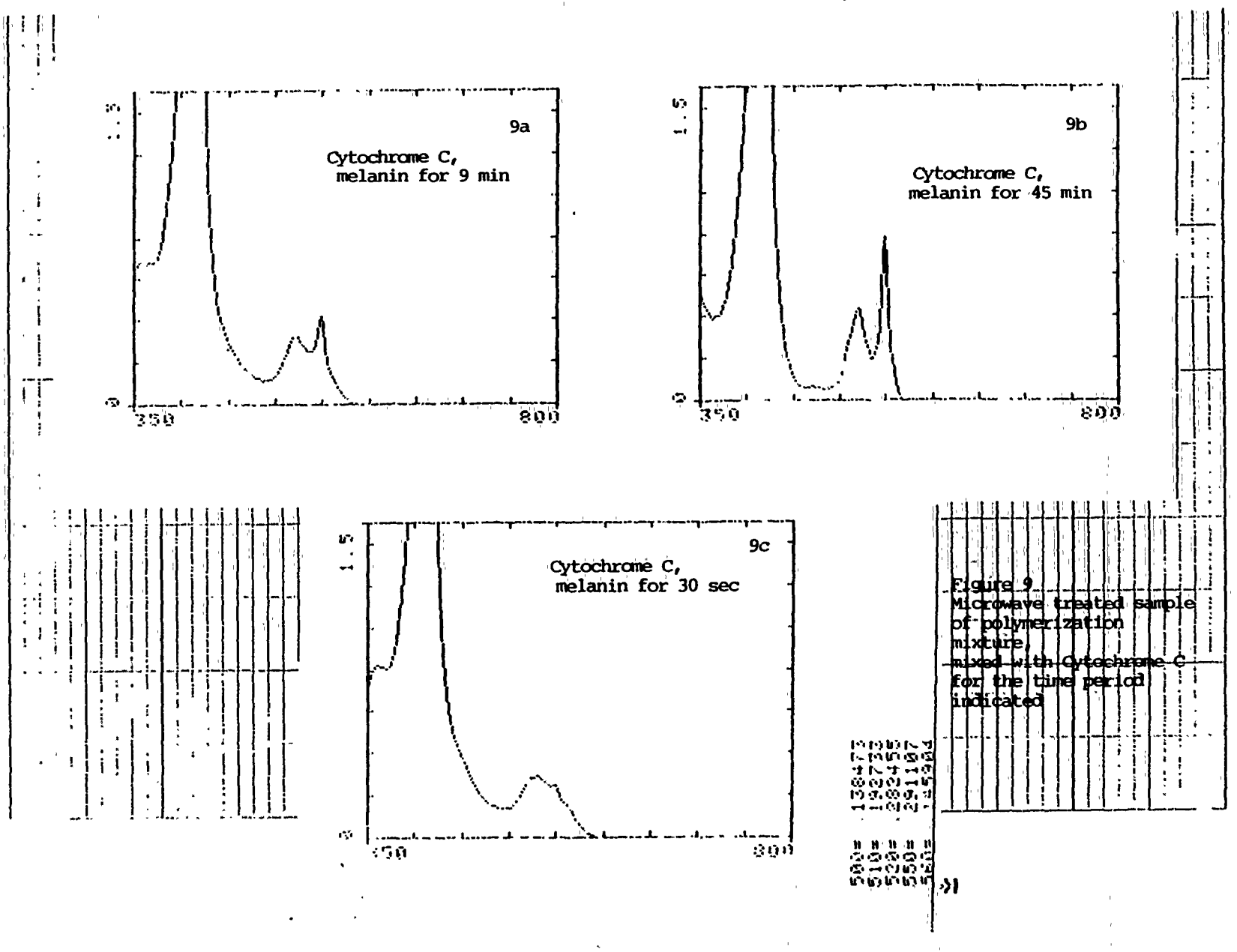


Figure 9
Microwave treated sample of polymerization mixture, mixed with Cytochrome C for the time period indicated

Table 1

EFFECTS OF MICROWAVE IRRADIATION ON THE REACTION BETWEEN MELANIN AND CYTOCHROME C

TREATMENT		$O.D^{550}$
A. Irradiation 2 hours after mixing reagents		
microwave (37 C)		0.765
sham irrad. (37 C)		0.761
B. The effect of mixing:		
irrad. melanin	+ normal cytC	0.402
sham irrad. melanin	+ normal cytC	0.430
C. The effect of mixing:		
melanin	+ irrad. cytC	0.520
irrad. melanin	+ irrad. cytC	0.507
sham irrad. melanin	+ irrad. cytC	0.531
D. The effect of mixing:		
melanin	+ sham irrad. cytC	0.576
irrad. melanin	+ sham irrad. cytC	0.561
sham irrad. melanin	+ sham irrad. cytC	0.564

Table 2
EFFECTS ON CELL GROWTH

Hours (a)	Inoculum x 1000	Cell Count x 1000			
		Control (b)	Sham (d)	RFR (c)	3-AT (e)
24					
	50	39	34	58	46
	100	102	60	86	122
48					
	50	89	109	74	93
	100	160	194	224	165
96					
	50	392	503	560	589
	100	620	693	810	647

(a) hours after treatment
(b) held in 37 C oven while the RF and Sham treatments were in progress
(c) cells received 2450 Hz, 30 min 37 degrees C
(d) same conditions as RF except no irradiation
(e) treated with 3-aminotyrosine

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FINAL REPORT

TWO-PHASE REGRESSION MODEL WITH APPLICATION

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USAF Researcher:	Joseph Fischer, Jr.

Date:	20 July 89
Contract No:	F49620-88-C-0053

TWO-PHASE REGRESSION MODEL WITH APPLICATION

by

Tze-San Lee

ABSTRACT

This research was motivated by a practical problem arising from the study of testing advanced anti-G valves to see if new manufactured valves meet the specific quality requirement. Since the data collected from the test results of thirty-one anti-G valves possess some special features including repeated measurements and possibly correlated errors, none of the two-phase regression models currently available in the literature is readily applicable to the prolem at hand. Based upon the results of this research, it is recommended that statistical tolerance intervals for two-phase nested linear model with autocorrelated errors shall provide adequate criterion for testing whether new anti-G valves produced by the manufacturer indeed meet the required quality standard.

ACKNOWLEDGEMENTS

This research is sponsored by the Air Force Systems Command and the Air Force Office of Scientific Research. Their generous support is gratefully acknowledged. Universal Energy Systems must be mentioned for their help to me in all administrative aspects of the program.

In addition, I wish to thank Mr. Joseph Fischer, Jr. and Ms. Carolyn Oakley who presented this challenging and exciting anti-G valve problem to me and assisted me tirelessly throughout every aspect of this research project. Also, I'd like to acknowledge Mr. Dan Bauer for the computer implementation of the algorithm developed in the theory of two-phase regression model with autocorrelated errors. The encouragement and help of Dr. Russel Burton clearly added to my enjoyable work at Brooks Air Force Base.

I. INTRODUCTION

The anti-G valve in fighter aircraft has been found to operate too slowly to counteract the rapid onset of G-force, possibly causing pilots of high-performance aircraft to black out, lose consciousness, and/or become fatigued. It is noticed that the time relationship to G-suit pressurization using the conventional anti-G valve is sigmoidal, having two relatively slow pressurization phases -- one early, and the other late -- in the suit-inflation schedule. These two slow phases were eliminated by preinflating the anti-G suit to 0.2 lbs/in^2 (psi) prior to an increase in G (called "Ready Pressure"); and by increasing the air flow through the anti-G valve (called "High Flow"). Based upon the notion of the High Flow Ready Pressure (HFRP), an advanced anti-G valve was developed by the US Air Force School of Aerospace Medicine (USAFSAM) Crew Technology Division. The HFRP anti-G valve increased the rate of the G-suit pressurization threefold. In addition, this valve was tested on eight F-15 pilots, using the centrifuge at the Naval Air Development Center, Warminster, PA. When this experimental valve was compared with the conventional anti-G valve now operational in the F-15 aircraft, the HFRP valve received a high degree of pilot acceptance because it had better valve response, reduced valve error scores, and allowed the pilots to tolerate high-G exposures with less effort. For details of the development, test, and evaluation of the HFRP anti-G valve for the F-15, see Burton, et al (1980).

Recently,31 HFRP anti-G valves were tested and the data on the G-suit pressures corresponding to different G-levels were collected. The statistical section of the Crew Technology

Division of the USAFSAM is involved with developing the confidence band on the G-suit pressures which shall later serve as the quality standard required for the HFRP-valve manufacturer.

My reserach interests are in the areas of regression analysis, multiple comparisons and survival analysis. In regression analysis, I am interested in solving the problem of multicollinearity, which is often encountered in observational studies. As a result of the multicollinearity, the least squares estimators for the unknown regression coefficients have unduly large variances. One of the alternatives to the least squares principle is to choose one estimator from a family of ridge estimators which has the smallest mean squared error. Lee-Campbell (1985) proposed a way to select such an optimum biased ridge estimator. A FORTRAN program for selecting the optimum ridge parameter is published in Lee (1987). Also, five deterministic ridge estimators were compared through a Monte Carlo study in Lee (1986). In multiple comparisons, I propose a new extension of Tukey's method to deal with the unbalanced data (Lee (1988)). In survival analysis, I am interested in the nonproportional hazard regression modeling. In particular, a two-phase regression model for the hazard function can be used to estimate the long term survival rate of patients with chronic diseases which surely has a nonproportional hazard function.

The problem of setting up the 95% confidence band on the G-suit pressures, which is the concern of the Crew Technology Division of the USAFSAM, falls in the area of regression modeling. In particular, it can be tackled by means of two-phase regression models. Because of my research background in regression analysis, I was asked to work on this problem.

II. OBJECTIVES OF THE RESEARCH EFFORT

Upon a closer examination on the data, it is found that the data on the G-suit pressures possess some special features. For example, for each of the 31 tested valves, continuous measurements of G-suit pressure were made on G level which was increased monotonically from 1G to 10G. Three replications of this test were done on each valve. The continuous recordings were digitized into 20 equally spaced G-levels, with their corresponding G-suit pressures.

The preliminary goals of our research effort are therefore set up as follows:

(1) Build a two-phase regression model with autocorrelated errors.

(2) Build a two-phase nested linear model which can take the repeated measurements into account.

(3) Find the simultaneous prediction intervals of future valves from serial measurements.

III. TWO-PHASE REGRESSION MODEL WITH AUTOCORRELATED ERRORS

Theory of the two-phase regression model with independent errors was developed in Hinkley (1969,1971) and Hudson (1966). For applications in Biology, see Sprent (1961). However, the two-phase regression model with independent errors is not applicable to the anti-G valve problem which has possibly correlated errors due to serial measurements. In this section, the two-phase regression model with autocorrelated errors is derived from the viewpoint of the frequentist.

Consider the two-phase regression model with autocorrelated errors as follows:

$$y_t = \begin{cases} a_1+b_1x_t + e_t, & t = 1, \ldots, m, \quad (3.1a) \\ a_2+b_2x_t + e_t, & t = m+1, \ldots, n; \quad (3.1b) \end{cases}$$

and

$$e_t = ce_{t-1} + \epsilon_t, \quad t = 2, \ldots, n, \quad |c| < 1, \quad (3.2)$$

where c is the autocorrelation coefficient and ϵ_t's denote the white noisy errors, i.e., ϵ_t's are independently, identically distributed (i.i.d.) with mean 0 and a common variance $\sigma^2 > 0$. It is further assumed that the underlying structural change of the regression model occurs in a continuous way so that the intersection point, r ,between two regression lines in Eqs. (3.1a-b) lies somewhere between two successive data points, that is,

$$x_m \leq r < x_{m+1}. \quad (3.3)$$

In practice, the value of r is not known and must be estimated from the data.

To estimate the unknown parameters a_1, a_2, b_1, b_2, c and r, the method of least squares is employed which is equivalent to solving the following constrained optimization problem:

$$\underset{a_1,a_2,b_1,b_2}{\text{Minimize}} \sum_{t=2}^{m}(y_t^* - (1-c)a_1 - b_1 x_t^*)^2 + (y_{m+1}^* - a_2 + ca_1 - b_2 x_{m+1} + cb_1 x_m)^2 + \sum_{t=m+2}^{n}(y_t^* - (1-c)a_2 - b_2 x_t^*)^2 \qquad (3.4a)$$

subject to

$$a_2 - a_1 + r(b_2 - b_1) = 0 , \qquad (3.4b)$$

Where x_t^* and y_t^* are obtained through the use of the autoregressive transformation as follows:

$$x_t^* = x_t - cx_{t-1}, \qquad (3.4c)$$

$$y_t^* = y_t - cy_{t-1}, \qquad (3.4d)$$

For the result of computational details, see Lee (1989).

IV. TWO-PHASE NESTED LINEAR MODEL WITH AUTOCORRELATED ERRORS

consider

$$y_{ijk} = \begin{cases} a_1 + b_1 x_{ijk} + d_{ijk}, & j = 1, \ldots, m, \\ & i = 1, \ldots, p, \; k = 1, \ldots, q, \quad (4.1a) \\ a_2 + b_2 x_{ijk} + d_{ijk}, & j = m+1, \ldots, n, \quad (4.1b) \end{cases}$$

$$x_{im_K} \leq r < x_{i,m+1,K}, \quad i = 1, \ldots, p, \quad k = 1, \ldots, q, \quad (4.2)$$

and

$$d_{ijk} = u_i + w_{j(k)} + e_{ijk}, \quad i = 1, \ldots, p, \; j = 1, \ldots, n, \; k = 1, \ldots, q, \quad (4.3)$$

where

y_{ijk} denotes the kth replicated measurement of the G-suit pressure at the jth G-level for the ith valve;

x_{ijk} denotes the kth replication of the jth G-level for the ith valve at which the observation y_{ijk} is obtained;

d_{ijk}, the random error associated with y_{ijk}, is assumed to be the sum of the random effect associated with the ith sampled valve (u_i), the kth replicated measurement for the jth G-level ($w_{j(k)}$), and the kth replication of the jth G-level of the ith valve (e_{ijk}).

Also, we assume that the random errors u_i and $w_{j(k)}$ are i.i.d. $N(0,\sigma_u^2)$ and $N(0,\sigma_w^2)$, respectively, and

$$e_{ijk} = ce_{ijk} + \epsilon_{ijk}, \quad |c| < 1, \quad i = 1, \ldots, p, \; j = 2, \ldots, n, \; k = 1, \ldots, q, \quad (4.4)$$

where $\{\epsilon_{ijk}\}$ are i.i.d. $N(0,\sigma_\epsilon^2)$.

We shall follow the approach of Fuller-Battese (1973) and

Pantula-Pollack (1985) to obtain the generalized least squares estimates of a_1, a_2, b_1, b_2 and consistent estimates of r , σ_u^2, σ_w^2, and σ_ϵ^2.

V. PREDICTION OF TWO-PHASE NESTED LINEAR MODEL

If only one new anti-G valve is produced, a prediction of its G-suit pressure based on a given G-level can be obtained by finding a prediction interval such that the G-suit pressure at the given G-level will lie in the interval with the preassigned probability, say 0.95. Unfortunately, it is often desired to obtain the prediction interval for each of the many new produced valves. Applying the approach for the one-valve case over and over again is incorrect since the prediction intervals of the many new produced valves are not independent. Furthermore, the number of the new produced valves are often unknown so that an exact probability statement is not possible.

Nonetheless, this problem of predicting the G-suit pressure of many (unknown) new anti-G valves can be solved by means of statistical tolerance intervals on the normal distributions of future observations, the intervals being probabilistically simultaneous in each possible value of the G-level. We shall follow the approach of Lieberman-Miller (1963) and apply it to the two-phase nested linear model with autocorrelated errors.

VI. RECOMMENDATIONS

Based upon the results of this research, it seems appropriate to make the following recommendations:

a. Theory of the two-phase regression model with autocorrelated errors was derived here from the standpoint of the frequentist. To apply the theory to the anti-G valve data, we implement it in two stages. In the first stage, we implement the algorithm for the model with independent errors and apply this model to the anti-G valve data by pooling the three replications at each of the 20 G-levels for all 31 valves together. After plotting the residuals from this model, it indicates that the assumption of independent errors is invalid. Currently, we move into the second stage by implementing an algorithm for the model with autocorrelated errors. However, this second stage of implementation is not completely done yet. It is anticipated that the work will be done in a week or two.

b. What we have accomplished this summer is only a first step towards the solution of the anti-G valve problem. Still,it is far from entirely solving the problem posed in Section I. Yet, we do have an idea how to tackle the whole problem as proposed in Sections IV and V. All we have to do is to continue our research effort along the paths mentioned in this report. I plan to apply for a mini-grant to pursue the follow-on work. Hopefully, a satisfactory solution to the problem of quality standards for anti-G valves will be obtained in a year or so.

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Final Report

System and Signal Analysis of VEP Data and Joystick Error Analysis

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USAF Researcher:	Captain Norman Barsalou
Date:	August 28,1989
Contract No:	F49620-88-C-0053

System and Signal Analysis of VEP Data and Joystick Error Analysis

by

Harold Longbotham,
Lionel Ramos,
and Joe Rea

ABSTRACT

One of the missions of SAM/RZV is the analysis of deterioration of the combat readiness of Air Force pilots due to flash blinding and laser eye damage. At RZV research is being conducted into methods of measuring visual acuity of unresponsive subjects using the visual evoked potential. Research will be conducted on the ability of pilots to perform eye tracking with artificial scatomas that simulate laser eye damage. Simultaneously work is in progress in image processing to model the visual field so that perturbations to it, due to laser damage may be simulated. Our research in this area resulted in the submission of two papers in image processing to the 1990 SPIE/SPSE conference, the submission of technical reports on analysis of joystick error and analysis of system error in VEP instrumentation, a literature search on robust methods (in statistics) that may prove applicable to VEP data analysis, and the outline of an expository paper on linear and nonlinear digital filtering and experimental design for data analysis with specific applications to VEP data.

Acknowledgements

This research was sponsored by the Air Force Systems Command and the Air Force Office of Scientific Research. We wish to thank them for their sponsorship and the chance to investigate problems of mutual interest. Universal Energy Systems must also be recommended for their professional execution of the administrative and directional aspects of this program.

We also wish to individually thank various members of the research team at SAM/RZV for their support. Dr. Harold Longbotham would like to thank Captain Barsalou and Lt. Col. Cartledge for spurring his investigations into new techniques of nonlinear digital filtering, robust biological data analysis, and image analysis. Dr. Longbotham would also like to thank Dr. Glickman (Krug International) and Peter Smith (British Royal Air Force) for many interesting conversations on the visual system. Lionel Ramos would like to thank Major McLin and Lt. Beneditz for their support in his development of a methodology for analysis of joy stick error. Joe Rea would like to thank Dr. Glickman and Doug Coffey, of Krug International, for their support in the analysis of system error of the VEP instrumentation. We would also like to thank Dr. Farrer, the group leader at RZV, for his continued support in the following research.

I. INTRODUCTION:

SAM/RZV has as a mission, the analysis and prevention of deterioration of the combat readiness of Air Force pilots due to flash blinding and laser eye damage. To this effect research has been conducted into Visual Evoked Potentials (VEP) [1,2,3,4] and will be conducted on the ability of pilots to perform eye tracking with artificial scatomas that simulate laser eye damage [5]. Simultaneously work is in progress in image processing to model the visual field so that perturbations to it, due to laser damage may be simulated [6].

A new technique for the measurement of visual acuity, the Sweep VEP, is currently being investigated for a faster appraisal of visual acuity. Due to the background of Dr. Longbotham in filtering and statistics, he was recruited to investigate possible data analysis techniques that might be applied. Questions have also been raised as to the system characteristics of the instrumentation used in the collection of the VEP data. Joe Rea and Dr. Longbotham, due to their electrical engineering background, have been recruited to measure the system response of the instrumentation used. In the eye tracker experiment that is to be performed by Major McLin it was deemed necessary to first measure any joystick error that could possibly interfere with the measurement of tracking error. Lionel Ramos and Dr. Longbotham, due to their electrical engineering experience, were recruited to investigate this problem.

In image analysis, the techniques of mathematical morphology have proven useful in the time domain, albeit root signal analysis (passbands) is still in its infancy. Dr. Longbotham, due to his research in one dimensional time domain signal analysis, was ask to aid Captain Barsalou in work in this area.

II. OBJECTIVE OF THE RESEARCH EFFORT:

The preliminary goals that brought us to SAM/RZV for the summer of 1989 were the characterization of joy stick error, the classification of the system response of the instrumentation used for collection of VEP data, the filtering of sweep VEP data, and analysis of morphological filters. In section III we outline a general methodology developed to measure the positional dependence of joy stick error. The complete description is too lengthy for this report and has been submitted as a technical report. Our classification of the system response of the instrumentation used for the collection of VEP data has not only resulted in a complete description of the magnitude and phase response of the complete system and each component, but has also resulted in a general methodology for usage in examining similar systems and the outlining of necessary changes in the present system if investigators are to eliminate phase response problems. These results are outlined in section IV, again a technical report is being submitted that outlines the methodology in complete detail. In section V we outline the various techniques which can be used to analyze sweep VEP data. This study is far from over and will be part of our ongoing proposal (RIP). In the time domain analysis of images, our research this summer has resulted in the submission of an abstract to the 1990 SPIE/SPSE conference. Section VI contains this abstract.

As research progressed this summer it was obvious that other goals were also appropriate. Lt. Col. Cartledge and Dr. Farrer have requested Dr. Longbotham to write up an introduction to noise analysis from both the frequency and statistical viewpoint that will outline current trends in robust and nonrobust techniques and their appropriate usage for VEP data analysis at RZV. The investigation into robust signal processing procedures have resulted in the submission of a second paper to the 1990

SPIE/SPSE conference for the use of these techniques in image processing. The outline of the introduction to noise analysis comprises section VII and the abstract for this second paper in contained in section VIII.

III. MEASUREMENT OF JOYSTICK ERROR:

A study "Visual Effects of Artificial Scatomas" is currently being undertaken at SAM/RZV. The purpose of this study is to investigate the ability of subjects with artificial scatomas (blind spots that simulate laser burns) to track targets with a joystick on a computer monitor. But before this could be accomplished one must separate the error in tracking due to the joystick from the total error recorded.

The basic goal of this segment of our project this summer was to determine if the system (joystick) error and the human error could be separated and if so to develope a general methodology to be followed in measuring the system error for any joystick and computer monitor system.

We succeeded in developing a general methodology (including software) that will not only measure the system error in the general system (as depicted in figure 1) but also will measure variances in the system error over five different areas of the screen (these areas are depicted in figure 2).

Full details of the systematic procedure for measurement of the system error along with the results when applied to our particular system may be found in the technical report "A Methodology for Separating Joystick Error from Human Adaption" on file at SAM/RZV. Here we will summarize the assumptions made, the general methodology and extensions that should be considered.

The computer generates a target on the screen and a cursor whose

position is manipulated by the joystick. The subject is then requested to place the cursor on the target and push the button when it is as close as possible. The computer then measures the distance from the cursor to the target and enters it as the error for that trial. Another target is now generated in the same area (figure 2) and the process is repeated (the number of data points recorded at each target is controlled by the investigator). The above experiment was repeated for distances from one to ten feet in increments of a foot. It was assumed that the error due to the adaptability of the human would vary linearly with the distance from the screen to the eye and the system error would be constant as the distance of the subject to the screen was varied (as is pictured in figure 3).

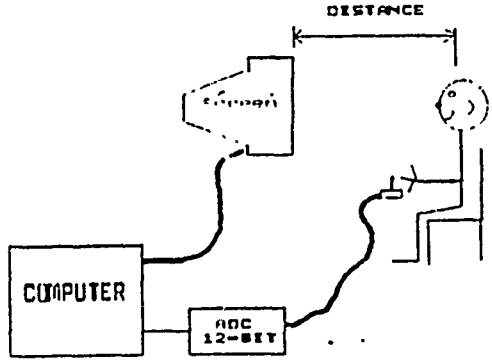


figure 1

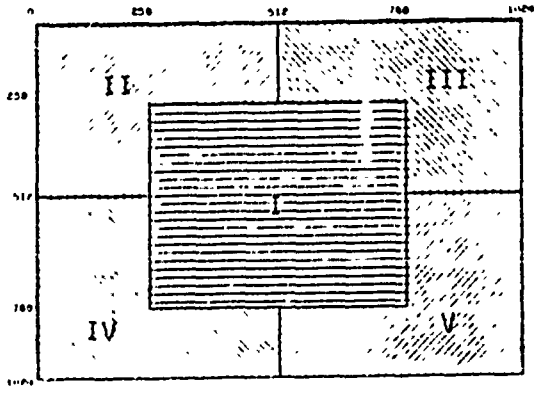


figure 2

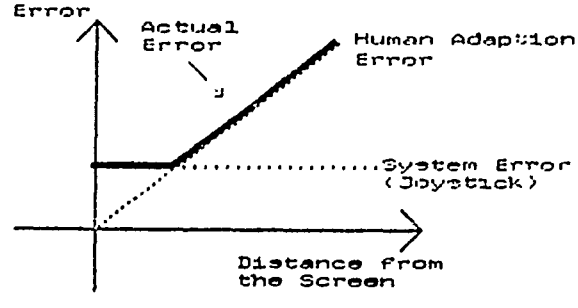


figure 3

Our assumptions proved to be true. For the system we investigated, the only distances important,i.e. the distances over which the error leveled off and became approximately constant, were those between 1 and 3 feet. The results would be much clearer if we had time to run the experiment from 1 to 3 feet in increments of 1/4 feet instead of increments of one foot. If one used a different system (and quite possibly subject since it should also be a function of visual acuity), it would be important to verify with a different system, the interval of distances that are of interest. We did note that there were outliers (we assumed due to fatigue since they were prone to come at the end of sessions). We eliminated those outside a standard deviation, but latter noted this was not the correct method. One needs to first determine the distribution of the noise, or to use distribution free methods. We did not have a chance to determine a good method for detection of variability in the system error for different regions of the screen. This needs to be done so the P.I. knows if variance in the error with respect to spatial coordinates is due to the system or subject. If there is a variance in the tracking error w.r.t. spatial coordinates, then one should see if there is a correlation between this error and the variance (spatially) in the tracking error. If there is a positive correlation we must find a way to subtract out the system error. The main source of error seemed to stem from head movement and eye fatigue. Therefore to eliminate as much error as possible we suggest that the experimental set up include a chin holder to fix distance to the screen more accurately, the experiment be run as efficiently as possible, and the subject made as comfortable as possible.

IV. ANALYSIS OF VEP INSTRUMENTATION:

The collection of VEP data involves the stimulus of the subjects eye

by an osciilatory stimulus and the recording of the subjects response (figure 4). Since the response, as seen by the surface mounted electrode is in the microvolt range, it is amplified before being sampled and stored in the computer for analysis and display. The system between the electrode and A/D converter is therefore to amplify the signal without changing it's inherent shape. Therefore we have been asked to investigate two questions about this portion of the system; is there any outside noise introduced and what effect does this part of the system have on the shape of the response. We were able to determine points where outside noise could be introduced and though this part of the system had an amplitude response that was flat over the region of interest, the phase response was not linear at the lower frequencies and could lead to distortion of the response for frequency components around 1 hz.

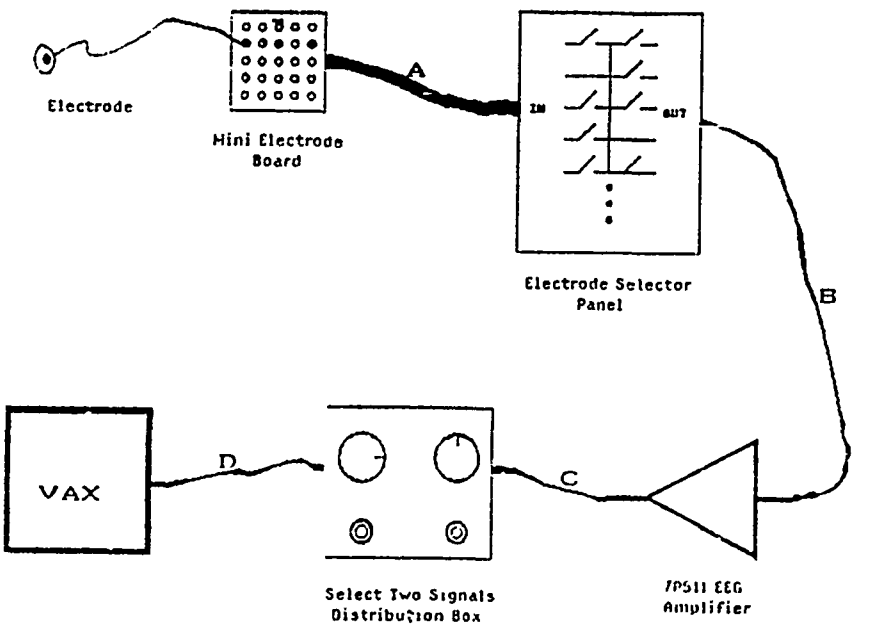


figure 4

Due to a restriction on the number of pages we may submit in this report we will only describe the system studied and our recommendations for improvement. In figure 5 we indicate how the bioelectric signals were replaced by test signals in the same frequency and amplitude range, the A/D converter and signal processor/display (VAX) were replaced by the HP35660A FFT Analyzer. In figure 6 the system analyzed is displayed.

The system studied has been shown to be susceptible to 60 hz and other time-dependent noise. This together with the amplifier manufacturer's recommendation to not use the 60 hz notch filter except in case of emergency (we assume this is because of the nonlinear characteristic introduced) leads us to make the following suggestions if the current system is to be used.

1. Use appropriate wire and cable.
    a. Electrode leads should be shielded.
    b. Cable B (figure 3 should be shielded.
    c. Cable A (figure 3) should be tested for crosstalk.
2. All coaxial cables should be terminated with proper coaxial connectors.
3. The distribution box and Vax should be enclosed with metal (shielded) panels.
4. Mil-C-39012 shorting plugs should be used to protect selected channels on the distribution box when not in use.

The system as a whole has a relatively flat amplitude characteristic. But the phase characteristic is not as linear. This suggests the signal may change shape between the electrode and the A/D converter. If one is to consider improving the characteristics of the system the following should be considered (listed in order of most beneficial effect):

1. Develop (we have been unable to find one currently available) an

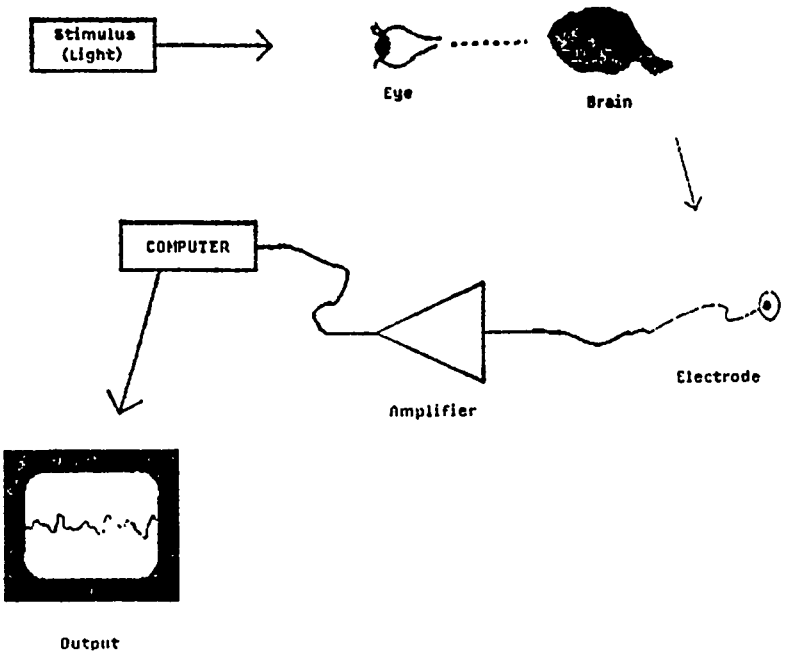


figure 5

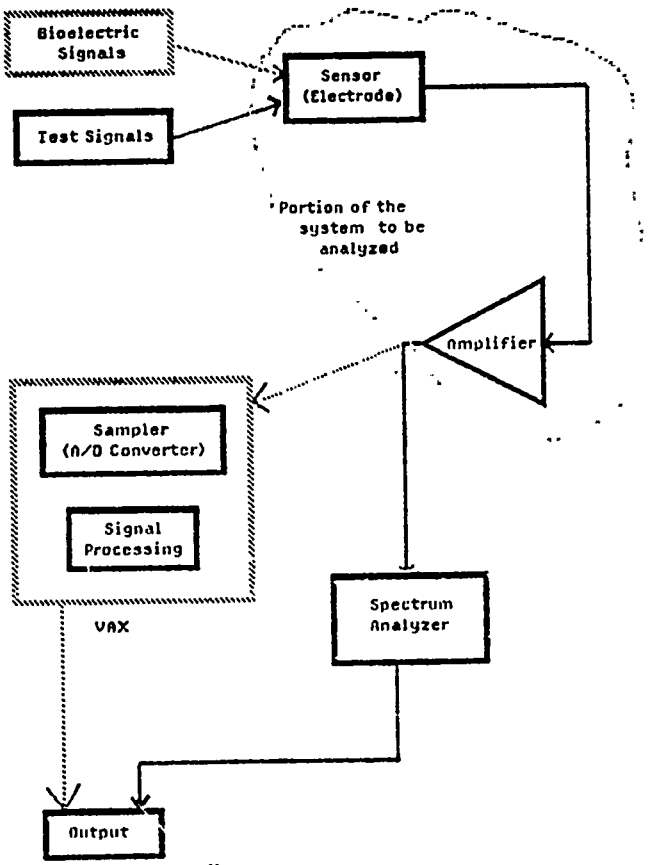


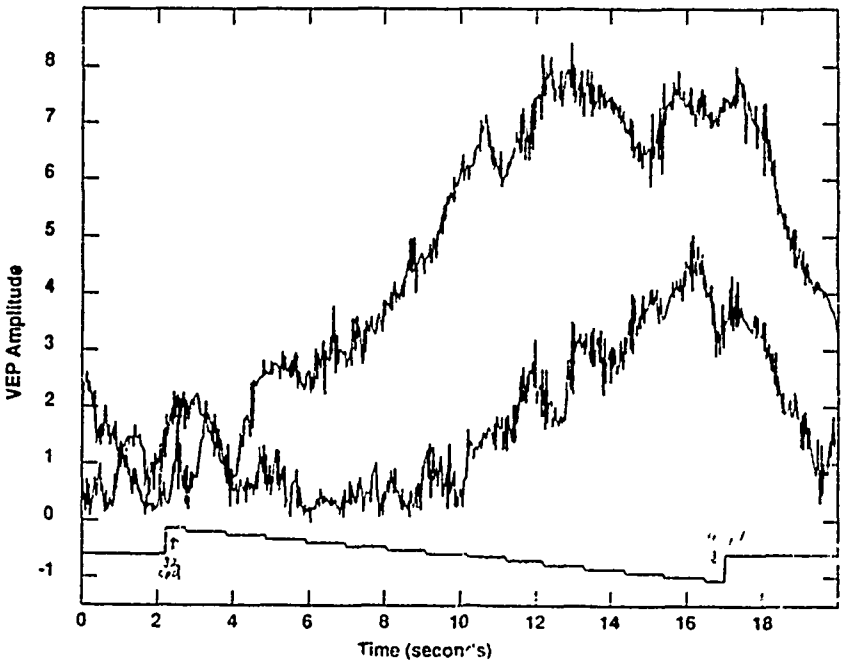
figure 6

electrode with a built in A/D converter. Then one can digitally amplify and design filters with completely linear phase.

2. Since the system (amplifier) has been characterized it may be possible to cancel the nonlinearity in the phase by inverse modeling the amplifier digitally. This may then cause difficulties for the P.I. since different filters are used at different times. One might be able to overcome this difficulty with an adaptive algorithim.
3. Use the HP35660A FFT Analyzer as the VEP data acquisition system. This should eliminate the need of the analog amplifier.
4. Investigate the use of phase sensitive analysis.
5. Use the HP35660A FFT Analyzer as a precursor to the current experiment, to determine if aberrant noise sources are present.
5. Look at bispectral analysis as a way of classifying the degree of linearity of the (any) system.

V. METHODS OF INFERRING VISUAL ACUITY FROM SWEEP VEP DATA:

Sweep VEP's are being developed as a rapid method for evaluation of visual acuity of unresponsive subjects. The data produced is very noisy as illustrated in figure 7. Current approaches to the estimation of visual acuity via sweep VEP data consist of an educated researcher simply stating as to where the response rises up out of the background noise. Sometimes this estimate is enhanced by averaging across experimental runs. We have researched several methods, outlined below, that may be automated to take out human judgement (and therefore any human bias). We suggest that each of these methods be tested for consistency and compared against the current procedures.



In the course of our investigation we have noticed the data curve (after onset of acuity) has a logarithmic shape. We can not be sure until more data sets are run, but if this proves true linear regression techniques may prove useful when applied to the data after exponential translation. Due to the noise in the data we also suggest robust procedures be applied. The following list contains several approaches that should be compared against each other.

(1) The current technique of estimation by the investigators[7,8].

(2) Using linear regression to estimate the baseline noise, estimate the parameters governing the transformed data, and then the point of intercept of the two curves [9,10].

(3) Robust regression [11], in the place of the linear regression in (2).

(4) Filtering of the raw data in time (types of filters should be both time domain and frequency domain) then applying methods (1), (2) and (3) separately [12,13,14,15].

(5) Filtering the data across experiments, as is currently done but using filters other than the average, then applying (1), (2), and (3) [12,13,14,15].

VI. PAPER ON TIME DOMAIN SIGNAL PROCESSING:

The following is the abstract of a paper submitted to the 1990 SPIE/SPSE conference, co-authored by Dr. Longbotham and Captain Barsalou. It has also been pointed out that these results should be extended to yield fixed points (or root signals) of morphological filters [16] and should result in more results in this area.

> Current research into the area of stack filters has demonstrated that when identical positive Boolean filters are "stacked " the output signals obey the stacking principal. It has also been shown that when the Boolean function at each level,B, is a rank order filter, the corresponding stack filter,S_B, is the same rank order filter. Root structure for these specific filters have also been discussed when the signal is finite and end effects are eliminated by padding.
>
> Maragos has used mathematical morphology to demonstrate that stack filters as defined above are finite max-min or min-max relations on the original quantized input values. We will extend this by demonstrating (without the use of mathematical morphology) a simple one-to-one and onto function that maps the class of positive Boolean functions onto the class of filters, S_B, such that S_B is the MAX of a set of MIN's operating on a subset of the binary windowed values. We also show there is a simple one-to-one and onto function that maps the class of positive Boolean functions onto the class of filters, S_B, such that S_B is the MIN of a set of MAX's operating on a subset of the binary windowed values. This allows us to determine the stack filter for any given positive Boolean filter B. Furthermore it serves to delineate what filters one may

implement with a stack filter.

The root structure for rank order filters when the class of signals is restricted to finite length signals with constantly padded input has been previously discussed. We have previously extended these results to include two-sided infinite length signals. In this paper roots to positive Boolean filters are discussed and it is shown how the inclusion of a monotone segment (i.e. padding) of certain length allows the inclusion of periodic solutions in the root structure.

It has been pointed out that it is unnecessary to restrict oneself to the stack filter definition above in order to develop digital filters that obey the threshold decomposition/stacking principles . A generalization may be made by letting the Boolean functions vary from level to level. We prove necessary and sufficient conditions on the variation of the Boolean filters from level to level in order for stacking to be upheld.

VII. INTRODUCTION TO NOISE, FILTERING, AND EXPERIMENTAL DESIGN :

The following is the tentative outline of a tutorial Col. Cartledge ask Dr. Longbotham to write that will help explain appropriate usage of linear, nonlinear, and robust filtering techniques when applied to VEP data.

1. Classification of Noise
   - additive
   - nonrandom
   - random (known and unknown)
   - random processes
   - other types
2. Filtering and Statistical Design for Noise
   - linear filters and nonrandom/periodic signals

statistical design for random noise and linear regression analysis
order statistic filters
order restricted statistical inference

3. Classification of Systems and Their Effects on Signals and Noise
linear system
nonlinear

4. More modern techniques that will have an impact on noise and system analysis of biological systems.
chaotic systems/signals
bispectral measure of nonlinearity
robust regression analysis
mathematical morphology
adaptive filtering
neural networks

The above paper is approximately 30% complete (20 pages completed) and will be completed as part of the ongoing research (RIP).

VIII. PAPER ON ROBUST TECHNIQUES IN IMAGE PROCESSING:

The following is the abstract of a paper on applications of robust signal processing to image analysis submitted by Dr. Longbotham and Captain Barsalou to the 1990 SPIE/SPSE conference.

The order statistic (OS) filters, from the median to the a-trimmed mean and including the OS filter, have gained popularity due to the ability to edit

outliers, yielding robust estimates. These filters all have one common defect. They assume the same "type" of outliers in each window and do not adjust to varying noise conditions. To be more explicit, the a-trimmed mean of length 2N+1 with filter coefficients having N/2 zeros on each end assumes there are N/2 outliers accentuated positively and N/2 accentuated negatively in each window.

In comparison let we will consider the least median of squares [LMS] filter, a generalized OS filter. The output of the LMS filter is the average of the N+1 "closest clustered" values in the window. Therefore if there are outliers as in the paragraph above the output will be the same as that of the corresponding a-trimmed mean. But the N outliers could be grouped differently, N-1 positively accentuated and N accentuated negatively for example, and still not change the output of the LMS filter as long as the N+1 nonoutlier group covers a smaller range of values than any other group of N+1 values. Therefore the LMS is an adaptive a-trimmed mean in that it has the same output as the a-trimmed mean under the outlier conditions the a-trimmed mean was designed for and adapts to varying outlier conditions in each window without any variance in the output. We will also discuss

various robust measures, their usage in impulsive noise situations, and the optimality of the LMS under them. Generalizations of the LMS filters and their applications to image processing will be discussed. We will then discuss real time implementation of these adaptive robust filters via stack filters.

IX. RECOMMENDATIONS:

Since there were three separate projects involved we thought it best if all recommendations were placed in their respective sections (the last part of sections III,IV, and V).

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1989 USAF/UES SUMMER FACULTY RESEARCH PROGRAM

GRADUATE STUDENT RESEARCH PROGRAM

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FINAL REPORT

HIGHER PLANT HORMONES EFFECT UPON CHLAMYDOMONAS PHOTOTAXIS

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Date: August 24, 1989

Contract No. F49620-88-C-0053

Higher Plant Hormones Effect Upon *Chlamydomonas* Phototaxis

by

Rex C. Moyer

ABSTRACT

The long term goal of this program is develop a way of testing drugs for their ability to improve night or long wave length vision of pilots and/or to shield their vision in certain wavelengths from laser or nuclear blast-induced flash-blindness. The specific goal of this summer's SFRP Fellowship was to determine if our recently developed phototaxis assay of *Chlamydomonas reinhardtii* CC-125 could be utilized to detect compounds which would stimulate phototaxis. The higher plant hormones Indole-3-acetic acid, Indole-3-butyric acid (auxins), Abscisic acid, Gibberellic acid, and the auxin-like weed killer, 2,4-Dichlorophenoxyacetic acid (2,4-D) were tested. This research showed that ethanol, in a final concentration of 0.28M does stimulate phototaxis. Indole-3-acetic acid and Indole-3-butyric acid stimulate phototaxis slightly more than ethanol alone. Thus it is not possible to determine at this point if they do stimulate phototaxis. Gibberellic acid, abscisic acid and 2,4-D, however, do significantly stimulate phototaxis over the ethanol controls.

It is safe to conclude that the phototaxis assay can rapidly and sensitively detect compounds which will stimulate phototaxis. This opens the possibility of testing drugs which may affect pilot vision. It also appears that this research may provide a rapid and sensitive bioassay for certain higher plant hormones as well as provide a new tool for the study of biochemical evolution in plants.

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My thanks to Dr. Jerome Schmidt, Chief of Microbiology, Division of Epidemiology for allowing us the use of their pH meter and to Dr. Vee Davison for aid in its use.

My personal thanks to my graduate students George Kim and Dagmar Fertl who formed an important part of our team and who worked hard this summer to accomplish our collective goals. I also thank Mr. Tod Romo, Trinity University undergraduate student for digitizing the cell size distribution curves.

Finally, my thanks to Dr. Russell Burton for his conscientious overseeing of the summer programs at Brooks, AFB. His "Brown Bag Seminars" and SAM Summer Seminars Series developed collegiality among the faculty and graduate student Fellows. My thanks also for his support of the seminar by Dr. John B. Phillips of Indiana University.

I. INTRODUCTION

The long term goal of this program is to find a way of testing drugs for their ability to improve night or long wave length vision of pilots. A literature survey pointed to extracts from the plants in the Panax, Eleutherococcus, and Schizandra genera as potential sources of these drugs. A major problem interfering with the attainment of this goal is that no simple inexpensive test system exists that can assess the pharmacological effect of a drug directly upon visual biochemical apparatus without complicating indirect effects. With the discovery that the phototactic unicellular algae, Chlamydomonas, employs rhodopsin, the human visual pigment, it becomes theoretically possible to study the effect of drugs on an isolated visual system. Any drugs that enhance or alter phototaxis in Chlamydomonas may be of interest in human vision.

II. OBJECTIVES OF THE RESEARCH EFFORT

The objectives of this component of the 1989 Summer Faculty Research Program (SFRP) was to utilize our previously developed phototaxis assay to assess if it was amendable to detecting compounds that would affect the phototactic response of Chlamydomonas. The compounds that were selected were higher plant hormones. Some higher plant hormones have been detected in algae, but it has not been demonstrated if the higher plant hormones play a role in algal physiology or not. This Final Report describes the results of experiments which suggest that some higher plant hormones do stimulate the phototactic response of Chlamydomonas to 500 nm light as compared to controls.

III. MATERIALS AND METHODS

A. Cultures:

1. Chlamydomonas reinhardtii strain CC-125 was obtained from Dr. Elizabeth Harris, Chlamydomonas Genetics Center, Duke University, Durham, N.C. and has been maintained in the Trinity University stock culture collection on HSA agar. CC-125 is a wild type mt+ from original wild type strain 137c.

B. **Culture Media:**

1. Trace Elements mix (1000x) from Elizabeth Harris, personal communication.

Trace Element	Weight (g)	ml dist. H_2O
Na_2 EDTA	50.0	250
$ZnSO_4.7H_2O$	22.0	100
H_3BO_3	11.4	200
$MnCl_2.4H_2O$	5.06	50
$FeSO_4.7H_2O$	4.99	50
$CoCl_2.6H_2O$	1.61	50
$CuSO_4.5H_2O$	1.57	50
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.10	50

Dissolve each salt in distilled or deionized water as indicated. Disodium EDTA must be dissolved in boiling water. Prepare $FeSO_4$ last since it oxidizes in solution. Mix all solutions except EDTA. Bring the mixed salts solution to a boil and then add the Na_2 EDTA solution. The mixture should turn green. When all the salts are dissolved, cool the solution in 70°C. Keeping the temperature at 70°C, adjust the pH to 6.7 with 80-90 ml of hot KOH (20%). Standardize the pH meter at 70°C also. Do not use NaOH to adjust the pH.

Let final solution stand for 1-2 weeks in a cotton stoppered flask. Shake the solution once a day. The solution should turn purple and leave a rust-brown precipitate. After two weeks, filter the solution through 2 layers of whatman #1 filter paper. Refrigerate or freeze in convenient aliquots.

2. Concentrated Beijerinck's solution:

Salt	wt (g/l)
NH_4Cl	100
$MgSO_4 . 7H_2O$	1
$CaCl_2 . 2H_2O$	2

3. Concentrated phosphate

Salt	wt (g/l)
K_2HPO_4	288
KH_2PO_4	144

4. Media Formulations

Constituent	HSA	HS
Conc. Beijerink's	5 ml	5 ml
Conc. Phosphate	5 ml	5 ml
Trace Elements	1 ml	1 ml
Sodium Acetate · ($3H_2O$)	2.0 gm	
Agar*	-	-
Distilled water	990 ml	990 ml

* Agar Conc. - ordinary agar plates 15 gm/l
agar slants 20 gm/l

C. ROUTINE MAINTENANCE OF CULTURES

Slants of 5 ml HSA were prepared in screw-capped glass tubes (pyrex No. 9825). The tubes with media were autoclaved at 121°C for 20 minutes then cooled in a slanted position. Cultures were transferred aseptically with an inoculating loop. All cultures grown on agar medium were incubated at 23°C in an environmental chamber with continuous lighting. Lighting was provided by 4 Sylvania 40W cool white bulbs placed 64 cm above the agar cultures. Cultures were transferred weekly.

D. Growth of Chlamydomonas Vegetative Cells.

HS or HSA Agar was made up in 500 ml batches and autoclaved for 20 min at 121°C in a 2 liter Erlenmeyer flask. The molten medium was transferred at 25 ml/plate to standard 100 x 15 mm plastic petri dishes. Plates were left undisturbed at room temperature in a laminar flow hood for the agar to solidify.

The algae were grown in a liquid medium overlay over an agar medium base, for example. 5 ml HS broth over a 25 ml HS agar

base. Cultures were inoculated with 10^6 algal cells/ml or more final concentration and yielded 150-600 million cells per plate after 2-3 days incubation.

E. Cell Harvest and Cell Counts

Cells were harvested from the broth overlay by adding fresh HS or HSA broth and suspending in cells in a final volume of 7.0 ml. This cell suspension was further diluted in HS broth to an OD600 of 0.5 to 1.0 in a 10 mm cuvette. Algal cell counts were made with a Model ZBI Coulter Cell Counter with attached Coulter Channelyzer II for obtaining cell size distributions. Culture samples were diluted roughly 1:400 into Isoton and counted four times and cell size distribution obtained according to directions supplied by the manufacturer.

F. Phototaxis Assays

Time scans (phototaxis assays) were performed as described previously (Schroeder, Taboada, and Moyer, Manuscript in preparation). The phototaxis assays were recorded as optical density at 500nm [OD(500)] during an 8 minute time scan of 3.0 ml of motile Chlamydomonas cells in a 10mm cuvette.

The difference between the OD(500) of the cells at the beginning of the time scan and the maximum OD(500) obtained (OD max-OD initial) was referred to as ΔOD(500).

G. Plant Hormones

The following plant hormones were purchased from Sigma and were made up at 0.01 M concentration in absolute ethanol and stored at -20°C in the dark.

Indole - 3 - Acetic Acid (IAA)
Indole - 3 - Butyric Acid (IBA)
Abscisic Acid (ABA)

Gibberellic Acid (A3)

2,4 - Dichlorophenoxyacetic Acid (2,4-D)

The plant hormones were added to 3.0 ml cell suspension in HS to a final concentration of 1.67 x 10^{-4}M to asses their effects upon phototaxis. Controls included HS broth and the equivalent volume of absolute ethanol alone.

H. Motility Assessment

A 10 µl sample was aseptically removed from a plate and placed on a cover slip and examined as a hanging drop under a microscope. The percentage of motile cells was estimated.

I. Digitizing Cell Size Distributions and Analysis of Population Fraction in Various Size Classes.

A Sun 4/110 Sparc Workstation with a CalComp 9300 digitizing tablet was used to digitize the cell size distribution profiles obtained from the Coulter Channelyzer II. Most of the interface routines, the tablet handling routines, and the interpolation routines were adapted from a program by Dr. Glenn Kroeger (Geology Department, Trinity University) to digitize seismic data. The data from the tablet was scaled to a user defined unit system and derotated according to an initially entered baseline. Interpolation between the sampled points was calculated using a hermite cubic spline algorithm.

First, two points along the baseline of the graph were digitized. Then the lower left corner, the zero, of both axes was digitized as a reference mark from which all other points were measured. A second reference mark along the X-axis was digitized and the number of units between that mark and the initial reference mark was used to scale all data entered on both the X and the Y axis. Comparison of the raw data retained from the tablet from a 1 cm square area for both axes showed a reasonable degree of

orthogonality between the two and no further correction on either axis was performed. Derotation was not performed, but the lengths of the calculated line segments, as calculated internally by the digitizing tablet were used.

The cell size profile was sampled by digitizing the initial point, then digitizing roughly each peak, trough and inflection point, and on segments of larger curves, evenly spaced data points were taken over the whole curve. Before any calculations were performed, the data was resampled via the spline interpolation. Each profile was resampled to a theoretical interval of 0.1 mm. Calculations for total area was performed by totaling the area (height) of each segment comprising the curve, and piecewise area calculations were made by totaling the segments under a given length of the curve.

IV. RESULTS

Effects of the Higher Plant Hormones, on Chlamydomonas phototaxis.

A 3 day old culture growing on a bilayer HS medium was harvested as described. The culture was diluted with HS to yield an OD(500)= 0.8. A phototaxis assay was performed on the fresh culture to serve as a base-line control. All seven control cell suspensions were time scanned sequentially. Then the control agent, or hormone was admixed. About 1-2 minutes transpired between the time the hormone was added and the phototaxis assay was begun. The time scans for each sample required 8 minutes. Each of the immediate post hormone phototaxis assays were then again assayed for phototaxis which yielded a time span of about 30 minutes from the time of addition of the hormone or control agent. The time scans were then repeated again on all the samples at about 4 hours after the hormone or control agent had been added. The results of this experiment are recorded as ΔOD(500) in Table 1. Those results are again expressed in a bar graph in Fig.1 as

percent of the control value before addition of hormone or control agent which was set at 100%. Several interesting observations emerge from this experiment. Absolute ethanol alone added to the 3.0 ml algal suspension has a final concentration of 0.28M (1.64% v/v) and significantly stimulates phototaxis as compared to the controls. It appears that the roughly equivalent stimulation of phototaxis by IAA is due to the ethanol and not the hormone. The stimulation of phototaxis by the hormones IBA, A3, 2,4-D and AbA are in excess of the stimulation by ethanol alone. The ethanol and the hormones produce their stimulatory effect within 8-minute time scan and appear to change the shape of the OD(500) vs time profile which represents the rate of influx of algae into the light path. The OD max of the OD(500) vs. time profile becomes shifted later in the time scan as shown in Table 2. It can be seen that the average time to reach OD(500) max is 2.13 min before ethanol or hormones in ethanol are added to the algae. The time to reach OD(500) max is delayed to about 7 minutes after ethanol or hormones in ethanol were added (the values of the HS control were omitted in the determination of the average values)

V. DISCUSSION

Higher plants synthesize hormones that influence the growth and differentiation of other regions of the plant. These plant hormones can be divided into five major categories: auxins (indole derivatives) and gibberellins (gibbane ring derivatives) which stimulate cell enlargement; cytokinins (adenine derivatives) which promote cell division; and absisicic acid and the gas ethylene, which inhibit growth during winter and unfavorable environmental conditions. These categories of activities of the plant hormones are only nominal since Leopold (1972) has recorded the existence of known effects of each of the five classes of plant hormones on most of the twelve different higher plant developmental processes.

The Nobel Prize-winning research of Sutherland showed that animal hormones catalyze various enzymic processes within target cells

through second messenger carriers. such as cyclic AMP. cyclic GMP, inositol triphosphate and cytoplasmic calcium. In contrast to animal hormones, plant hormones do not utilize cyclic nucieotides as second messengers even though it is found in plants. In contrast, inositol triphosphate and cytoplasmic calcium do serve as second messages in higher plants (Leopold, 1987).

Most studies of hormones in plants have focused on angiosperms. The farther down the presumed evolutionary tree one looks, the fewer actual chemical identifications of putative hormones one sees. A number of algal extracts have showed auxin-like or gibberellin-like activity when tested in angiosperm bioassays, however, remarkably few of the chemicals responsible have been identified. Even fewer reports demonstrate that chemicals acting as hormones in angiosperms actually function as hormones in algae. Jacobs (1985) in a review, describes gibberellin-like activity from extracts of the multicellular, differentiated green alga, Caulerpa paspaloides when they were tested in the lettuce-hypocotyl and Avena - leaf bioassays. A few seed-plant hormones have been demonstrated by mass spectrometry and other advanced methods in lower plants. The major auxin, Indole-3-acetic acid has been demonstrated in the green alga Caulerpa (Jacobs et al, 1985), and phenylacetic acid in the green alga, Enteromorpha (reviewed by Jacobs, 1985). The current status of information is that the angiosperm hormone IAA is present in at least one genus of green algae and has hormone-like action there. In a classic paper, Chorin-Kirsh and Mayer (1964) showed that IAA and ethylenediamine tetraacetic acid (EDTA) stimulated phototaxis in motile but non-phototactic Chlamydomonas snowiae cultures. The optimal pH of the IAA effect was between 5.4 and 5.8. The optimal concentration of IAA was between 10^{-4} and 10^{-5}M, with little or no response at 10^{-6}M. The investigators also showed that indole butyric acid, indole propionic acid, naphthalene acetic acid, and 2, 4 dichlorophenoxyacetic acid (2,4-D) were also effective in stimulating phototaxis.

The famous and profitable weed killer, 2, 4-D has chemical

properties close enought to endogenous auxin to stimulate plant growth but is chemically different enough to escape the endogenous auxin regulating systems. The herbicide, 2, 4-D affects dicots much more than monocots and is therefore used to kill broad-leaf dicot weeds. The mechanism of action of 2, 4-D is generally unknown although it is widely known to have auxin activity and has been misleadingly stated to cause a plant to "grow itself to death". The common effect of 2, 4-D on plants is to induce deformed growth and epinastic effects that arise from the common property of all auxins to enchance ethylene production. Ethylene is well known for causing epinasty. Since 2, 4-D is indirectly responsible for nastic plant movements and tropisms, it was logical to test its effect on phototaxis in Chlamydomonas.

Bertagnolli and Nadakavukaren (1974) studied the physiological responses of the green microalga, Chlorella pyrenoidosa to 2,4-D. At an optimum level of 5×10^{-4}M, 2,4-D stimulated oxygen uptake and production and glycollate accumulation, but did not alter size, dry weight, degree of synchrony, pigment content of cells, nor ethylene production. The probable target of the 2,4-D in Chlorella were the chloroplasts and it could be interfering with photorespiratory processes.

The data collected in this study show that ethanol at a final concentration 0.28M (1.64% v/v) stimulate ΔOD(500) of Chlamydomonas 60-70% over the ΔOD(500) of the cells before ethanol addition. In view of this stimulation by the alcohol alone and a similar level of phototaxis enhancement by IAA and IBA, I conclude that the IAA and IBA do not significantly stimulate phototaxis in Chlamydomonas. Gibberellic acid, 2,4-D and abscisic however do significantly stimulate phototaxis over the stimulation by ethanol alone. The A3, 2,4-D, and ABA all stimulated phototaxis within the 1 minute period after the hormone was admixed and the 8-minute time scan. Maximal stimulation was observed after 30 minutes, but the effect was still strong after four hours of contact with these three hormones.

Although these experiments were carefully done, I feel these experiments need to be repeated but using more concentrated hormone in ethanol solutions so that the volume of ethanol added to the 3 ml cuvette of cells is only 5-10 µl instead of 50µl. A 5µl volume of hormone should lower the ethanol final concentration to 0.028M which aught not to stimulate phototaxis. It is also important to test several hormone concentrations on phototaxis and to determine the effect of pH on the process.

These studies have demonstrated a hormone-like stimulation of phototaxis by GA3, 2,4-D, ABA, and perhaps IBA, but not IAA. These studies do accomplish our goal of showing that our phototaxis assay can be used to detect compounds that will stimulate algal phototaxis. These studies also stimulate the intriguing question on biochemical evolution: did the angiosperm hormones only appear as chemicals after the vascular plants evolved on land, or did the vascular plants evolve hormonal uses for chemicals that were already present, but not functioning as hormones in evolutionary earlier and simpler forms?
These studies also open up some interesting and potentially valuable future avenues for exploration. This technique may provide a rapid, sensitive and inexpensive bioassay for certain plant hormones. Standard plant hormone bioassays may require hours or days where as this assay was completed in 9 minutes and was attained maximal sensitivity in 38 minutes. Specific chemical analysis of plant hormones may require expensive mass spectrography and gas chromatography.

VI. RECOMMENDATIONS

I recommend that this avenue of research be continued. This research together with previous research, I feel satisfies Air Force objectives in developing rapid and sensitive screening technique for detecting compounds that may help (or hinder) human vision. This research appears to provide a rapid and sensitive bioassay for certain plant hormones and, with some additional research, may

provide a model for probing the relationship in vision between light-responsive compounds and cyclic nucleotides. Finally, it could provide a new approach to the understanding of biochemical evolution.

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Table 1. **Effect of Higher Hormones on Chlamydomonas Phototaxis Immediately, 30 Minutes, and 240 Minutes after Addition of the Hormones.** [1]

	OD500 (-)1min	OD500 (+)1 min	OD500 (+)30 min	OD500 (+)240 min
HS Control	.3164	-	.3664	.2924
ETOH Control	.3354	.3824	.5470	.5755
IAA	.4117	.6471	.6304	.6326
IBA	.3648	.6470	.7503	.6612
A3	.3258	.5816	.7730	.6806
2,4D	.2819	.6231	.7613	.6473
AbA	.3118	.5891	.7607	.6543

[1]The final concentration of hormone added in each case was 1.67×10^{-4} M HS control, HS broth added of the same volume (50 µl) as the volume of hormone added;

ETOH control, 50µl absolute ethanol added;

IAA, Indole-3-acetic acid, in abs. ETOH (50µl)

IBA, Indole-3-butyric acid in abs. ETOH (50µl)

A3, Gibberellic acid in abs. ETOH (50µl)

2,4-D, 2,4 - Dichlorophenoxyacetic acid in abs. ETOH (50µl)

AbA, Abscisic acid in abs. ETOH (50µl)

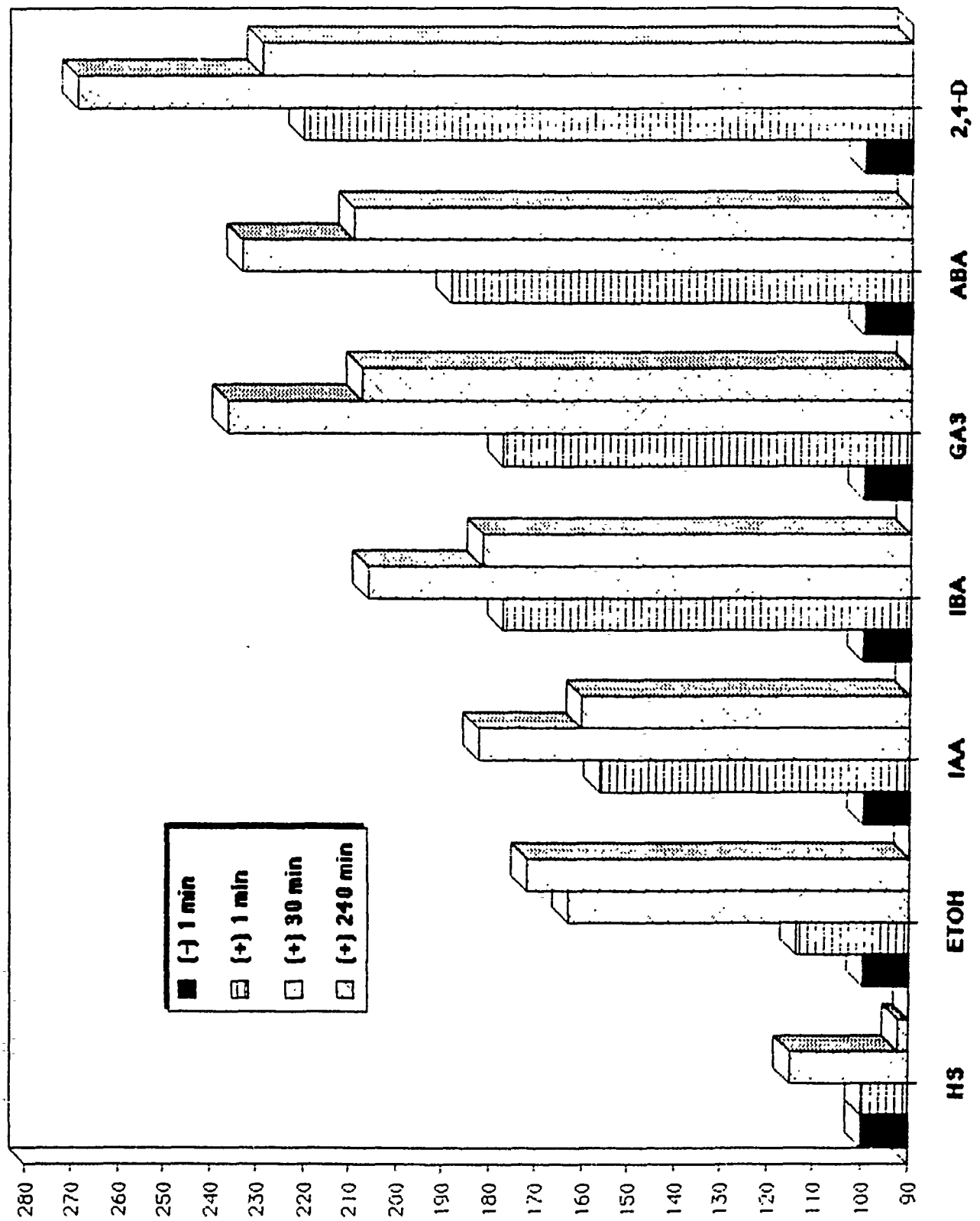
Table 2. Effect of Ethanol and Hormones Dissolved in Ethanol upon the Time to Reach OD500 max in the Phototaxis Assay Time Scan.[1]

Time To Reach OD500 Max in 8-Minute Time Scan.

Substance Added	OD500 Max (-) 1 min	OD500 Max (+) 1 min	OD500 Max (+) 30 min	OD500 Max (+) 240 min
HS Broth	6.46	-	2.56	6.53
ETOH	1.80	5.90	5.46	8.0
IAA	2.60	7.50	6.06	8.00
IBA	2.60	6.43	7.06	7.46
A3	2.25	7.80	6.80	7.46
2,4D	2.00	8.00	8.00	8.00
AbA	1.53	8.00	8.00	8.00
Mean (w/o HS)	2.13	7.27	6.90	7.82

[1]The hormone final concentration in each case was 1.67×10^{-4}M; the ethanol final concentration was 0.28M; abbreviations are the same as Table 1.

Fig. 1. Effect of higher plant hormones on Chlamydomonas phototaxis, immediately, 30 minutes, and 240 minutes after addition of the hormones. A phototaxis assay at 500nm was performed on a freshly harvested algal culture. One minute later the control agent or hormone was added (hormone final conc. was 1.67×10^{-7}M). Within 1 minute another phototaxis assay was initiated (Time scan requires 3 minutes). Thirty minutes later another phototaxis assay was performed and again 240 minutes later. The data were collected as OD(500) max - OD(500) initial [OD(500)] but are presented here as percent of the phototaxis performed on the culture before the control agent or hormone was added. The initial (-) 1 min phototaxis assay was represented as 100% and the other assays performed after the hormone was added to the cells are related in percent to the (-) 1 min. assay.


(-) 1 min
(+) 1 min
(+) 30 min
(+) 240 min
HS
ETOH
IAA
IBA
GA3
ABA
2,4-D
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
% OF CONTROL

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FINAL REPORT

"Influence of radio frequency radiation on psychotropic drug effects"

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INFLUENCE OF RADIOFREQUENCY RADIATION ON PSYCHOTROPIC DRUG EFFECTS

Raymond Quock

ABSTRACT

The increasing use of microwave-emitting radar and communications equipment has generated great interest in possible bioeffects of radio-frequency radiation (RFR). Part of the research effort involves studying potential RFR-induced changes in psychotropic drug effects. The present investigation was conducted to ascertain the influences of various specific absorption rates (SARs) of RFR at two different frequencies upon behavioral effects of the benzodiazepine chlordiazepoxide. Male ICR mice were tested in a mouse staircase paradigm following RFR exposure (4, 12 or 36 W/kg SAR, 1800 or 4700 MHz) and/or pretreatment with chlordiazepoxide (8, 16 or 32 mg/kg). The findings indicate that certain levels of RFR are indeed capable of influencing effects of chlordiazepoxide, mainly reduction of sedative and/or anxiolytic effects of the drug. These data suggest that RFR exposure bioeffects include alteration of psychotropic drug effects.

ACKNOWLEDGEMENTS

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Thanks are also due to a great many people in the Radiation Sciences Division at the United States Air Force School of Aerospace Medicine (USAFSAM): Mr. James H. Merritt and Dr. B. Jon Klauenberg for giving me guidance and the benefit of their experience; Mr. R. Richard Bixby for conducting the necessary dosimetry studies; Dr. Melvin R. Frei for his interest and wise counsel; and Mr. William Hurt and the military personnel of the Directed Energy Bioeffects Laboratory, notably SSgt. Stanley Carter, who made sure I always had animals to work with, and A1C Keith Tickle, SSgt. Ernie Ferguson and SSgt. George (Al) Johnson, who assisted in the majority of experiments described in this report.

I. INTRODUCTION

There is increasing deployment and utilization by the USAF of microwave-emitting radar and communications equipment. The bioeffects of radiofrequency (RF) electromagnetic fields are still poorly understood. There is an intensive ongoing effort in the Radiation Sciences Division of USAFSAM to examine the potential health hazards of microwave radiation to personnel who operate such equipment. The objective of such activity is to better define safety standards for exposure to RF radiation.

Benzodiazepine drugs such as diazepam (Valium^{R}) and chlordiazepoxide (Librium^{R}) are widely used in medicine for their sedative/hypnotic, skeletal muscle relaxant, anxiolytic and anticonvulsant properties. It is conceivable that subjects who use such medications may include personnel who man microwave-emitting systems. Whether exposure to RF radiation might alter effects of such psychotropic drugs is uncertain and was addressed in this research.

II. OBJECTIVES OF THE RESEARCH EFFORT

The aim of this research was to ascertain whether microwave radiation might alter behavioral effects of chlordiazepoxide in the mouse staircase test.

The mouse staircase test is a simple and efficient paradigm for assessing sedative and anxiolytic effects of benzodiazepines (Simiand et al., 1984; Quock et al., 1987). In this test, the endpoints are the

number of steps ascended and the number of rears during an animal's initial 3 min in an enclosed staircase, consisting of 5 identical (2.5 cm high, 10 cm wide and 7.5 cm deep), ascending steps. Step-climbing is indicative of locomotor activity and drug suppression of step-climbing reflects a sedative drug effect. On the other hand, rearing behavior is indicative of anxiety and drug suppression of rearing reflects an antianxiety drug effect. Benzodiazepines in increasing doses will decrease the number of rears without affecting the number of rears, though, at high doses, both step-climbing and rearing will be suppressed.

This research was designed to determine whether sedative and/or antianxiety effects of chlordiazepoxide might be sensitive to alteration by various RF exposure conditions, namely specific absorption rate (SARs) of 4, 12 and 36 W/Kg at 2 different frequencies, 1800 MHz (resonance frequency for 30-g mice) and 4700 MHz (supraresonance frequency). Animals were tested in pairs. Each pair of mice were subject to the following sequence of treatments:



A. Each pair of mice treated IP with one of 3 doses of chlordiazepoxide (8, 16 or 32 mg/kg) or vehicle (physiological saline).

B. Each pair of mice placed inside cylindrical plexiglass restrainers 20 min after pretreatment and 5 min prior to microwave exposure. During these 5 min, the restrainers are placed inside the anechoic chamber at their pre-designated exposure positions in front of the horn.

C. Each pair of mice exposed to microwave radiation for 5 min.

D. Each pair of mice placed into individual staircases immediately after microwave exposure (30 min after pretreatment), activity of each mouse in the staircase is videotaped for 3 min. The tapes are later reviewed and the number of steps ascended and of rears exhibited by each mouse counted.

III. RESULTS (See TABLES 1-2 and FIGURES 1-4)

A. In sham-irradiated mice, vehicle pretreatment results in 27-33 steps ascended and rears. Increasing doses of chlordiazepoxide will sequentially increase step-climbing without affecting rearing (8 mg/kg); increase step-climbing while decreasing rearing (16 mg/kg); and decrease both step-climbing and rearing (32 mg/kg).

B. At 1800 MHz microwave exposure, increasing SAR's of 4, 12 and 36 W/kg generally did not significantly alter step-climbing and rearing behavior of mice treated with vehicle or 8 or 16 mg/kg chlordiazepoxide. However, mice pretreated with 32 mg/kg chlordiazepoxide exhibited significantly greater step-climbing at 12 and 36 W/kg SAR and significantly greater rearing at 36 W/kg SAR.

C. At 4700 MHz microwave exposure, increasing SAR's of 4, 12 and 36 W/kg generally did not significantly alter step-climbing and rearing behavior of mice treated with vehicle or 8 or 16 mg/Kg chlordiazepoxide. However, mice pretreated with 32 mg/kg chlordiazepoxide exhibited significantly greater step-climbing at all SAR's and significantly greater rearing at 36 W/kg SAR.

D. If the basic premise of the staircase test remains consistent under RF radiation, the tendency for the SAR-related increase in steps ascended by mice treated with 32 mg/kg chlordiazepoxide would be interpreted to mean that RF might reduce the sedative side effect of chlordiazepoxide. The relative intactness of the reduced rears in mice receiving 32 mg/kg chlordiazepoxide and exposed to RF suggests that RF does not alter the antianxiety effect of chlordiazepoxide (save exposure to 36 W/kg of 4700 MHz, which suggests RF antagonism of the antianxiety effect of 32 mg/kg chlordiazepoxide).

IV. RECOMMENDATIONS

The findings of this study are, of course, preliminary. There is indication that certain frequencies of RF radiation might indeed alter effects of certain doses of chlordiazepoxide in the mouse staircase test. Sedative and antianxiety effects of chlordiazepoxide appear to be diminished by exposure to high levels of RF radiation. Alternatively, microwave-induced heating may induce a thermal stress and evoke changes in thermoregulatory behavior. Additional research is recommended along the following lines:

A. Exposure conditions

1. Different frequencies
2. Longer times for exposure
3. Higher SAR's
4. Do test in field under RF

B. Paradigm

1. Species difference? Rats more susceptible to RF effect?
2. Does heating cause behavioral thermoregulatory responses (e.g. escape attempts) that invalidate interpretation of the meaning of steps and rears?
3. Tactical consideration - styrofoam staircase needs to be painted with quinine to prevent gnawing and perhaps lined with plastic to prevent climbing (by digging claws into walls).

. . .

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LEGENDS OF TABLES

Interaction between drug treatment (8, 16, or 32 mg/kg chlordiazepoxide or saline vehicle) and RF radiation exposure (SAR 4, 12 or 36 W/kg or sham) on animal performance in the mouse staircase test. Each cell represents one of 16 possible combinations of drug treatment and RF radiation exposure. Each pair of numbers in each cell represents the number of steps ascended and the number of rears, respectively, exhibited by an individual mouse during 3 min of observation. Below the broken line in each cell is indicated the mean number of steps ascended ± SEM on the top line and the mean number of rears ± SEM on the bottom line.

TABLE 1. 1800 MHz (resonance frequency)

TABLE 2. 4700 MHz (supraresonant frequency)

TABLE 1

	SHAM		SAR 4 W/kg		SAR 12 W/kg		SAR 36 W/kg	
Saline Vehicle	44-26	23-37	11-13	26-34	28-33	52-36	41-31	35-18
	38-29	32-36	38-26	37-40	37-21	34-32	72-30	32-22
	10-21	30-33	35-27	28-29	15-19	32-15	43-22	42-31
	29-30	28-33	45-37	37-29	8-13	30-27	53-41	6-15
	10-27	30-18	26-26	32-26	32-23	41-31	25-22	13-19
	36-23	35-25	37-28	27-16	32-20	45-24	36-33	39-27
	28.8 ± 3.0		31.6 ± 2.5		32.2 ± 3.4		36.4 ± 5.0	
	28.2 ± 1.7		27.6 ± 2.2		24.5 ± 2.1		25.9 ± 2.2	
Chlordiazepoxide 8 mg/kg	22-15	43-17	23-27	9-18	38-34	43-31	62-42	38-17
	64-24	33-25	69-46	21-21	61-32	76-39	36-30	36-43
	34-14	20-30	38-17	43-27	43-23	69-31	40-23	41-38
	66-42	23-21	6-19	57-25	25-32	38-20	49-39	87-32
	54-26	31-12	9-7	41-9	10-14	27-26	14-21	38-22
	48-23	48-27	42-25	55-29	46-29	60-38	32-24	66-38
	40.5 ± 4.6		34.4 ± 6.0		44.7 ± 5.6		44.9 ± 5.5	
	23.0 ± 2.4		22.5 ± 2.9		29.1 ± 2.1		30.8 ± 2.6	
Chlordiazepoxide 16 mg/kg	42-26	54-22	26-14	35-21	49-24	36-22	33-46	42-13
	4-7	64-27	67-30	40-27	45-28	65-33	83-20	30-9
	10-10	36-24	26-27	14-12	6-8	54-30	22-32	38-35
	40-21	27-6	28-20	80-36	23-22	71-16	63-32	31-12
	21-26	50-15	18-15	39-17	11-9	51-31	13-18	39-26
	42-22	54-16	93-38	54-8	33-12	40-12	25-17	33-11
	37.0 ± 5.3		43.3 ± 7.2		40.3 ± 5.8		37.7 ± 5.4	
	18.5 ± 2.2		22.1 ± 2.8		20.6 ± 2.6		22.6 ± 3.3	
Chlordiazepoxide 32 mg/kg	6-9	16-4	15-10	0-6	0-0	27-9	37-15	34-13
	6-7	21-2	28-3	12-9	34-17	14-8	33-11	16-10
	17-8	30-21	5-4	17-9	36-25	47-17	6-4	13-3
	19-10	2-3	0-0	47-20	10-10	14-5	10-15	48-13
	0-0	19-8	0-0	24-15	5-9	15-4	20-12	40-15
	4-4	0-0	7-8	32-10	0-0	46-15	20-11	36-10
	11.7 ± 2.8		15.6 ± 4.3		20.7 ± 4.9		26.1 ± 3.9	
	6.3 ± 1.7		7.8 ± 1.7		9.9 ± 2.2		11.0 ± 1.1	

TABLE 2

	SHAM		SAR 4 W/kg		SAR 12 W/kg		SAR 36 W/kg	
Saline Vehicle	46-34	18-14	49-35	21-30	46-30	45-27	43-31	33-18
	22-21	28-35	34-29	24-24	32-35	42-30	27-25	37-33
	29-24	27-25	26-7	25-24	12-24	43-28	33-21	43-31
	44-36	42-27	34-26	23-15	36-32	31-27	34-22	48-22
	35-29		34-25		21-17		28-23	
	35-29		37-24		44-32		31-22	
	32.6 ± 3.0		30.7 ± 2.7		35.2 ± 3.6		35.7 ± 2.2	
	27.4 ± 2.2		23.9 ± 2.5		28.2 ± 1.6		24.8 ± 1.6	
Chlordiazepoxide 8 mg/kg	53-41	56-38	58-21	40-44	40-29	58-34	60-38	54-33
	44-26	43-15	61-35	54-18	65-33	86-24	30-16	41-22
	50-37	48-31	10-24	35-19	55-24	55-37	47-28	51-27
	40-29	50-31	58-27	33-16	49-30	55-23	55-39	56-43
	34-27		39-28		10-17		57-31	
	34-13		35-29		42-24		46-26	
	45.2 ± 2.4		42.3 ± 5.0		51.5 ± 6.2		49.7 ± 2.8	
	28.8 ± 2.9		26.1 ± 2.7		27.5 ± 1.9		30.3 ± 2.6	
Chlordiazepoxide 16 mg/kg	50-32	19-13	66-29	36-25	11-12	27-26	41-19	34-13
	45-39	45-12	57-38	42-26	86-37	43-19	52-20	49-27
	39-23	64-20	49-36	8-2	26-14	31-15	70-26	35-20
	38-12	30-12	14-7	73-26	47-27	50-26	49-34	48-24
	27-18		35-21		20-14		28-18	
	43-30		56-18		44-14		33-25	
	40.0 ± 4.0		43.6 ± 6.7		38.5 ± 6.6		43.9 ± 3.9	
	21.1 ± 3.1		22.8 ± 3.6		20.6 ± 2.6		22.6 ± 1.8	
Chlordiazepoxide 32 mg/kg	12-30	1-1	22-0	16-7	25-9	15-0	45-28	35-25
	22-10	15-6	20-8	39-2	47-16	35-8	60-24	51-24
	20-13	26-3	18-8	5-2	9-8	34-15	52-27	31-9
	22-11	15-7	40-14	30-7	36-12	23-8	39-27	47-11
	0-0		35-26		32-10		65-31	
	17-16		16-4		27-16		89-28	
	15.0 ± 2.7		24.1 ± 3.6		28.3 ± 3.5		51.4 ± 5.4	
	9.7 ± 2.8		7.8 ± 2.4		10.2 ± 1.5		23.4 ± 2.3	

LEGENDS OF FIGURES

Behavioral effects (solid symbols, number of steps ascended; open symbols, number of rears) induced by chlordiazepoxide in mice exposed to microwave radiation (circles, sham; upright triangles, 4 W/kg; squares, 12 W/kg; inverted triangles, 36 W/kg). Each symbol represents the mean number of steps or rears; vertical lines represent the SEM.

FIGURE 1. 1800 MHz (resonance frequency)

FIGURE 2. 1800 MHz (resonance frequency)

FIGURE 3. 4700 MHz (supraresonant frequency)

FIGURE 4. 4700 MHz (supraresonant frequency)

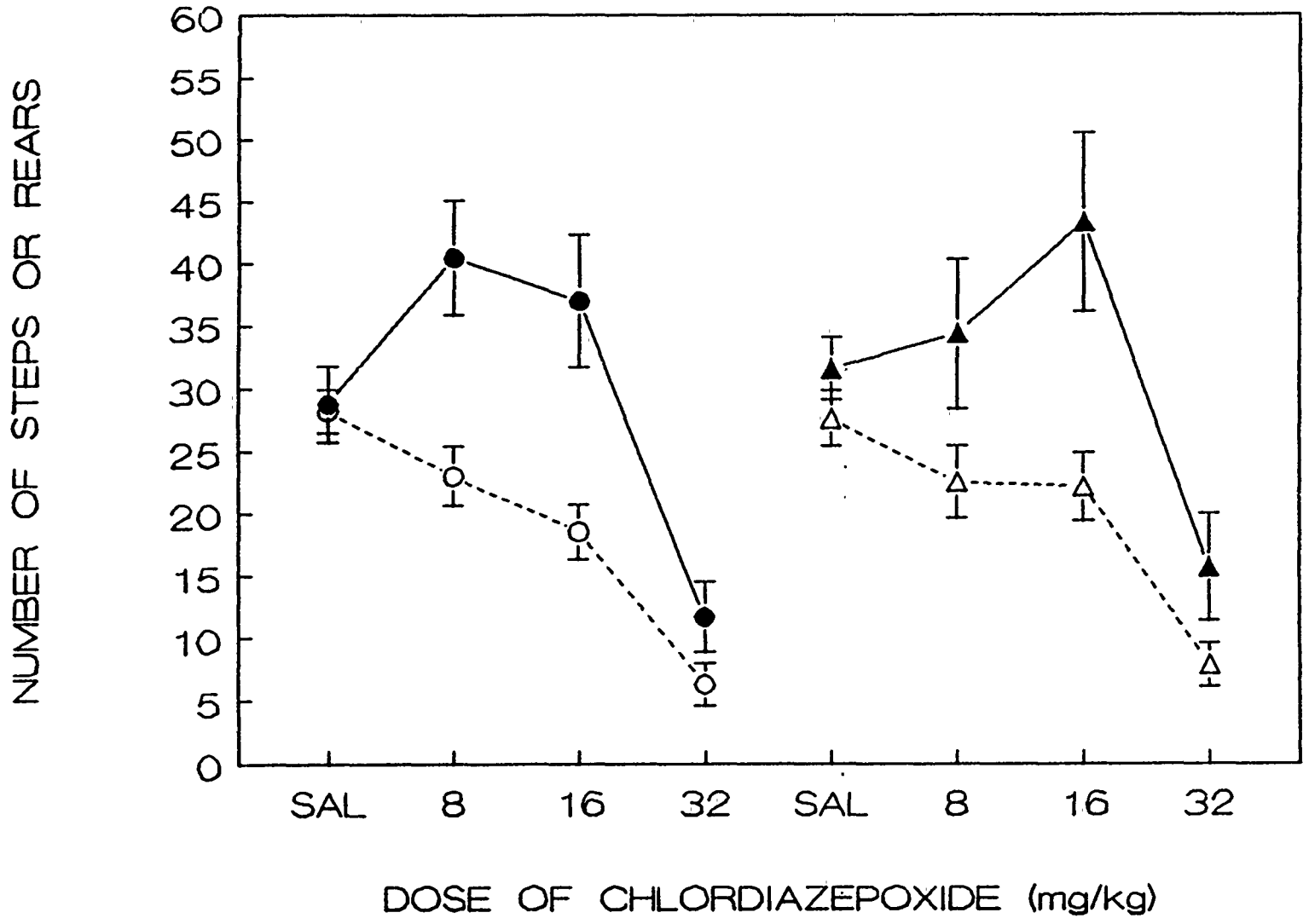

NUMBER OF STEPS OR REARS
60
55
50
45
40
35
30
25
20
15
10
5
0
SAL
8
16
32
SAL
8
16
32
DOSE OF CHLORDIAZEPOXIDE (mg/kg)

FIGURE 1

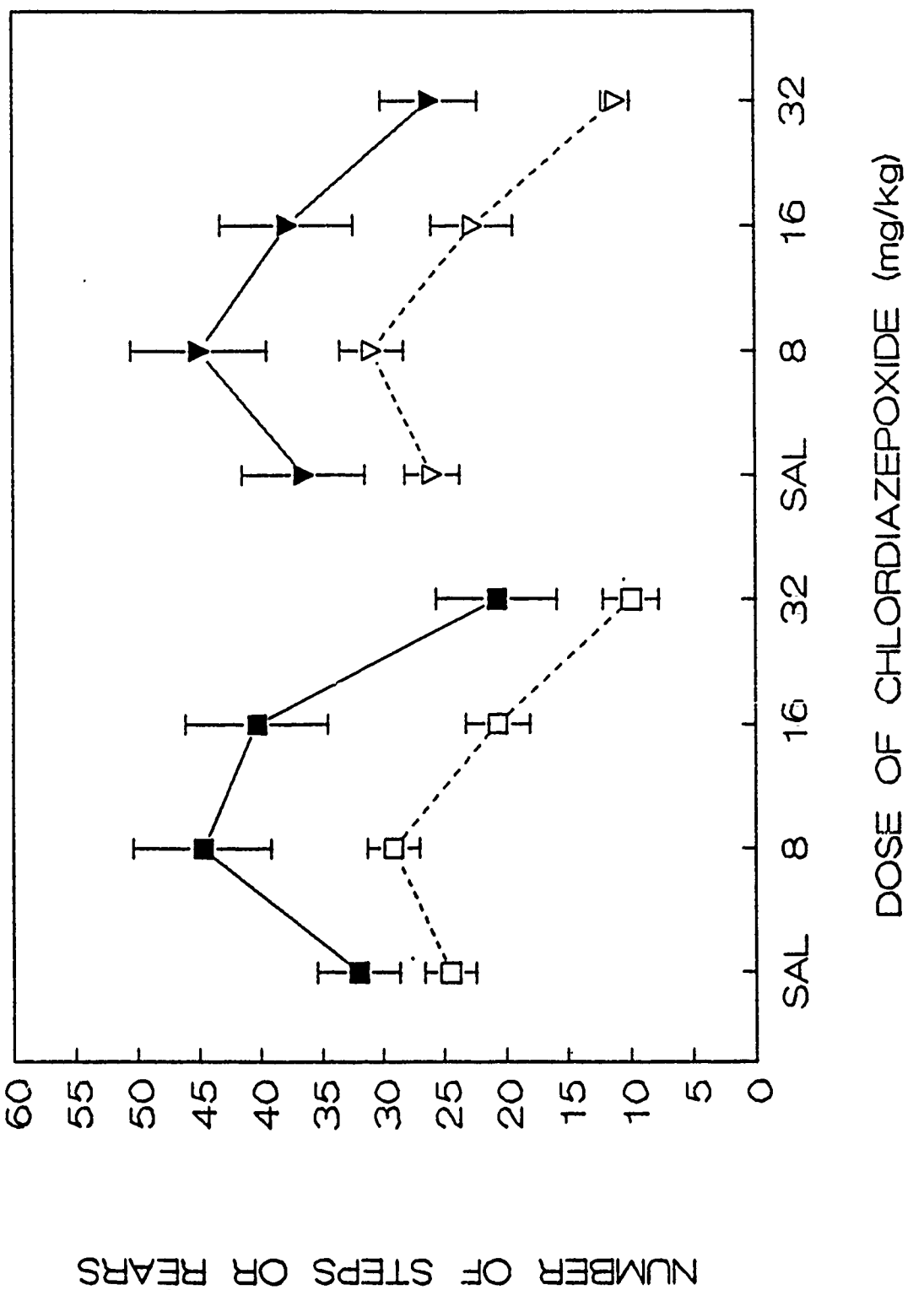
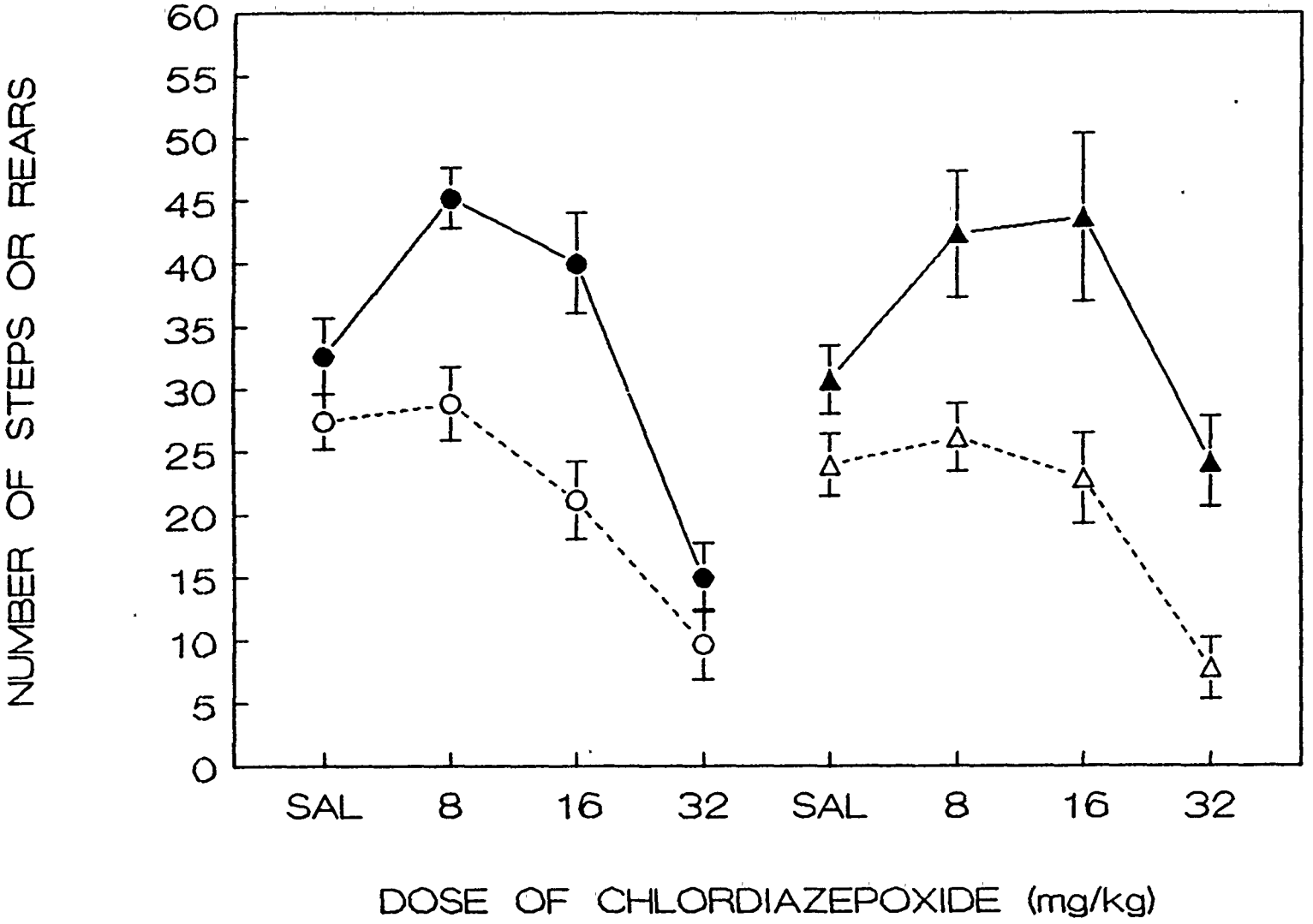

NUMBER OF STEPS OR REARS
60
55
50
45
40
35
30
25
20
15
10
5
0
SAL
8
16
32
SAL
8
16
32
DOSE OF CHLORDIAZEPOXIDE (mg/kg)

FIGURE 2


NUMBER OF STEPS OR REARS
60
55
50
45
40
35
30
25
20
15
10
5
0
SAL
8
16
32
SAL
8
16
32
DOSE OF CHLORDIAZEPOXIDE (mg/kg)

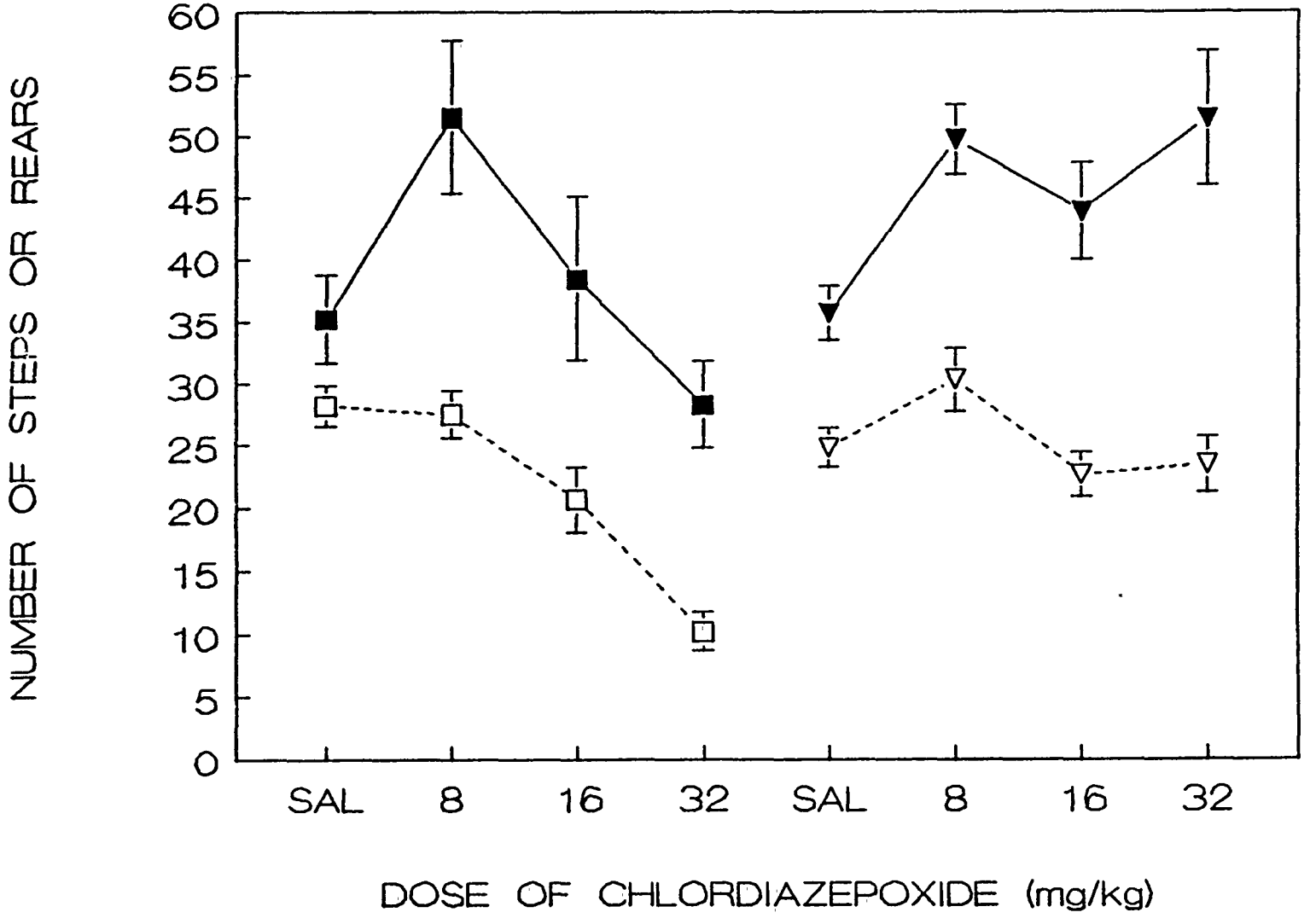

NUMBER OF STEPS OR REARS
60
55
50
45
40
35
30
25
20
15
10
5
0
SAL
8
16
32
SAL
8
16
32
DOSE OF CHLORDIAZEPOXIDE (mg/kg)

FIGURE 4

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FINAL REPORT

Porous Glass as Bed Material in a Pressure Swing Adsorption Unit Used for Air Purification

Prepared by:	Paul O. Scheie, Ph.D.
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USAF Researcher:	Kenneth Ikels

Date:	11 Aug 89
Contract No:	F49620-88-C-0053

POROUS GLASS AS BED MATERIAL IN A PRESSURE SWING ADSORPTION UNIT USED FOR AIR PURIFICATION

by

Paul O. Scheie

ABSTRACT

Removal of water vapor from air was attempted using granulated porous glass as bed material in a laboratory scale pressure swing adsorption unit. It eventually was possible to produce 1 liter/min of effluent with a dew point of -86° C, or 70ppb water vapor, from input air having a dew point up to +15°C, 2000 ppm, or 50% RH. This performance compares favorably with that of molecular sieve beds.

ACKNOWLEDGEMENTS

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research as well as Universal Energy Systems for their efficient administration of the Summer Faculty Research Program.

My experience was a most enjoyable and rewarding one; a consequence of interacting with some wonderful people. The entire VNL Branch headed by Colonel John Bomar was most cooperative and hospitable. Dr. Kenneth Ikels provided a truly exceptional working atmosphere as well as expert guidance. Aaron Shakocius supplied a willing pair of hands as well as useful suggestions. Dr. Russell Burton's efforts to organize both a weekly lunch meeting and a weekly seminar provided for added enrichment.

I. INTRODUCTION

Air purification is an increasingly major environmental concern as it affects life-support systems as well as the operation of highly technical equipment. One of the most common contaminants of air is water vapor. Currently, there are two major approaches to the separation of gas and vapor molecules from air. One involves a static system, such as a membrane or a filter, that depends either on selective permeability or selective adsorption. The other, called pressure swing adsorption (PSA), is a dynamic system based on selective adsorption at different pressures.

Typical bed materials for PSA units include activated carbon, molecular sieve, activated alumina, and silica gel. Porous ("thirsty") glass is one of the numerous materials that can be used as a static membrane filter. Dr. Kenneth Ikels suggested that if porous glass were granulated it might serve as a new type of bed material for PSA units used in air purification.

My work as a summer faculty member at Brooks AFB during the summer of 1988 consisted of a study using molecular sieve material in a PSA unit to produce, from air, a product low in oxygen concentration. In addition, my research at my home institution has involved the adsorption of liquids on glass surfaces.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The objective of this project was to assess the applicability of granulated, porous glass as bed material in a laboratory-scale PSA unit as applied to air purification. Primary emphasis was placed on determining the degree to which water vapor could be removed from compressed air.

III.

The project consisted of varying a) the length of metal tubes (1-inch, OD) to contain the granulated glass, b) cycle times, and c) product flows, in order to produce as dry effluent as possible from compressed breathing air. Input flows were measured with an electronic flow meter; product flows were measured with a rotameter; gas concentrations were monitored with a Perkin Elmer medical gas analyzer; and water content and temperatures were measured with Panametric System 1 moisture-content probes calibrated from a dew point of +20^{o}C to -110oC . Reasonably dry air (dew point < -60^{o} C) could be obtained with beds as short as 12 inches, cycle times from 12 sec to 48 sec, and product flows up to 5 slpm. Driest air was produced with 27 inch stainless steel pipes for beds and no aluminum or copper in the PSA unit. Product flow rates were kept below 3 slpm. Cycle times between 16s and 48s seemed to work equally well, although the oxygen concentration

in the effluent dropped to near 20% at the shorter times. The minimum dew point achieved was -90°C which is less than 60 ppb of water vapor. This value was reached after the unit had been in operation for two weeks during normal working hours, generally with input air having a dew point of -50°C. Occasional challenges with input air with a range of dew points between +10°C and +20°C for up to 6 hours seemed to have no affect on the product.

IV. RECOMMENDATIONS:

a. The use of granulated porous glass as a desiccant should be pursued with more specific attention to the engineering aspects.

b The use of a PSA unit containing porous glass as bed material should be investigated for possible use in other air purification applications.

c. Additional properties of porous glass such as heat of adsorption and adsorption isotherms should be obtained.

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FINAL REPORT

MODELS FOR RFR BIOLOGICAL EFFECTS

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Date:	September 8, 1989
Contract No.:	F49620-88-C-0053

MODELS FOR RFR BIOLOGICAL EFFECTS

by

Ronald L. Seaman

ABSTRACT

Efforts involved three activities related to action of radiofrequency radiation (RFR) pulses on biological systems. First, development of a nonmammalian model for RFR sensory effects was initiated. Electrophysiological techniques were adapted for use on a frog preparation exposed to RFR. Second, preliminary observations of RFR effects on biological free radicals were made. A special imaging system was used to view light created by a compound developed at SAM for detecting free radicals. Third, consultation was provided to a new Air Force contractor. Plans for RFR exposure schemes and biological preparations were discussed with faculty of the University of Texas - San Antonio.

Acknowledgements

Efforts were performed during the period of 3 July through 8 September 1989 under the Summer Faculty Program sponsored by the U.S. Air Force Systems Command and Office of Scientific Research. The program was administered by Universal Energy Systems, Inc. of Dayton, Ohio.

Dr. Russell R. Burton, Chief Scientist of the School of Aerospace Medicine (SAM) facilitated access to resources available at Brooks Air Force Base. Mr. James H. Merritt, Dr. David N. Erwin, and Dr. Jonathan L. Kiel of the Radiation Sciences Division (RZ), Radiation Physics Branch (RZP) gave direction to the efforts. Dr. Melvin R. Frei, of Trinity University and a SAM/RZP contractor, was extremely helpful in providing practical advice and locating experimental apparatus and supplies. The SAM/RZ technical staff assisted in implementing experimental approaches.

Although many resources were available in SAM/RZP, additional instrumentation and services were required for the unique research efforts. An Etymotic ER-3A Earphone set; Grass P16 and P18 Preamplifiers with battery chargers; and an IBM AT Personal Computer, peripherals, and software were on loan to the Principal Investigator from Louisiana Tech University. The university also shared the Principal Investigator's salary.

At Brooks Air Force Base, Lt. Weisman of Bioenvironmental Engineering (CLINIC/SGPB) measured laboratory noise levels. Majs. Fairman and Seibert of the Occupational and Environmental Health Laboratory, Health Surveillance Division, Industrial Hygiene Branch (OEHL/EHI) performed measurements and analyses of acoustic stimuli. Mr. Earl Cook, a contractor in the Crew Technology Division, Systems Engineering Branch of the School (SAM/VNS), loaned an accelerometer to the efforts.

I. INTRODUCTION:

The biological effects of pulsed radio-frequency radiation (RFR) are largely unknown. Although some effects can be predicted on the basis of heating by average power, the basis may not hold for extrapolating from effects observed for continuous wave (CW) RFR. This is especially true for RFR pulses with low duty cycle.

The Radiation Sciences Division, Radiation Physics Branch of the U.S. Air Force School of Aerospace Medicine (SAM/RZP) is particularly concerned with biological effects of pulsed, as well as other, RFR. Information is needed by SAM/RZP to aid in setting RFR exposure standards.

The research experience of the Principal Investigator includes studies of RFR/microwave biological effects. Effects of CW and pulsed RFR had been studied at different times using abdominal neural ganglia of Aplysia and aggregates of cultured chick cardiac cells. Responses to CW RFR in the vestibular system and to pulsed RFR in the auditory system had been recorded from the cat medullary nucleus. The studies involved adapting sensitive electrophysiological techniques to use during RFR exposure. Research interests include cellular and molecular actions of RFR on biological systems and sensory transduction mechanisms. During the summer faculty program, the experience and interests were applied to the study of pulsed RFR effects on biological systems.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The preliminary research objectives agreed upon during the pre-summer visit involved the development of a nonmammalian model for sensory and central nervous system (CNS) effects of RFR. The frog was selected as the experimental animal because of its previous use in sensory research. The objectives included recording behavioral and electrophysiological end-points for detection of RFR actions, which were predictable from previous work on microwave hearing and sound production by RFR pulses. Because animals were not available until the seventh of ten weeks, the research objectives were revised during the summer.

Two objectives were added during the summer. One additional objective was to observe the effect of pulsed RFR on a chemical system designed to indicate the presence of free radicals. It was decided to conduct a preliminary experiment to see whether RFR pulses caused a detectable event in the system. At the request of Dr. Erwin, a second additional objective was to consult with faculty of the University of Texas - San Antonio who were starting research on RFR biological effects.

In addition, related activities were also undertaken during the summer. Contacts were made with researchers in several SAM divisions to better understand their activities. Statistical analysis was discussed with staff of SAM/RZP, the Analysis Branch of RZ. The excellent library facilities at SAM and the University of Texas Health Science Center at San Antonio were used several times a week.

III. NONMAMMALIAN MODEL FOR RFR SENSORY AND CNS EFFECTS:

A. Background:

It is well known that RFR can heat biological tissue, with heating capability proportional to time-average deposited RFR energy. Responses of biological systems with known temperature dependencies can be predicted on the basis of the temperature change caused by absorbed RFR.

Unlike the CW case, the maximum, or peak, power of pulsed RFR can be many times the average power. Effects of the high peak power can be envisioned without significant general heating for processes which (1) can respond to the brief, intense RFR electromagnetic fields; (2) are nonlinear with intensity, obviating extrapolation from low powers; and (3) derive from rapid, although brief, temperature transients.

The Air Force uses pulsed RFR in a number of radar systems. Pulsed RFR is also being considered for use in directed energy weapons. Air Force personnel and civilians thus might encounter pulsed RFR in a variety of situations. Consequently, it becomes important to understand the biological effects of pulsed RFR in order to determine safe levels of exposure. Adaptation of conventional biological preparations for application of RFR provides ways to study mechanisms of RFR-pulse action on biological systems.

B. Approach:

The frog was selected for development as a model for study of pulsed RFR effects on basic sensory processes and the CNS. The frog inner ear is fairly well understood and is used as a model transduction organ of auditory, vestibular, and seismic stimuli [Capranica, 1976; Hetherington, 1988; Hillman, 1976; Koyama et al., 1982; Narins & Lewis, 1984; Precht,

1976; Wilczynski & Capranica, 1984]. During the summer it was planned to record potentials evoked by acoustic and RFR stimuli as an initial step in the development of the model.

A key requirement for later recording of potentials during RFR free-field exposure is the use of nonperturbing, RFR-insensitive conductors in the fields to avoid possible distortion of the RFR fields, secondary stimulation of the preparation, and recording artifacts. Therefore, initial efforts were aimed at developing techniques using a commercially available carbon-loaded Teflon conductor (CTC) to record biological potentials. Having an insulating jacket of Nylon, CTCs had been used successfully by others to record cochlear microphonics in guinea pigs and cats [Chou & Guy, 1979a] and EKG in rats [Frei et al., 1988].

Bullfrogs (*Rana catesbeiana*) were obtained from a New Jersey vendor and maintained in a laboratory aquarium. For experiments, frogs were initially anesthetized with sodium pentobarbital (37.5 mg/kg) and ketamine (75 mg/kg). Injections were made with different syringes into large muscles of different hindlegs. A satisfactory level of anesthesia was maintained with subsequent ketamine injections.

After toe-pinch and eye-touch reflexes were no longer present, the animal was placed on a gauze-covered foam pad on a work table. The preparation was inside a shielded room which afforded a degree of sound and electrical isolation. After implantation of CTCs as described below, a tube was positioned close to each tympanum to deliver clicks with Etymotic ER-3A Earphones.

Recording from several sites was attempted, with CTCs inserted into small holes in skin or frontoparietal and exoccipital bones. Based on a

literature report on *Rana pipiens* [Corwin et al., 1982], midline head locations were initially tried as active sites and the left leg as the ground-electrode site. However, a larger signal-to-noise ratio was obtained by placing one active electrode laterally, with insertion through a small skin incision immediately posterior to the cartilaginous posterior wall of the otic cavity. It was possible to insert the electrode up to 1 cm under muscle along the wall without additional surgery. No bleeding or gross tissue trauma was observed to occur from electrode insertion.

C. Findings:

Acoustic and RFR evoked potentials were successfully recorded at room temperature using nonperturbing CTCs. It was found that evoked potentials could be recorded in response to acoustic stimuli applied to a variety of locations -- ipsilateral, contralateral, and bilateral tympana; ipsilateral leg; contralateral body wall -- and to the supporting substrate. Potentials evoked by RFR applied with a probe made from semi-rigid coaxial cable inserted into the otic cavity just posterior to the columella were usually similar to those evoked by airborne acoustic stimuli.

Several technical procedures were developed to use the nonperturbing CTCs for recording. Conductor ends stripped of Nylon insulation were connected directly to preamplifier terminal posts. Contact of the ground electrode with the frog was made subcutaneously by inserting a stripped end through a small skin incision on the back. Active electrodes, to the "+" and "-" preamplifier inputs, were found to work best with about 0.5 mm of extended conductor at the implanted end. Longer extensions reduced recorded potential amplitudes. Shorter extensions were found satisfactory for short-term recordings, but shrinkage of the center conductor material

during an experiment changed the CTC tip configuration. When the center conductor was initially cut flush with the insulation, it was recessed approximately 0.5-0.8 mm after implantation for 3-4 hours. The shrinkage was presumably due to the fluid observed between the center conductor and the insulation for 1-2 cm at the end of the electrode.

The changes in implanted CTCs impact the recording of electrical potentials in several ways. The electrical contact with tissue would be expected to change over time, possibly leading to high effective impedance and a consequent decrease in signal-to-noise ratio or to loss of contact. Also, the chemistry at the implantation site would change as fluid and solutes are taken up, possibly leading to altered physiology. Although not a factor in the experiments with local application of RFR, the fluid uptake would also increase the effective conductivity of the CTC. The last factor would be especially important in using CTCs to record potentials in free-field exposure conditions.

Two RFR-specific aspects of the experiments reduced measurement sensitivity by causing increased background electrical noise. First, with everything else constant, use of the high-impedance CTCs increased noise amplitude several times over that seen with use of metallic conductors. Second, contact of the coaxial-probe center conductor with tissue caused a many-fold increase in noise. In the latter case, ground conductors had to be re-arranged without disturbing the preparation.

A significant frog behavior unrelated to RFR was observed to result from thumps delivered to the substrate on which a frog rested. A sufficiently intense thump resulted in a reflexive whole-body startle response. It has been reported that the frog does not startle to acoustic

stimuli [Landis & Hunt, 1939; Yerkes, 1905]. Although not studied in detail, the response seems analogous to startle in mammals [Davis, 1984; Landis & Hunt, 1939].

D. Relationship to previous knowledge:

The potentials evoked by acoustic/vibration stimuli were consistent with reported frog nervous system responses to inner ear stimulation [Corwin et al., 1982; Hall & Feng, 1988; Loftus-Hills & Johnstone, 1970; Mudry & Capranica, 1987]. Most previous work involved recording from the eighth nerve or the CNS. To my knowledge, the work reported here was the first use of lateral and midline electrode positions to record auditory evoked potentials in the amphibian, although similar placements of surface electrodes are common in human work [e.g., Katz, 1985; Rintelmann, 1979].

The RFR evoked potentials are certainly the first to be reported in the amphibian. Previous electrophysiology in microwave hearing studies was done with guinea pigs and cats [e.g., Chou & Guy, 1979b; Chou et al., 1982; Guy et al., 1975; Lin et al., 1979; Seaman & Lebovitz, 1987, 1989; Wilson & Joines, 1985]. Other experiments related to microwave hearing involved rat behavior [Hjeresen et al., 1979; Johnson et al., 1976] and human psychophysics [Chou et al., 1982; Frey & Messenger, 1973; Guy et al., 1975; Tyazhelov et al., 1979]. Thus, the potentials are the first RFR responses observed in a nonmammalian sensory system.

The evoked potentials are evidence that RFR pulses are sensed by the frog nervous system. The potentials indicate that the pulses (1) create a sound or vibration sensed by the frog inner ear or (2) act directly on the sensory transduction apparatus in the inner ear.

IV. EFFECTS OF RFR PULSES ON CHEMICAL SYSTEM

A. Background:

The chemical compound diazoluminomelanin (DALM) had been developed at SAM/RZP for use as an RFR microdosimeter [Kiel et al., 1989]. The compound emits light as a function of temperature without substantial disturbance of RFR electromagnetic fields in an exposed solution. The rate of RFR energy deposition is calculated from the rate of temperature rise and the specific heat of the solution.

The spatial distribution of light emitted by DALM, and consequently the distribution of deposited RFR energy, is detected with a specially constructed imaging system in SAM/RZP. In its normal operating mode, the system is capable of exposing a sample in a 2-ml spectrophotometer cuvette to moderately intense CW RFR.

B. Approach:

A coaxial probe similar to the one used in frog experiments was used to deposit pulsed RFR in solutions containing DALM. With the exception of using this delivery method, the system was operated in its normal manner. Light from the cuvette was sensed and displayed in false color on video monitors.

C. Findings:

Light flashes were seen occurring in certain solutions at the open end of the coaxial probe. When they occurred, the flashes were coincident with RFR pulses. Direct visual observation of the cuvette during RFR pulse delivery revealed a yellow-orange flash generated at the probe tip. A sharp click could also be heard to accompany the flash.

D. Relationship to previous knowledge:

Because of the newness of the DALM method for detection of RFR actions, there are few previous observations with which to compare the above observations of light production. It is known that DALM light emissions are temperature sensitive and possibly also responsive in some way to RFR electromagnetic fields themselves.

The observed production of sound by RFR pulses is consistent with previous experimental work with lossy dielectric materials [Foster & Finch, 1974; Guy et al., 1975; Lin, 1978; Olsen & Lin, 1983; Seaman & Burdette, 1980; Sharp et al., 1974]. However, the production of sound in the solutions seemed to depend specifically on the presence of DALM. The dielectric properties of DALM solutions are not known precisely at this time, but the influence of DALM on average dielectric properties might be expected to be minor for the small concentrations used.

Possible actions of the RFR pulses include temperature effects on the chemical reaction rate constants. However, this is unlikely because of the short pulse duration. Direct effects on the reactants would also be reflected in changes in effective rate constants. A possible site of action is the free-electron content of the DALM. Because of the local deposition of RFR energy using the coaxial probe, it can be expected that secondary interactions between exposed and unexposed regions of the solution occur.

V. CONSULTATIONS WITH THE UNIVERSITY OF TEXAS - SAN ANTONIO (UTSA)

Discussions were held with Drs. Armstrong, Senseman, and Nash regarding RFR equipment, biological preparations, and biological endpoints. The UTSA group's capabilities and expertise in neuroscience and biophysics are being applied to the study of RFR biological effects.

The majority of discussion involved the required microwave/RFR equipment and the development of appropriate RFR exposure devices for the anticipated biological experiments. Early specification of RFR frequency, modulation, and power level was stressed for definition of RFR equipment needs. It was noted that SAM/RZP may be able to loan certain RFR equipment. To avoid extensive development efforts, it was agreeable to UTSA to use an open-ended coaxial cable for RFR exposure by adapting published techniques [Seaman et al., 1989]. One adaptation scheme discussed was to contact the preparation dish from the side to allow an unobstructed optical path which is necessary in certain preparations. However, use of the adaptation will depend on several technical details associated with the preparation itself.

VI. RECOMMENDATIONS:

The preliminary experiments on the frog inner ear should be extended into an in-depth study of the RFR actions causing the observed responses. The frog provides a well-studied inner ear with sensory processes representative of those in other animals. Knowledge of the mechanisms of action would lead to a better understanding of the actions on other systems, in particular on human sensory and central nervous systems.

Further development of technique to reduce noise in recordings will be needed in order to use CTC electrodes and the coaxial probe routinely in RFR studies. A systematic study of the fluid uptake by CTCs and its effects on electrode characteristics is recommended. Related to the study, a method of preparing the CTC tip to avoid fluid uptake needs to be developed. At the same time, methods to shape the CTC tip for optimal recording geometry can be studied.

Results of the preliminary experiments on DALM lead to the recommendation to study further the phenomena of light and sound production by RFR pulses. A systematic study in which DALM and its chemical environment are controlled should reveal information related to mechanisms of action. Related studies could be done using the closely related compound melanin, which is found in many biological tissues. Productive studies could be done using only sound as the observed endpoint.

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FINAL REPORT

AN INVESTIGATION OF DIOXIN HALF-LIFE HETEROGENEITY IN HUMANS BASED ON TWO MEASUREMENTS PER SUBJECT

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USAF Researcher:	Joel E. Michalek
Date:	August 30, 1989
Contract No:	F49620-88-C-0053

AN INVESTIGATION OF DIOXIN HALF-LIFE HETEROGENEITY IN HUMANS BASED ON TWO MEASUREMENTS PER SUBJECT

by

Ram C. Tripathi

Division of Mathematics, Computer Science, and Statistics

The University of Texas at San Antonio

30 August, 1989

Abstract

In this research, heterogeneity of the half-life of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) in humans is investigated with respect to weight changes. This investigation is based on mixed-effects linear models with two repeated measurements per subject. The model can accommodate any number of covariates. By including relevant covariates and interaction terms in the model, this approach produces adjusted estimate of half-life of TCDD, and through the interaction term, an assessment of half-life heterogeneity in Ranch Hand Vietnam veterans. Some recommendations are made to further refine this estimate.

ACKNOWLEDGEMENTS

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsoring this research. I also thank the Universal Energy Systems for their help and concerns in the administrative and directional aspects of this program.

I thank Dr. Joel Michalek and his staff of the Biometrics Branch of the Epidemiology Division for providing me all the facilities I needed to carry on this research. The help of Mr. Alton Rahe and Mr. Thomas White in computational aspect of the project was very helpful and is gratefully acknowledged. I also wish to thank Dr. Burton who took extra efforts to supervise this program at Brooks Air Force Base and kept all SFRP visitors in touch with each other and in touch with the program.

I. INTRODUCTION

Environmental and occupational exposures of individuals to potentially harmful chemicals have prompted researchers to launch large scale epidemiologic studies including the estimation of half-life of various compounds in humans. As a part of the Air Force Health Study, the Biometrics Branch of the Epidemiology Division of the USAF School of Aerospace Medicine at Brooks Air Force Base is interested in estimating the half-life of TCDD (the toxic contaminant in Agent Orange called 2,3,7,8-tetrachlorodibenzo-p-dioxin used as a defoliant during the Vietnam War) in Ranch Hand Vietnam veterans. Due to limited data on toxic compounds in humans, estimates of half-life developed thus far have been based on only two observations per subject. Additionally, none of these estimates accommodates adjustments for potentially important covariates.

Due to a breakthrough in chemistry, scientists now are able to measure the TCDD levels in human serums accurately although at high costs. Utilizing these measurements along with some relevant covariate information, the Air Force is interested in developing better estimates of the half-life of TCDD.

My research interests are in the areas of mathematical statistics which encompasses estimation, testing and model building related to linear models. This together with my past collaboration with the Air Force Health Study contributed to my selection in this program and to my subsequent assignment to the Biometrics Branch of the Epidemiology Division.

II. OBJECTIVES OF THE RESEARCH EFFORTS:

The methods available so far to estimate the half-life of toxic compounds in humans are based on only two measurements per subject and can not accommodate any covariate information. Additionally, due to continued medical follow-up of Ranch Hand personnel, it is feasible to acquire more measurements per subject which, along with covariate information, can produce improved estimates of half-life of TCDD.

My assignment as a participant in the 1989 Summer Faculty Research Program was to develop statistical models which can utilize two or more measurements per subject and thereby produce improved estimates of the half-life of TCDD. Another primary aspect of the assignment was to identify some relevant covariates and include them in the model so that heterogeneity of half-life can be statistically assessed.

During the course of this research it was further decided that it would be valuable information to relate the new estimates based on two or more measurements per subject with the existing ones which are based on only two measurements, and to compare their performance. It was also decided to investigate the relative importance of each additional measurement. However, due to lack of time, this latter aspect will be proposed for funding under the Mini Grant Program.

III. REVIEW:

When, as is the case here, the initial dose is unknown, the only methods available for estimating half-life are given by Phillips (1989) and Michalek et. al. (1989). Both of these methods assume the first order kinetics for the concentration, C_t, at time t:

$$C_t = C_0 e^{-\lambda t} \tag{1}$$

where C_0 is the initial concentration, and λ is an unknown decay rate. Based on (1), the true population half-life is

$$t_{1/2} = \frac{\ell n\, 2}{\lambda}. \tag{2}$$

When two measurements per subject are available, according to Phillips, one computes $\hat{t}_{1/2,i} = \frac{(\Delta t)_i \ell n\, 2}{\ell n(C_{t_i}/C_{t_i+(\Delta t)_i})}$ for the i^{th} individual, $i = 1, 2, ..., N$, where C_{t_i} and $C_{t_i+(\Delta t)_i}$ are the concentrations at t_i and $t_i + (\Delta t)_i$ years since exposure. Phillip's estimate is the median of the N sample half-life estimates $\hat{t}_{1/2,i}, i = 1, 2, ..., N$. The weaknesses of this estimate are i) it is ad-hoc, ii) it is restricted to two measurements per subject, iii) it can not accommodate covariate information.

Michalek et. al. (1989) proposed a maximum likelihood estimate of half-life based on two-measurements per subject that accommodates analytical error and a threshold value that conditions the sample so as to ensure first order kinetics. Specifically, their modeling assumptions are as follows:

Let C_t be the concentration in an individual at a calendar time t. Then , the model assumed for $Y(t)$, the observed concentration at time t, is

$$Y(t) = C_t(1 + rX) + B$$

where X is a unit normal random variable representing analytical error, r is the analytic coefficient of variation and B is a background level assumed normally distributed with mean b and variance r^2b^2. The variables C_t, X and B are assumed mutually independent. The coefficient of variation r and the mean value b are assumed known. An observation taken Δt years later denoted as

$$Y(t + \Delta t) = C_{t+\Delta t}(1 + rZ) + B$$

where Z is standard normal and $C_{t+\Delta t}$, Z and B are mutually independent. It is further assumed that the observations are restricted to ranges considerably in excess of background levels. For this purpose, let $k, k > b$, denote a fixed level of concentration above the background level. According to this approach, inference regarding λ is based on the joint conditional density of $Y(t)$ and $Y(t+\Delta t)$ given that $Y(t) > k$ and $Y(t+\Delta t) > k$, denoted by $f_\lambda(y_1, y_2|Y(t) > k, Y(t + \Delta t) > k)$. On denoting the conditional sample, conditioned on $Y(t) > k$ and $Y(t + \Delta t) > k$ of size N by $\{y_i(t_i), y_i(t_i + (\Delta t)_i), (\Delta t)_i, i = 1, ...N\}$, and letting $y_{1i} = y_i(t_i), y_{2i} = y_i(t_i + (\Delta t)_i), i = 1, 2, ..., N$, inference on λ is based on maximizing the likelihood

$$L(\lambda) = \prod_{i=1}^{N} f_\lambda(y_{1i}, y_{2i}|Y_{1i} > k, Y_{2i} > k). \tag{3}$$

The conditional density f_λ can be written as

$$f_\lambda(y_1, y_2|Y_1 > k, Y_2 > k) = \frac{e^{\lambda\Delta t}\int_0^\infty g(c)\phi(\beta, \delta)/(D_1D_2)dc}{\int_0^\infty g(c)\int_\alpha^\infty\int_\gamma^\infty \phi(x, z)dxdzdc} \tag{4}$$

where D_1, D_2, α and γ are constants which depend upon r, b, k, y_1 and y_2, g is the density of C_t and ϕ is the standard bivariate normal density with correlation coefficient $\frac{r^2b^2\varsigma}{D_1D_2}$ with $\varsigma = e^{\lambda\Delta t}$.

The maximum likelihood estimate $\hat{\lambda}$ of λ is obtained by maximizing (3) numerically. Utilizing the large sample properties of the maximum likelihood estimates, this method also provides approximate confidence intervals for λ and hence for $t_{1/2}$. The estimate of λ obtained by this procedure is quite precise as it utilizes valuable information regarding the concentration measurements.

However, as is obvious from (4), extension of this procedure to more than 2 measurements per subject would involve computing higher dimensional integrals in the maximization process of the corresponding likelihood function. This would be numerically intractable. The method can accommodate covariate information, however.

IV. APPROACH

Motivation: Consider the first-order kinetics model

$$C_t = C_0 e^{-\lambda t} \tag{5}$$

where C_t is the concentration t years after exposure, C_0 is initial exposure, and λ is a constant but unknown decay rate. On taking natural logarithm of both sides of (5) we get

$$\begin{aligned} \ell n C_t &= \ell n C_0 - \lambda t \\ \textit{or} & \\ y_t &= \alpha + \beta \end{aligned} \tag{6}$$

where $y_t = \ell n C_t, \alpha = \ell n C_0$ and $\beta = -\lambda$. Since α is the natural logarithm of initial exposure, it is unobservable and hence random for every subject, whereas, $\beta = -\lambda$ is an unknown constant. Thus, (6) can be regarded as a motivating equation for a model which can accommodate multiple measurements per subject as well as covariates. Such a model is known as mixed-effects linear model with repeated measures and is described below:

Mixed-Effects Linear Model with Repeated Measures:

Denote by $\mathbf{y}_i' = (y_{1i}, y_{2i}, ..., y_{Ji})$ as a vector of J measurements of log-concentrations on the i^{th} individual, $i = 1, 2, ..., N$. Consider the model

$$\mathbf{y}_i = \mathbf{X}_i\beta + \mathbf{Z}_i\alpha_i + \epsilon_i$$

where $\mathbf{X}_i$ and $\mathbf{Z}_i$ are known $J \times (p+1)$ and $J \times q$ design matrices, β is a vector of $p+1$ fixed effects to be estimated, and α_i and ϵ_i are random vectors of dimension q and J, such that

$$\alpha_i \sim N(\mathbf{0}, \sigma^2\mathbf{D})$$
$$\epsilon_i \sim N(\mathbf{0}, \sigma^2\mathbf{I}_J).$$

Here, $\mathbf{D}$ is a $q \times q$ positive semi-definite matrix of unknown constants to be estimated and $\mathbf{I}_J$ is $J \times J$ identity matrix. It follows that conditionally

$$\mathbf{y}_i|\alpha_i \sim N(\mathbf{X}_i\beta + \mathbf{Z}_i\alpha_i, \sigma^2\mathbf{I}_J)$$

and marginally

$$\mathbf{y}_i \sim N(\mathbf{X}_i\beta, \Sigma_i)$$

where $\Sigma_i = \sigma^2\mathbf{V}_i$ with $\mathbf{V}_i = \mathbf{I}_J + \mathbf{Z}_i\mathbf{D}\mathbf{Z}_i', i = 1, 2, ..., N$. Also $\text{cov}(\mathbf{y}_i, \mathbf{y}_j) = \mathbf{0}$ for $i \neq j$. If we let $\mathbf{y}' = (\mathbf{y}_1', \mathbf{y}_2', ..., \mathbf{y}_N'), \mathbf{X}' = [\mathbf{X}_1', \mathbf{X}_2', ..., \mathbf{X}_N'], \mathbf{Z} = \text{Diag}(\mathbf{Z}_1, \mathbf{Z}_2, ..., \mathbf{Z}_N), \alpha' = (\alpha_1', \alpha_2', ..., \alpha_N'), \epsilon' = (\epsilon_1', \epsilon_2', ..., \epsilon_N'), \Sigma = \text{Diag}(\Sigma_1, \Sigma_2, ..., \Sigma_N)$, then we can write the grand model as

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}\alpha + \epsilon$$

where $\mathbf{y}$ is a $NJ \times 1$ observable vector, $\mathbf{X}$ is a $NJ \times (p+1)$ known matrix, and $\mathbf{Z}$ is $NJ \times N$ known matrix α and ϵ are, respectively $Nq \times 1$ and $NJ \times 1$ unobservable random vectors. Σ is a $NJ \times NJ$ unconditional covariance matrix of $\mathbf{y}$.

Let θ denote a vector of the unique parameters of $\mathbf{D}$. Then the full log-likelihood can be written as (ignoring the constant term)

$$\ell_F(\beta,\sigma,\theta|\mathbf{y}_i,i=1,...,N) = -\frac{1}{2}\sum_{i=1}^{N}\ell n|\sigma^2\mathbf{V}_i| - \frac{1}{2\sigma^2}\sum_{i=1}^{N}\mathbf{r}_i'\mathbf{V}_i^{-1}\mathbf{r}_i \tag{7}$$

with $\mathbf{r}_i = \mathbf{y}_i - \mathbf{X}_i\beta$.

Estimates of β,σ and θ can be obtained by maximizing (7) with respect to these parameters. However, a criticism of these (maximum likelihood) estimators is that they are biased downward (Lindstrom and Bates (1988)) because they do not take into account the loss in degrees of freedom from the estimation of β. The restricted maximum likelihood method (REML) corrects for this by defining the estimates of the variance components as the maximizers of the log-likelihood based on $NJ - p - 1$ linearly independent error contrasts. The restricted maximum likelihood (REML) estimates of β,σ and θ may be obtained by maximizing the restricted log-likelihood (ignoring the constant term)

$$\ell_R(\beta,\sigma,\theta|\mathbf{y}_i,i=1,2,...,N) = -\frac{1}{2}\ell n|\sigma^2\sum_{i=1}^{N}\mathbf{X}_i'\mathbf{V}_i^{-1}\mathbf{X}_i| + \ell_F(\beta,\sigma,\theta|\mathbf{y}_i,i=1,2,...,N). \tag{8}$$

The computations can be simplified by noting that

$$\hat{\sigma}^2_{REML}(\beta,\theta) = \frac{1}{NJ-p-1}\sum_{i=1}^{N}\mathbf{r}_i'\mathbf{V}_i^{-1}\mathbf{r}_i \tag{9}$$

and, by substituting it in (8), we get the profile log-likelihood of β, θ:

$$\begin{aligned}\mathrm{pr}(\beta,\theta|\mathbf{y}_i,i=1,2,...,N) = &-\frac{1}{2}\ell n|\sum_{i=1}^{N}\mathbf{X}_i'\mathbf{V}_i^{-1}\mathbf{X}_i| - \frac{1}{2}\sum_{i=1}^{N}\ell n|\mathbf{V}_i| \\ &-\frac{1}{2}(NJ-p-1)\ell n\left(\sum_{i=1}^{N}\mathbf{r}_i'\mathbf{V}_i^{-1}\mathbf{r}_i\right).\end{aligned} \tag{10}$$

The REML estimates of β and θ are obtained by maximizing (10) with respect to β and θ. The REML estimate of σ^2 is obtained by substituting the estimates of β and θ in (9).

III. ESTIMATING HALF-LIFE OF TCDD IN THE AIR FORCE VIETNAM VETERANS.

As described in Michalek et. al. (1989), the United States Air Force in collaboration with the Centers for Disease Control has obtained data on the concentrations of TCDD in parts per trillion (ppt) during 1982 and 1987 on 36 Ranch Hand Vietnam veterans (after ignoring the data on two veterans whose concentrations were less than 10 ppt.). Let C_{1i} and C_{2i} denote the concentrations of the i^{th} individual during 1982 and 1987 respectively. The actual times when the observations are taken may vary from subject to subject; denote these by t_{1i} and t_{2i} respectively. In order to assess the effect of weight on the estimate of half-life, the weights of these subjects in kilograms were also recorded during the Vietnam tour and at times t_{1i} and t_{2i}. Let these weights be denoted by W_{0i}, W_{1i} and W_{2i} respectively.

As stated in Michalek et. al. (1989), the log-concentrations are approximately normally distributed, and since a background level of 4 ppt of TCDD is found in the comparison population, we define

$$y_{ji} = \ell n(C_{ji} - 4), \quad j = 1,2$$
$$i = 1,2,...,36.$$

For assessing the effect of weight change on half-life, it was decided to include the relative weight change in the model which is defined as

$$w_{ji} = \frac{W_{ji} - W_{0i}}{W_{0i}}, j = 1,2; i = 1,2,...,36.$$

In order to apply the mixed-effects model to this data, we now make the notation precise for a model containing time, relative weight change and the interaction term.

Let

$$\mathbf{y}_i = \begin{bmatrix} y_{1i} \\ y_{2i} \end{bmatrix}$$

$$\mathbf{X}_i = \begin{bmatrix} 1 & t_{1i} & w_{1i} & t_{1i}w_{1i} \\ 1 & t_{2i} & w_{2i} & t_{2i}w_{2i} \end{bmatrix}$$

$$\mathbf{Z}_i = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$$

$$\epsilon_i = \begin{bmatrix} \epsilon_{1i} \\ \epsilon_{2i} \end{bmatrix}, i = 1, 2, ..., 36$$

$$\beta = \begin{bmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{bmatrix}.$$

Hence, for the i^{th} subject, we can write the model as

$$\mathbf{y}_i = \mathbf{X}_i\beta + \mathbf{Z}_i\alpha_i + \epsilon_i. \tag{11}$$

We also assume that

$$\epsilon_i \sim N(0, \sigma^2\mathbf{I}_2)$$

where $\mathbf{I}_2$ is the identity matrix of size 2, and

$$\alpha_i \sim N(0, \sigma^2\theta)$$

where $\theta > 0$ is an unknown parameter. This gives

$$\mathbf{y}_i|\alpha_i \sim N(\mathbf{X}_i\beta + \mathbf{Z}_i\alpha_i, \sigma^2\mathbf{I}_2)$$

and

$$\mathbf{y}_i \sim N(\mathbf{X}_i\beta, \Sigma_i)$$

where $\Sigma_i = \sigma^2\mathbf{V}_i$ with $\mathbf{V}_i = \begin{bmatrix} 1+\theta & \theta \\ \theta & 1+\theta \end{bmatrix}$ and $\mathbf{y}_1, \mathbf{y}_2, ..., \mathbf{y}_{36}$ are independently distributed. If we define

$$
\begin{aligned}
\mathbf{y}' &= [\mathbf{y}_1', \mathbf{y}_2', ..., \mathbf{y}_{36}'] \\
\mathbf{X}' &= \left[\mathbf{X}_1', \mathbf{X}_2', ..., \mathbf{X}_{36}'\right] \\
\mathbf{Z} &= \text{Diag}(\mathbf{Z}_1, \mathbf{Z}_2, ..., \mathbf{Z}_{36}) \\
\alpha' &= (\alpha_1, \alpha_2, ..., \alpha_{36}), \\
\epsilon' &= (\epsilon_1', \epsilon_2', ..., \epsilon_{36}') \\
\mathbf{V} &= \text{Diag}(\mathbf{V}_1, \mathbf{V}_2, ..., \mathbf{V}_{36})
\end{aligned}
$$

we can write the grand model as

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{Z}\alpha + \epsilon. \tag{12}$$

Then from the previous assumptions,

$$\mathbf{y}|\alpha \sim N(X\beta + Z\alpha, \sigma^2\mathbf{I}_{72})$$

and

$$\mathbf{y} \sim N(\mathbf{X}\beta, \Sigma),$$

with $\Sigma = \sigma^2\mathbf{V}$.

The REML estimate of β, σ^2 and θ can be obtained as mentioned earlier. Since, $t_{1/2} = \frac{\ell n 2}{\lambda}$ and $\lambda = -\beta_1$ where β_1 is the coefficient of time in (11), the REML estimate of $t_{1/2}$ is given by $\hat{t}_{1/2} = \frac{-\ell n 2}{\hat{\beta}_1}$ where $\hat{\beta}_1$, is REML estimate of β_1. From the computational procedure, one can also find an estimate $\hat{\sigma}_{\hat{\beta}_1}$ of $\sigma_{\hat{\beta}_1}$, the standard error of $\hat{\beta}_1$. Then, utilizing the large sample properties of the maximum likelihood estimates, one can construct $100(1-\alpha)\%$ confidence interval for β_1, which in turn, produces a corresponding confidence interval for $t_{1/2}$.

The above model was fitted to this data set using BMDP5V where this procedure has recently become available. These calculations were also checked independently by Mr. Tom White who programmed the algorithm given by Lindstrom and Bates (1988),

or equivalently, (10) and (9). The evaluation of the model parameters appears in the following tables:

Table 1

Parameter	Estimate	Asymptotic Standard Error	z	p-value
Constant	5.786	0.288	20.050	0.000
Time (t)	-0.088	0.014	-6.251	0.000
Relative weight change (w)	-1.859	1.281	-1.451	0.147
Interaction w× t	0.042	0.061	0.692	0.489

Table 2

Parameter	Estimate	Asymptotic Standard Error	z	p-value
Constant	5.671	0.236	24.035	0.000
t	-0.082	0.011	-7.335	0.000
w	-1.046	0.506	-2.068	0.038

Table 1 shows that the interaction between time and relative weight change does not affect the log-concentration of TCDD (p=0.489). Hence, λ and $t_{1/2}$, do not change significantly with weight. When this interaction term is removed, as shown in Table 2, both the time and the relative weight change become significant predictors of log-concentration of TCDD. This model gives the coefficient of time adjusted for relative weight change. Estimates of half-life as obtained by these models with and without adjustments, and as obtained by other authors are given in Table 3. This table also includes approximate 95% confidence intervals for $t_{1/2}$ in each case.

Table 3

Comparison of estimates of half-life from different methods

Method	$\hat{t}_{1/2}$ (in yrs.)	95% Confidence interval
Pirkle et. al. (1)	7.10	(5.8, 9.6)*
Michalek et. al. (2)	7.54	(6.19, 9.65)
Mixed-Linear Model:		
Unadjusted (3)	7.56	(6.10, 9.95)
Adjusted for relative weight change (4)	8.41	(6.61,11.56)
Adjusted for relative weight change and its interaction with time (5)	7.85	(5.98, 11.44)

* 90% confidence interval.

From Table 3 it is clear that the estimates of half-life of TCDD in the Air Force Vietnam veterans involved in the Ranch Hand obtained by the above methods except (1) are all of the same order of magnitude.

RECOMMENDATIONS

a) New and Existing Methods:

As mentioned earlier, the results based on Method (1) are crude as the method is ad-hoc and is not based on any optimal consideration. This method therefore should not be used. Method (2) on the other hand uses very important information regarding the analytic coefficient of variation as well as the joint log-normality of the concentrations. This method utilizes the optimal maximum likelihood method, hence the estimates have desirable properties. Method (2) can be further improved, in the case of two measurements per subject, by modeling half-life in terms of time and some other relevant covariates. Its only disadvantage is the computational difficulty when more than two measurements would be available on each subject. In view of this, the

method proposed here for estimating half-life of TCDD based on the mixed-linear model is preferable because it can easily accommodate any number of measurements and any number of covariates per subject. As the number of measurements and the number of covariates increase, no new software would need to be developed.

b) **Future work:**

i) Data: The estimates reported here are based on only 36 subjects. In order to ascertain the effect of weight change on half-life more accurately, this data set needs to be expanded to include more subjects and possibly more covariates.

ii) **Theory:** It is important to investigate the relationships between the estimates of half-life based on the methods (1) - (4). It is also important to compare their relative performance, using the maximum likelihood estimates as the standard. It would be useful to construct an adjusted half-life estimate based on Method (1) which uses median.

It is also important to refine the maximum likelihood estimate of half-life based on method (2) by directly modeling half-life in terms of some relevant covariates in the case of two measurement per subject. It would also be of interest to refine the adjusted half-life estimates based on mixed-linear model by incorporating the analytical coefficient of variation. This information might improve the precision of the estimate. Another important aspect of future research is to evaluate the contribution of each successive future measurement in the light of the number of measurements available at any point of time. This information would be very valuable in view of the expenses involved with each measurement.

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Final Report

"Temperature Effects on Erythrocyte Sedimentation Rates in Whole Blood and on Erythrocyte and Platelet Volumes"

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"Temperature Effects on Erythrocyte Sedimentation Rates in Whole Blood and on Erythrocyte and Platelet Volumes"

by

W. Drost-Hansen
and John Lafferty

ABSTRACT

Erythrocyte sedimentation rates in whole, anticoagulated blood have been measured over a wide temperature range (26°C to 53°C) at closely spaced intervals (0.7°C or 0.9°C). Anomalous sedimentation rates are frequently observed near 30°-32°C, and at 45° (±1°)C the sedimentation rate decreases abruptly and dramatically in all sample studies (human, baboon, pig, dog, goat). The temperatures of the two anomalies coincide with the temperatures at which vicinal (i.e., interfacially modified) water is known to undergo structural transitions. In blood from humans, baboon and pig the mean cell volume (MCV) goes through a maximum around 42°-43°C, followed by a minimum near 45°C and a dramatic, large and narrow peak around 49°-50°C. Mean platelet volumes (MPV) for humans, baboons, and pigs generally go through a broad minimum around body temperature (37°-38°C) followed by a peak around 46°C. The values for MCV and MPV (and the temperatures at which the maximum and minimum occur) are not affected by the transmembrane enzyme blockers Digoxin or Verapamil. (A small effect on initial sedimentation rates is observed with Verapamil. After 2 or more hours of incubation, this effect disappears.) The heat-induced changes in MCV and MPV show notable hysteresis: the values of these parameters remain unchanged after subsquent incubation at 24°C for at least 2 hours. Sedimentation rate measurements have also been made at 25°C and 37°C on blood from 40 healthy, normal individuals and 30 patients with known pathologies. On the basis of the preliminary data analysis it has not yet been possible to determine if the temperature coefficient of the sedimentation rate based on these data may contain diagnostically useful information not revealed by the standard, room-temperature ESR.

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I. INTRODUCTION

The Erythrocyte Sedimentation Rate (ESR) in whole blood has been widely used clinically for about 60 years. It remains one of the easiest, simplest and cheapest diagnostic parameters ever devised. Regrettably, its diagnostic value is limited and as a result the ESR is currently enjoying far less favor than in earlier decades. This is particularly true because of the recent widespread availability of highly sophisticated (and generally expensive) automatic cell counters. On the other hand, in less sophisticated settings - and perhaps particularly in underdeveloped countries - the ESR may continue to provide useful diagnostic information as it is highly economical (benefit/cost ratio is high.)

My interest in ESR measurements was aroused by a general interest in the structure and properties of interfacial, aqueous systems. For more than three decades I have been studying the nature and extent of the structural changes which occur when water is adjacent to a solid surface. Such structurally modified water is referred to as "vicinal water." While most of the initial work was concerned with water at physico-chemically well-defined, inorganic surfaces, it has become possible to extend such inquiries to intra- and extra-cellular water (see for instance, Drost-Hansen and Clegg, 1979, or Drost-Hansen, 1971). In the course of our general studies of vicinal water, we have investigated the hindered settling of particulate matter (such as polymer latex particles and mineral suspensions). As the result of these studies, it became obvious that vicinal water might also play an important, and so far unrecognized, role in the settling of erythrocytes in whole blood.

II. OBJECTIVES

I. The primary objective of the proposed research was to determine the temperature effects on the sedimentation rate of erythrocytes in whole blood. Very early in the study it also became obvious that a parallel need existed for thermal data on cell volumes of erythrocytes (and of platelets.)

II. The secondary objective of the proposed study was to investigate the possibility that the temperature coefficient of the sedimentation rate may contain useful, diagnostic information: As it is both easy and cheap to perform a sedimentation rate measurement, it was tempting to suggest that such determinations be made at two temperatures (for instance, 37° and 25°C) rather than merely at room temperature. The cost of setting up two (rather than one) Wintrobe tubes is minimal, the additional time required to read two

tubes (rather than only one) is likewise minimal. Thus, the only added laboratory requirement is the availability of an incubator (or a constant temperature bath) set at a higher temperature. Most clinical labs already have such equipment, for instance 37°C incubator ovens. Thus, in view of the promising economics, many measurements were made to determine the possibility that the temperature coefficient of the sed rate might provide additional useful clinical information at very low cost.

III. EXPERIMENTAL METHODS

The sedimentation rate of erythrocytes in whole blood has only rarely been measured over a wide temperature range and, as with most biological variables, only infrequently measured at closely spaced temperature intervals.

To facilitate making measurements at closely spaced temperature intervals, a Linear Temperature Gradient Incubator (TGI - i.e., a "polythermostat") has been developed by the Senior Author and his collaborators. (A detailed description may be found in Drost-Hansen, 1981.) A number of commercial models of the TGI have been available for some time; however, these units are mostly designed for studies on systems requiring agitation, such as bacterial growth, cell cultures, enzyme reactions and adsorption studies. The TGI unit employed in the present study is "homemade" and allows for arranging two sets of 30 standard-sized sample tubes in rigid, vertical positions making it highly useful for sedimentation studies.

As with the commercial, more sophisticated models, the principle is the same: a heavy, rectangular aluminum bar (8 x 20 x 150 cm, weighing about 100 kg) is carefully insulated in a support box. One end of the bar is provided with electric heating elements and a thermoregulator to maintain any preselected, constant temperature. The heat is dissipated by conduction through the bar; at the cold end a plastic chamber serves as a reservoir for a circulating cold liquid, the temperature of which is kept constant. As lateral heat losses from the bar are kept to a minimum, an essentially linear temperature gradient is set up in the bar. Along the length of the bar are two sets of 30 "wells," equidistantly spaced. Each well is large enough to accommodate a standard culture tube (15cm long and 18 mm diameter). The long term stability (constancy) of the TGI has proven highly satisfactory: except possibly for wells #30 and #1, temperature fluctuations in any well over a six week period have been within 0.1°C., or less. Considering that a thermal gradient of about 0.4°C exists across each well, fluctuations of ±0.1°C in any well are insignificant.

Hematology parameters were determined with a Baker System 9000 Cell Counter which is calibrated for both human and animal blood samples. In repeat experiments, the reproducibility was found to be excellent, usually within 1% in cell numbers (both RBC and platelets) and better than 1% in cell volume measurements.

In the present study standard Wintrobe tubes were placed directly in the wells of the TGI using rubber stoppers assuring as nearly vertical positioning as practical. In separate experiments with the Wintrobe tubes, the rate of temperature equilibration was determined: after about 15 minutes the temperature of the blood has essentially reached the equilibrium temperature. As most sedimentation experiments were carried out over periods of three to four hours, the initial lack of equilibration (at the higher temperatures) is not significant.

For sedimentation and all volume measurements using the TGI EDTA was used as anticoagulant. A 0.25 molar stock solution of disodium EDTA was added to the freshly drawn blood; 1ml EDTA stock solution per 50 ml blood - i.e., a 2% dilution of the whole blood. For the sedimentation rate comparison experiments (at room temperature and 37°C) standard, commercial 5 ml "purple top" "Vacutainer" tubes were used.

IV. VICINAL WATER CHARACTERISTICS

As expected from earlier results in similar types of experiments with well-defined inorganic and organic colloid particles (Drost-Hansen, 1981 and Drost-Hansen et al., 1987) the present study has clearly demonstrated that vicinal (i.e., interfacially modified) water plays a major role in the physiological responses of blood to temperature. Both cell volumes and sedimentation behavior are affected. For this reason it is useful to call attention to some characteristics of vicinal water.

Previous studies have led to estimates for the geometric extent of the water structure changes induced by proximity to an interface as well as allowed determination of various properties of vicinal water. The range of vicinal water is likely 20 to 50 molecular diameters (i.e., about 50 to 150A). The most surprising aspect of vicinal water is the occurrence of no less than four critical temperature ranges where the properties of vicinal water change abruptly. The four ranges are: 14°-16°C(T_1): 29°-32°C(T_2); 44°-46°C(T_3), and 59°-62°C(T_4). (Drost-Hansen 1971, Drost-Hansen 1978) . Equally surprising is the observation that the specific, detailed chemical nature of the solid surfaces does not appear to affect greatly the establishment of the vicinal water. (This is referred to as the "Paradoxical Effect" -P.E.) In view of the P.E., it is not surprising that cell-associated water frequently also displays the characteristics of vicinal water with all the manifestations of such, including notable, abrupt changes in properties near the thermal anomalies, i.e., near 15°, 30°, 45° and 60°C.

V. RESULTS

A. SEDIMENTATION RATE DATA AND SEDIMENTATION EQUATIONS

Figure 1 shows a typical set of sedimentation rate data. The ordinate is the distance, h, which the plasma/red cell interface has moved (at various time intervals) as a function of the well number in the TGI. The well number and the temperature are essentially linearly proportional. Some typical values for the corresponding temperatures are indicated on the graphs. Temperatures "in between" may be calculated by linear interpolation.

Overall, the distance, h, from the top of the plasma meniscus to the sediment interface, at any given time, increases with temperature up to about 45°C - in other words, the sedimentation rate increases. As the viscosity of all low molecular weight liquids generally decreases with increasing temperature, the overall increase in sedimentation rate is hardly surprising. In the first series of measurements made (May 25 through June 6, 1989) attention was focused on the effects of temperature on the sedimentation rate around 30°C as this temperature is known to affect the properties of vicinal water in a highly non-linear manner. Consistent with this notion, anomalies in the sedimentation rate are frequently seen near 30°(to 32°)C - though not invariably. Sometimes the anomaly is more readily seen when the logarithm of h is plotted as a function of temperature. Figure 2 shows such a graph for a typical set of sedimentation data, after 3 hours of sedimentation. A distinct anomaly is seen around 31°C and similar results have frequently been obtained. (The method of presenting the data is log h vs T°C is without any theoretical foundation. However, it is easily seen that the approach resembles an Arrhenius-type analysis as if the sedimentation rate, dh/dt, depended exponentially on the absolute temperature. As discussed by the present author (Drost-Hansen, 1973), for measurements around 30°C the reciprocal of the absolute temperature, T, in Kelvin is nearly a linear function of T°C - quite by accident. Thus, plotting log (rate data) versus T°C is almost as valid as plotting against the reciprocal absolute temperature.

While deceptively straight line segments - and distinct anomalies between these segments - are obtained in the present study, one must guard against unwarranted generalizations in interpretation. Thus, even under the best of circumstances, the Arrhenius expression is approximately valid only when <u>one</u> mechanism plays the rate controlling role. In the case of sedimenting erythrocytes various processes must be assumed to play major roles: various intracellular regulatory processes, membrane bilayer lipid processes, and temperature effects on

the concentration, nature and hydration of the plasma proteins. The fact remains, however, that some dramatic change (or changes) occurs at or near 30°(to 32°)C.

The effect of cell volume on sedimentation rate is usually discussed in terms of the hematocrit, and a rich literature exists on this subject. Because of time limitations, it was not possible to pursue the specific effects of MCV on sed rate in any detail. Instead, as discussed in the Appendix, we have analyzed the most likely sites of action of vicinal water on the sed rate.

B. 45°C SEDIMENTATION RATE ANOMALY

Dramatic changes in rates of sedimentation have been observed for temperatures around 45°C (±1°C). Figure 3 shows two such examples. It is important to note that this behavior has been observed on all samples studies, including those from other mammals (baboon, pig, dog, goat).

Measurements of sed rates and cell volumes have been made at temperatures up to 53°C in order to elucidate the crucial temperature range of 44° to 46°C (the T_3 transition of vicinal water.) However, it cannot be emphasized too strongly that temperatures above 45°C are "unphysiological" as no mammal (or bird) is able to withstand temperatures above this critical level (see Drost-Hansen, 1971). On the other hand, recent clinical efforts to treat malignancies by hyperthermia - at temperatures approaching 42 to 43°C - make it highly desirable to study this temperature range most carefully.

C. MEAN CELL VOLUMES

Mean Cell Volumes (MCV) (of erythrocytes) and Mean Platelet Volumes (MPV) have been determined over the temperature range from 26° to 53°C, using a Baker Series 9000 Hematology Counter.

Figure 4 and Figure 5 show typical sets of data, respectively MCV (in cubic microns, μ^3) and MPV (μ^3). In Figure 4 note the distinct, but relatively broad maximum in MCV near 42°C followed by a somewhat sharp minimum and, in turn, a very large, and very sharp peak around 49°C. These features have been observed in all of our measurements on human blood. Furthermore, the temperatures observed for the extrema do not vary from one individual to another.

Similar bimodal MCV curves have been obtained on blood from another primate (a baboon) as well as from pigs.. On the other hand, blood from dogs and goats does not show the bimodality: in the case of dogs only the sharp peak at 49°C is observed while in the case of goats no maximum is observed - only a smooth rise

in MCV (from 36°C to 51°C) of about 5%. That the blood of the primates and pig show similarities may perhaps not be too surprising as the pig has been found to be a highly useful animal model mimicking human behavior in many cardiovascular respects.

The thermally induced volume changes in both erythrocytes and platelets are "remembered" for relatively long time periods - at least for two hours. Specifically, the anomalous cell volumes, induced by incubating the blood in the TGI for three hours, remain essentially unchanged following subsequent incubation at room temperature (24.5 ± 1°C) for two hours. (In one experiment: after three hours of incubation at room temperature.) The effect is likely to occur in vivo as well as in vitro; in that case cell volume changes in a patient may persist for several hours after acute febrile episodes (and hyperthermia treatment.) Likewise, in patients whose body-temperature has been severely lowered it may take hours for the cell volumes to return to normal. Very likely the exposure to high temperatures generates "heat shock proteins" which, once formed, may persist for extended periods of time. It is also possible that vicinal water may play a role in cell volume regulation and may in fact contribute to the "volume memory effect:" vicinal water is known to exhibit notable hysteresis, at least in some cases (Drost-Hansen, 1971, Drost-Hansen 1978); once "disturbed" -- broken down for instance by heating above one of the transition temperatures -- the original vicinal water structure may take hours to reform (upon return to the original temperature.) The mechanisms involved are not understood and are in fact most surprising in view of enormous speed with which bulk water structures break down and reform (small fractions of nanoseconds).

In view of the large and unusual cell volumes observed at higher temperatures it was of interest to examine the corresponding hematocrit values. Thus, in a separate series of experiments the hematocrit readings obtained from the Baker System 9000 Counter (equal to the cell volume (MCV) times the number of cells) were compared to hematocrit readings made by the micropipette centrifuge method. As discussed above the RBC retain their modified volumes for at least two hours after being brought to room temperature; thus no error is introduced by the use of the slightly slower centrifugal determination of the hematocrit. Indeed, at all temperatures checked the two values agreed, usually within 1 or 2%. This lends further credence to the claim that notable (and unexpected) cell volume changes are induced by temperature variations.

D. EFFECTS OF TRANSMEMBRANE TRANSPORT BLOCKERS

Because of the unexpected effects on temperature on both sedimentation rates and cell volumes, it was of interest to determine if transmembrane transport enzymes play a role in these thermal effects.

Two series of measurements were made in which a cardioglycoside (Digoxin) was added to whole blood prior to incubation at the various temperatures. The concentration used was 100ng/ml, or 50 to 100 times normal therapeutic levels. No differences were seen in either MCV or MPV after 3 hours of incubation nor in rate of sedimentation. Thus, it is concluded that the anomalous thermal responses of the cell volumes do not reflect an influence on the active ATPase mediated Na^+/K^+ transmembrane transport!

In a similar manner, we have measured sedimentation rates and cell volumes in the presence of Verapamil. This drug is known to affect Ca^{++} transmembrane transport, and hence it was of particular interest to see if platelet cell volumes were affected. The concentration used was 100 ug/ml blood or approximately 50 to 100 times normal, therapeutic levels. For both MCV and MPV, no differences were observed between Verapamil treated and control samples. In two cases, the initial sed rates after 1.0 and 2.0 hrs., were reduced by about 30%. However, after 3 hrs. of sedimentation this difference disappeared!

In a separate set of experiments, we have measured sedimentation rates and cell volumes of blood from normal, healthy individuals in the simultaneous presence of Digoxin and Verapamil (using same concentrations as before). The initial transient effect of Verapamil on sedimentation rate was observed but again this effect disappeared after 2 hours of incubation and again, no volume changes in MCV and MPV were observed.

VI POSSIBLE ORIGIN OF ANOMALOUS SEDIMENTATION RATES

There is no simple, obvious explanation for the thermal anomalies found in the sedimentation rate data at 30-32°C and at 45°C. However, it is most likely that the anomalies reflect changes induced by transitions in the vicinal water but the mechanism by which this happens is not at all clear.

Figure 6 shows a typical comparison of the MCV versus temperature together with the sediment height. The parallel behavior between h and MCV over the range of temperature from 32°C to 44°C is striking. On the other hand, there is no correlation whatever between the two variables above 44°C.

As indicated above it seems inescapable that the anomalous temperature effects observed are due to the vicinal water transitions. However, it is difficult or impossible at this time to pinpoint the "site (or sites) of action" of the vicinal waters structure changes, but some speculations are presented in the Appendix.

VII CLINICAL ASPECT

A. TEMPERATURE EFFECTS

In the present study, extensive measurements have been made of the temperature effects on the hindered settling of red blood cells (RBC) in whole, anti-coagulated blood: data have been obtained on blood from healthy, normal humans and blood from dog, goat, pig and baboon.

Previously published papers have generally not paid great attention to temperature effects on blood sedimentation rates other than warnings not to run sedimentation rate determinations on blood recently removed from a refrigerator. Apart from this, however, little attention has been paid to the effects of temperature on the ESR. One of the most frequently cited papers on the subject is by Manley (1957) who measured the sedimentation rates at temperatures ranging from 12.8° to 35°C., using Westergren tubes with blood from 33 individuals. On the basis of these data a nomogram was constructed to allow for corrections to be made to the ESR when not performed at "room temperature," which in the study cited, was taken to be 18°C. For want of better data the nomogram has been quoted frequently, for instance, in the text "Laboratory Medicine Hematology" by J.B. Miale (1977). It is interesting to note that the blood samples used by Manley were not obtained from healthy, "normal" individuals but from patients being treated for pulmonary tuberculosis (!) and for this reason alone the general validity of the data may well be questioned.

Far more recently Linhoft, Larsen and Nielsen (in Denmark) (1985) have published an interesting, short note on ESR determinations. These authors measured sedimentation rates at 23, 28, 32 and 37°C using Westergreen and Wintrobe tubes as well as plastic sedimentation tubes. From the observed results the temperature coefficient of the sedimentation rate, i.e., d(ESR)/dT was calculated:

	ESR range : mm/hr			
	<50	50-100	>100	
Westergren	0.62	0.19	0.61	glass
Wintrobe	0.41	-	-	glass
Linhoft,et al	1.72	2.20	1.08	plastic

It is distressing to note in the Table the vastly different results, all affected by temperature, of the various specific methods used: the material of which the sedimentation rate tubes were made apparently also plays an important role.

B. TEMPERATURE COEFFICIENT OF ESR; CLINICAL DATA

The objective of the second part of our study was to investigate if additional clinically useful information might be revealed form measurements of the temperature coefficient of the sedimentation rate between room temperature (24.5 $\pm$0.5°C) and at 37°C. All measurements in this report have been made using Wintrobe tubes. A total of about 70 such determinations have been made consisting of 40 measurements on "normal", healthy subjects and 30 patients with known pathologies. The normals consisted of seven selected adults from the Laboratory and the rest were young (17 -20 year old) Air Force recruits, all assumed to be in excellent physical health.

Data Analysis

The value of the classical ESR rests largely on the vast historic data base which surely must include the experience gleaned from reports on a million or more patients. In our ten week study only a minute sampling was possible. Thus, we are not in a position to formulate any generally valid conclusions.

The first approach chosen for our data handling was to calculate the relative increase in sedimentation rate per degree C. This quantity (q) is defined as

$$q = \frac{1}{\nu_{25}} (\nu_{37} - \nu_{25}) \cdot 100 \cdot \frac{1}{\Delta T} (\%) \qquad (1)$$

where ν_{25} = rate of settling at 25°
ν_{37} = rate of settling at 37°

and ΔT = difference between high and low temperature, usually 37° and 25°C,
i.e., $\Delta T = 12^{\circ}C$

ν_{25} and ν_{37} are respectively equal to the distance, h, moves by the plasma/compacting cell interface in <u>one hour</u>, at the two temperatures. Thus, v_{25} is by definition the standard, "classical" ESR.

Rewriting eq. (1) in terms of the distance, h, which the plasma/cell interface has moved, one obtains

$$q = -\frac{h_{37}-h_{25}}{h_{25}} \cdot \frac{100}{12} = 8.3\,\frac{h_{37}-h_{25}}{h_{25}}\ (\%) \qquad (2)$$

For normal individuals q is usually in the range of 4 to 5% per degree while many patients (mostly with various types of cancers) have notably lower values, say 0.5 to 3% per degree. Even for normal, healthy individuals there is a large amount of scatter in the q-values; it is not difficult to see the reason for this: in the case of a very low sedimentation rate, h_{25} is small and in fact often close to zero. Thus, as h_{25} occurs in the denominator, q may become very large. Furthermore, a difference in sedimentation rate such as between 1mm or 2mm in one hour corresponds to a difference in q of a factor of 2. Clinically, the large q-value in these cases provides no new additional evidence, - it merely reflects a very low ("normal") sedimentation rate.

It is of interest, however, that qualitatively the q-values obtained on a population of patients do fall in a lower range than the values on normal, healthy individuals. It is possible that this may convey some diagnostically useful information. However, we have not yet completed our analysis of the available data.

We have explored the possible use of a number of other parameters to characterize the effects of temperature on the sedimentation rate. One of the simplest such is the difference, D, between the sedimentation rates at 37° and 25°C; i.e., $h_{37} - h_{25}$. It is obvious that D is related to q, and hence many of the limitations on q apply as well to D. As with the q-values discussed above the D-values seem to fall in two different ranges, generally being (far) larger for patients than for normals: Expressed in mm (after 0.5 hrs of sedimentation) D is in the range of 0 to 7 mm for the normals, but mostly 5 to 23 mm for the patients studied. Without more detailed information this may merely reflect the enhanced sed rate expected in patients with serious diseases. The final compaction volumes have been recorded. In nearly all cases, the final volume of the compacted cells is larger at 37°C than at 25°C. This is consistent with our finding that the MCV increases with temperature over the range 25° to 37°C.

So far, the examination of the temperature effects on sedimentation rate has proven disappointing from a clinical point of view. However, it is intend to pursue this approach because of its potential benefits as a cheap way of acquiring additional clinical information.

VIII SUMMARY AND CONCLUSIONS

A. Blood sedimentation rate measurements have been made over a large range of temperatures (21° to 53°C) at closely spaced intervals (0.7 to 0.9°C).

B. Over the range of temperature from 21° to 44.5° ± 0.5°C the sedimentation rate of blood (from normal, healthy individuals) generally increases about 3.8% per °C, corresponding roughly to an apparent energy of activation of 6.3 kcal/mole.

C. Frequently but not invariably, an anomaly occurs in the sedimentation rate around 30° to 32°. It is known that at this temperature (T_2) vicinal water undergoes an abrupt structural transition which has previously been shown to affect many cell physiological parameters and probably affects the hydration of macromolecular solutes in aqueous systems as well. The "site of action" of the vicinal water which is responsible for the sedimentation rate anomaly is not known: it may reflect changes in the RBC cell interior, the lipid bilayer membrane, the RBC cell surface or the nature, concentration and vicinal hydration characteristics of the plasma solutes (most likely various proteins, especially albumin and fibrinogen.)

D. A sudden decrease in RBC sedimentation rate occurs at 45 ±0.5°C in all samples studied. The transition from slowly increasing sedimentation rate to a dramatic drop in rate occurs over a very narrow temperature interval: 0.5 (to 1.0)°C. Exceedingly abrupt changes in the properties of biological systems usually reflect cooperative (collective) phase transitions. The lipids of some cell membranes are known to exhibit such phase behavior; however, in the case of the 45°C anomaly it is far more likely that this reflects the phase transition in vicinal water, namely T_3, which occurs at 44° to 46°C.

E. Comparison human/mammals: Anomalous sedimentation rates have been seen over the same temperature range for blood from humans, baboons, pigs, dogs and goats. Qualitatively the features of the sed rate curves as a function of temperature are the same, in particular the abrupt drop in sed rate above 45 (± 1)°C.

F. RBC and platelet volumes have been measured as a function of temperature, (primarily over the range 26° to 53°C).

1. In samples of blood from normal, healthy individuals, RBC mean volumes (MCV) go through two distinct maxima, respectively near 42-43° and 49°C. The peak around 49°C is exceedingly sharp, the "half-width" being of the order of 1 to 2°C; the minimum between the two peaks occurs at 44-45°C. It is likely that the 44-45°C minimum and/or the two peaks in MCV reflect rate controlling roles of vicinal water in the cell regulation processes.
2. Mean platelet volumes (MPV) go through a relatively broad minimum around 37°C, i.e., a minimum centered near the normal body temperature. This is followed by a relatively narrow peak centered around 45°C and sometimes an even sharper peak centered around 48°C with a deep minimum near 46.5-47.5°C.
3. Once heated, the volume changes of both the RBC and platelets are "remembered" for at least two hours. Thus, samples removed from the TGI after three hours of incubation continue to exhibit the anomalous volume characteristics after at least two hours storage at room temperature (24.5 ± 1°C). The effect may be caused by "heat shock" proteins from the plasma or the erythrocytes (and platelets.) Note in this connection also that vicinal water frequently exhibit notable hystersis (Drost-Hansen, 1971, 1978); upon cooling, "disturbed" vicinal water structures may reform only very slowly ("half-lives" of the order of hours).
4. Transmembrane Pump Blockers - Digoxin - a cardioglycoside known to interfere with the Na^+/K^+ transmembrane enzyme transport - in a concentration of 100 ng/ml does not affect the sed rate nor the MCV and MPV. Verapamil, known to interfere with Ca^{++} regulation likewise does not affect the MCV or MPV significantly at a concentration of 100 μg/ml. There is, however, an initial, temporary reduction in sed rate. This effect disappears after 2-3 hrs. of incubation.

G. As discussed previously by many authors, the kinetics of blood settling is remarkably complex. The results obtained in the present study suggest that the complexity may be ever greater than previously recognized and must

surely reveal a critical role of vicinal water in various hematological parameters.

IX RECOMMENDATIONS

It is recommended that the following processes (or parameters) be studied further:

A. Effects of temperature on erythrocyte and platelet cell-physiological properties, particularly volume relationships.

B. Effects of temperature on RBC sedimentation rates, over a wide temperature interval (say 0^{o} to $50^{o}C$).

C. Effects of RBC volumes and hematocrit on blood sedimentation rates.

D. Effects of temperature on cell transport systems as affected by various specific enzyme blockers.

E. Effects of temperature on blood sedimentation rates in normal individuals compared to patients with known pathologies, i.e., a continued search for diagnostic information not presently obtained in the standard room temperature ESR.

F. The role of vicinal water in RBC and platelet physiology particularly near 30^{o} and $45^{o}C$.

G. The implications for hemodynamics of the unusual RBC and platelet volumes induced by elevated temperatures: applications to vascular bed flow characteristics, heat transfer, effects of hysteresis of volumes and role in hyperthermia therapy of malignancies.

H. Direct measurements of viscosity of whole blood, washed and resuspended cells and plasma as a function of temperature over a wide range of temperatures and at closely spaced temperature intervals (as function of shear rate).

I. Develop improved nomogram for correcting ESR data to a "standard," reference temperature.

APPENDIX

The use of the sedimentation equation discussed below is primarily for the purpose of illustrating the likely dynamics of settling. Considering a dilute (!) suspension of spheres (!) in a strictly newtonian (!) suspending medium, Batchelor [1972] expressed the velocity of hindered settling (ν_{sed} in terms of the velocity given by the Stokes equation ν_{st}) for the fall of a single particle and the volume fraction, ø, of the spheres.

$$\nu_{sed} = \nu_{st}\,(1\text{-}K\text{ø}) \tag{3}$$

where

$$\nu_{st} = \frac{2(\varrho_\rho - \varrho o)\, r^2 g}{9\eta} \tag{4}$$

in which

K	=	a hydrodynamic constant
ϱ_ρ	=	density of settling particle
ϱo	=	density of suspending medium
r	=	radius of particle
g	=	acceleration of gravity
η	=	viscosity of suspending medium

In the analysis the sedimentation data of RBC in whole blood I have accepted the validity of the Batchelor equation but have modified it to include a "form factor", f, to account for the biconcave disk shape of the erythrocyte as opposed to spherical shape. The term Kø is taken to be proportional to the hematocrit. The equation probably does reflect in a critical way the effects of the "effective radius" of the RBC as well as the critical role of the factors $(\rho_{rbc} - \rho_{plasma})$, ø , r and η_{plasma}. On the other hand, the Batchelor equation is strictly valid only for rigid sphere, for volume fractions < 0.02 and for a pure newtonian suspending medium. None of these restrictions are fulfilled in the sedimenting of whole blood.

No doubt all of the parameters discussed above are influenced by temperature but it is unlikely that any of them (with the possible exception of η_{plasma}) would change sufficiently abruptly nor sufficiently much to make the sedimentation velocity drop dramatically over a one or two degree interval. On the other hand, the difference terms in eq(1) (to be denoted $\Delta\rho$ and Δø) may play a critical role:

$$\Delta\rho = \rho_{rbc} - \rho_{plasma} \tag{5}$$

$$\Delta\text{ø} = 1 - K\text{ø} \tag{6}$$

If the regulation of the transmembrane water and solute flow is impaired due to a structural change of the vicinal water, then $\Delta\rho$ may decrease notably and the sedimentation rate thus go towards zero. Similarly, if cell volume control becomes impaired due to the vicinal water structural transitions, the RBC may swell (as is indeed observed) and K$\emptyset$ (~ MCV times number of cells) may approach 1 and thus $\Delta\emptyset = 1\text{-}K\emptyset \rightarrow 0$, again reducing the sedimentation rate rather abruptly.

Finally, it is conceivable that the plasma viscosity may change drastically near 45°C as discussed below; on the other hand one would have expected that any notable abrupt change in η_{plasma} at 45°C would have been observed and reported before, yet I am not aware of any papers to this effect. However, in at least one case, dramatic and exceedingly abrupt changes in rheological properties have been reported in a study on a highly concentrated, aqueous protein system. Montejano et al. (1983) describes the temperature dependence of the molecules of rigidity (viscosity) of fish-meal gels. Figure 7 shows a typical example; note the pronounced anomaly (minimum) near 45°C, somewhat resembling the anomaly at that temperature in the sedimentation rate (see, for instance, Figure 6). Obviously the protein concentration in the blood plasma is far lower than in the fish-gel, but the fact remains that rheological changes may be abrupt and sometimes dramatic and no doubt reflect changes in the vicinal water.

In summary, from about 33°C to 45°C the primary rate controlling feature may be the factor, r^2, while above the critical temperature (T_3) either η_{plasma} and/or the difference terms ($\Delta\rho$ and $\Delta\emptyset$) may be the rate determining factors.

Consider eq (4) for the sedimentation rate of spheres. Before proceeding it must be stressed again that we are attempting to use this expression far outside the ranges for which it was devised: a) the RBC are not rigid spheres, b) the concentration (roughly the hematocrit) is far higher than the 0.02 volume fraction which is an approximate upper limit for the calculated sedimentation rate, c) the non-newtonian behavior of the plasma is ignored. Furthermore, neither ρ_{plasma}, ρ_{rbc}, η_{plasma}, the form factor (f) nor the "effective equivalent RBC radius" are known. Nonetheless, it is likely that eq(3 and 4) do convey some features which are generally correct.

Below the 45° transition point the sedimentation height follows rather closely, but not exactly, the MCV. This suggests that the primary rate-controlling variable is related to the MCV: note that in eq(4) the sedimentation rate depends on r^2; pushing the limits of interpretation of eq(4) the "effective radius" of the erythrocyte must be approximately proportional to $(MCV)^{2/3}$. Seeking an explanation for the abrupt drop

in sedimentation rate above 45°C, all of the factors in eq(4) may play a role (i.e., ρ_{rbc}, ρ_{plasma}, r_{rbc}, η_{plasma}, and ϕ).

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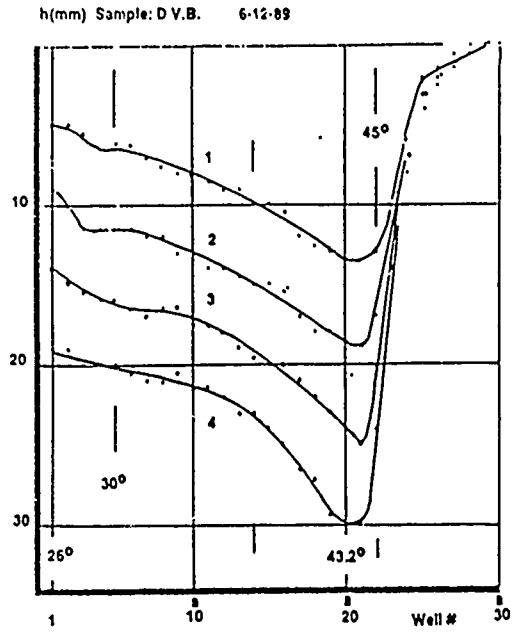


Fig 1 Distance, h, sedimented by erythrocytes in whole blood as a function of well number in the TGI (and hence of temperature.) Curves 1, 2, 3 and 4 respectively after 1, 2, 2.5 and 4 hours. Note indications of an anomaly near 30°C and the abrupt decrease above 44 5°C.

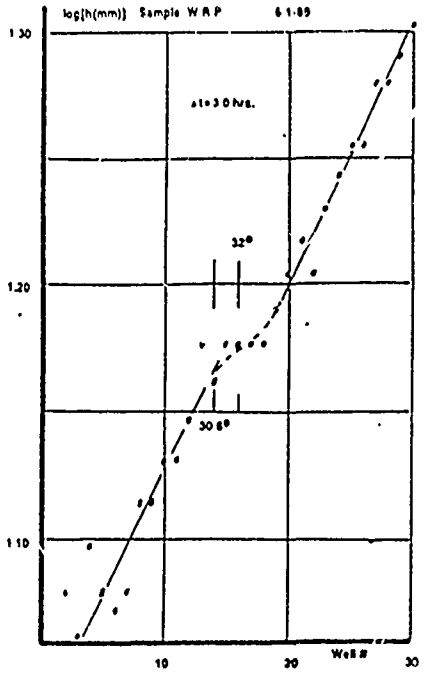


Fig 2 Log (distance sedimented) versus well number (proportional to temperature) After 3 0 hrs note anomaly near 30-32°C.

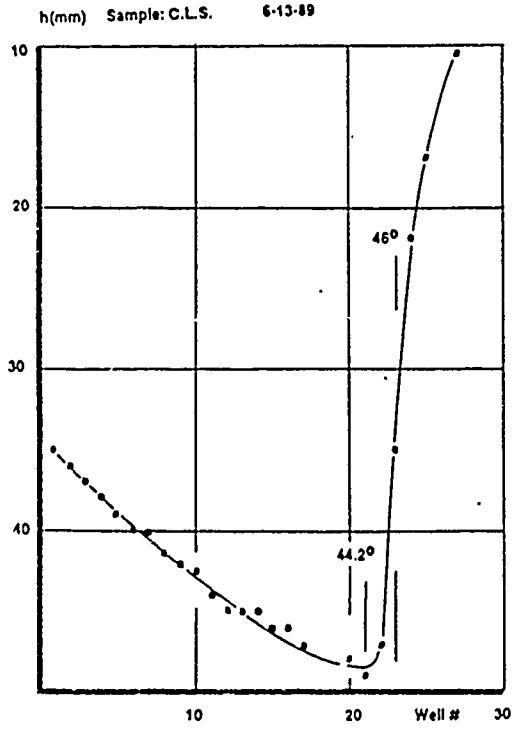


Fig 3a Distance (h) sedimented as a function of well number (and hence temperature) after 1.5 hrs Note abrupt decrease above 45°C.

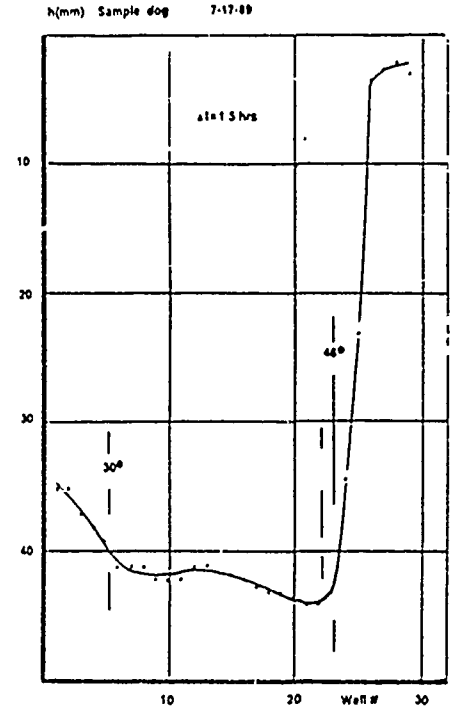


Fig 3b Same as for Fig 3a but with blood from dog Note abrupt decrease above 46°C.