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UNITED STATES AIR FORCE

SUMMER FACULTY RESEARCH PROGRAM

1988

PROGRAM TECHNICAL REPORT

UNIVERSAL ENERGY SYSTEMS, INC.

VOLUME IV OF IV

Program Director, UES
Rodney C. Darrah

Program Administrator, UES
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Lt. Col. Claude Cavender

Submitted to

Air Force Office of Scientific Research

Bolling Air Force Base

Washington, DC

December 1988

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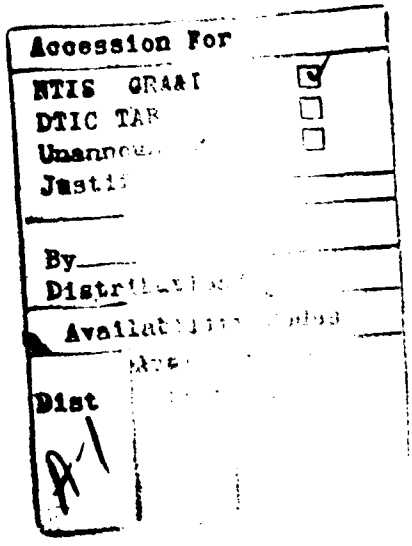
JOAN M. BOGGS
Chief, Technical Information Division

USAF Graduate Student Research Program (1988)

8 Technical Reports Enclosed

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# PREFACE

The United States Air Force Summer Faculty Research 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 1988 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 1988, 153-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.

## LIST OF 1988 PARTICIPANTS

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Ibrahim A. Ahmad<br>Professor and Director<br>Division of Statistics<br>Dept. of Math Sciences<br>Northern Illinois University<br>DeKalb, IL 60115<br>(815) 753-6739 | Degree: Ph.D., Statistics, 1975<br>Specialty: Statistics and Operations Research<br>Assigned: Armament Laboratory |
| Dr. Robert J. Arenz<br>Professor<br>Dept. of Mechanical Engineering<br>Gonzaga University<br>Spokane, WA 99258<br>(509) 328-4220 | Degree: Ph.D., Aeronautical Eng., 1964<br>Specialty: Solid Mech.<br>Assigned: Materials Laboratory |
| Dr. Lucia M. Babcock<br>Assistant Professor<br>Dept. of Chemistry<br>Louisiana State University<br>Choppin Hall<br>Baton Rouge, LA 70803<br>(504) 388-3239 | Degree: Ph.D., Chemistry, 1978<br>Specialty: Gas Phase Ion-Molecule Chem.<br>Assigned: Air Force Geophysics Lab. |
| Dr. Praphulla K. Bajpai<br>Professor<br>Dept. of Biology<br>University of Dayton<br>300 College Park<br>Dayton, OH 45469<br>(513) 229-3029 | Degree: Ph.D., Animal Physiology, 1965<br>Specialty: Physiology and Biomaterials<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. Stephen D. Baker<br>Professor<br>Dept. of Physics<br>Rice University<br>Houston, TX 77251-1892<br>(713) 527-8101 | Degree: Ph.D., Physics, 1963<br>Specialty: Nuclear Physics<br>Assigned: Air Force Geophysics Lab. |
| Dr. Pradip M. Bakshi<br>Research Professor<br>Dept. of Physics<br>Boston College<br>Chestnut Hill, MA 02167<br>(617) 552-3585 | Degree: Ph.D., Theoretical Physics, 1962<br>Specialty: Quantum Theory<br>Assigned: Air Force Geophysics Lab. |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Shankar S. Bale<br>Professor<br>Dept. of Science and Math<br>Saint Paul's University<br>Lawrenceville, VA 23868<br>(804) 848-3111 | Degree: Ph.D., Genetics, 1971<br>Specialty: Toxicology-Cytogenetics<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Mr. Beryl L. Barber<br>Assistant Professor<br>Dept. of Electronics Eng.<br>Oregon Institute of Technology<br>3201 Campus Drive<br>Klamath Falls, OR 97601-8801<br>(503) 882-6890 | Degree: MSEE, Electrical Eng., 1961<br>Specialty: RF/Microwave Components<br>Assigned: Rome Air Development Center |
| Dr. Bryan R. Becker<br>Assistant Professor<br>Dept. of Aerospace Engineering<br>University of Missouri<br>600 West Mechanic<br>Independence, MO 64050-1799<br>(816) 276-1279 | Degree: Ph.D., Eng. Science, 1979<br>Specialty: Computational Fluid Dynamics<br>Assigned: Aero Propulsion Laboratory |
| Dr. Reuben Benumof<br>Professor<br>Dept. of Applied Sciences<br>College of Staten Island<br>130 Stuyvesant Pl.<br>Staten Island, NY 10301<br>(718) 390-7973 | Degree: Ph.D., Physics, 1945<br>Specialty: Semiconductor Physics<br>Assigned: Air Force Geophysics Lab. |
| Mr. George N. Bratton<br>Associate Professor<br>Dept. of Math and Comp. Science<br>Austin State Peay State Univ.<br>P O Box 8343<br>Clarksville, TN 37044<br>(615) 648-7834 | Degree: Ed.D., Mathematics Ed., 1977<br>Specialty: Statistics<br>Assigned: Electronics Systems Division |
| Dr. Dan R. Bruss<br>Assistant Professor<br>Dept. of Physical Sciences<br>Albany College of Pharmacy<br>106 New Scotland Avenue<br>Albany, NY 12208<br>(518) 445-7225 | Degree: Ph.D., Chemistry, 1985<br>Specialty: Physical Organic Chemistry<br>Assigned: Frank J. Seiler Research Lab. |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Ronald Bulbulian<br>Associate Professor<br>Dept. of Health, Physical Education and Recreation<br>University of Kentucky<br>Seaton 100<br>Lexington, KY 40506<br>(606) 257-7904 | Degree: Ph.D., Physiology, 1980<br>Specialty: Exercise Physiology<br>Assigned: School of Aerospace Medicine |
| Dr. Charles M. Bump<br>Assistant Professor<br>Dept. of Chemistry<br>Hampton University<br>P O Box 6483<br>Hampton, VA 23668<br>(804) 727-5330 | Degree: Ph.D. Organic Chemistry, 1979<br>Specialty: Organic Synthesis<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. John A. Burke, Jr.<br>Professor<br>Dept. of Chemistry<br>Trinity University<br>715 Stadium Drive<br>San Antonio, TX 78284<br>(512) 736-7316 | Degree: Ph.D., Chemistry, 1963<br>Specialty: Inorganic Compounds<br>Assigned: School of Aerospace Medicine |
| Mr. Mike Burlakoff<br>Assistant Professor<br>Dept. of Computer Science<br>Southwest Missouri State Univ.<br>901 S. National<br>Springfield, MO 65804<br>(417) 836-5930 | Degree: MS., Math, Computer Sci., 1965<br>Specialty: Computer Science<br>Assigned: Avionics Laboratory |
| Dr. Larry W. Byrd<br>Assistant Professor<br>Dept. of Mechanical Engineering<br>Arkansas State University<br>P O Box 1080<br>State University, AR 72467-1080<br>(501) 972-3421 | Degree: Ph.D., Mechanical Eng., 1984<br>Specialty: Mechanical Engineering<br>Assigned: Flight Dynamics Laboratory |
| Dr. Clarence Calder<br>Associate Professor<br>Dept. of Mechanical Engineering<br>Oregon State University<br>Corvallis, OR 97331<br>(503) 754-2427 | Degree: Ph.D., Mechanical Eng., 1969<br>Specialty: Stress Wave Propagation<br>Assigned: Astronautics Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Richard T. Carlin<br>Assistant Professor<br>Dept. of Chemistry<br>Polytechnic University<br>333 Jay St.<br>Brooklyn, NY 11201<br>(718) 260-3339 | Degree: Ph.D., Chemistry, 1983<br>Specialty: Inorganic Chemistry<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. Gene O. Carlisle<br>Professor<br>Dept. of Chemistry and Physics<br>West Texas State University<br>Canyon, TX 79016<br>(806) 656-2282 | Degree: Ph.D., Inorganic Chem., 1969<br>Specialty: Coordination Chemistry<br>Assigned: Materials Laboratory |
| Dr. Patricia Carlson<br>Professor<br>Dept. of Humanities<br>Rose-Hulman Institute of Tech.<br>5500 Wabash<br>Terre Haute, IN 47803<br>(812) 877-1511 | Degree: Ph.D., Language & Lit., 1973<br>Specialty: Document Design<br>Assigned: Human Resources Laboratory:<br>Logistics & Human Factors Div. |
| Dr. David R. Cecil<br>Professor<br>Dept. of Mathematics<br>Texas A&I University<br>Campus Box 172<br>Kingsville, TX 78363<br>(512) 592-1839 | Degree: Ph.D., Mathematics, 1962<br>Specialty: Algebra (Finite Fields)<br>Assigned: Wilford Hall Medical Center |
| Dr. Wayne A. Charlie<br>Associate Professor<br>Dept. of Civil Engineering<br>Colorado State University<br>Fort Collins, CO 80523<br>(303) 491-8584 | Degree: Ph.D., Civil Engineering, 1975<br>Specialty: Geotechnical Engineering<br>Assigned: Engineering & Services Center |
| Dr. Steven C. Chiesa<br>Assistant Professor<br>Dept. of Civil Engineering<br>Santa Clara University<br>Santa Clara, CA 95053<br>(408) 554-4697 | Degree: Ph.D., Civil Eng., 1982<br>Specialty: Biological Waste Treatment<br>Assigned: Occupational and Environment<br>Health Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Karen C. Chou<br>Associate Professor<br>Dept. of Civil Engineering<br>Syracuse University<br>Syracuse, NY 13244-1190<br>(315) 423-3314 | Degree: Ph.D., Structural Eng., 1983<br>Specialty: Structural Engineering<br>Assigned: Flight Dynamics Laboratory |
| Dr. Phillip A. Christiansen<br>Associate Professor<br>Dept. of Chemistry<br>Clarkson University<br>Potsdam, NY 13676<br>(315) 268-4099 | Degree: Ph.D., Physical Chem., 1978<br>Specialty: Physical Chemistry<br>Assigned: Astronautics Laboratory |
| Dr. Keith A. Christianson<br>Assistant Professor<br>Dept. of Electrical Engineering<br>University of Maine<br>Orono, ME 04469<br>(207) 581-2244 | Degree: Ph.D., Materials Science and Engineering, 1985<br>Specialty: Electronic Materials<br>Assigned: Rome Air Development Center |
| Dr. Mingking K. Chyu<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>Carnegie Mellon University<br>Pittsburgh, PA 15213<br>(412) 268-3658 | Degree: Ph.D., Heat Transfer, 1986<br>Specialty: Heat Transfer<br>Assigned: Aero Propulsion Laboratory |
| Dr. Jerry D. Clark<br>Assistant Professor<br>Dept. of Physics<br>Wright State University<br>248 Fawcett Hall<br>Dayton, OH 45435<br>(513) 426-3917 | Degree: Ph.D., Physics, 1982<br>Specialty: Atomic Physics<br>Assigned: Aero Propulsion Laboratory |
| Dr. Lane Clark<br>Assistant Professor<br>Dept. of Mathematics<br>University of New Mexico<br>Albuquerque, NM 87106<br>(505) 277-2104 | Degree: Ph.D., Mathematics, 1980<br>Specialty: Graph Theory<br>Assigned: Weapons Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Donald F. Collins<br>Faculty in Physics<br>Dept. of Physics<br>Warren Wilson College<br>Swannanoa, NC 28778<br>(704) 298-3325 | Degree: Ph.D., Physics, 1970<br>Specialty: Optics, Image Processing<br>Assigned: Air Force Geophysics Lab. |
| Dr. Susan T. Collins<br>Assistant Professor<br>Dept. of Chemistry<br>California State University<br>18111 Nordhoff Street<br>Northridge, CA 91330<br>(818) 885-3367 | Degree: Ph.D., Physical Chem., 1981<br>Specialty: Matrix Isolation Spectroscopy<br>Assigned: Astronautics Laboratory |
| Dr. Charles D. Covington<br>Assistant Professor<br>Dept. of Electrical Engineering<br>University of Arkansas<br>Bell Engineering Center 3217<br>Fayetteville, AR 72701<br>(501) 575-6583 | Degree: Ph.D., Electrical Eng., 1984<br>Specialty: Digital Signal Processing<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. Parvis Dadras<br>Professor<br>Dept. of Mech. Systems Eng.<br>Wright State University<br>Dayton, OH 45435<br>(513) 873-2944 | Degree: Ph.D., Mechanical Eng., 1972<br>Specialty: Mechanics of Materials<br>Assigned: Materials Laboratory |
| Dr. John F. Dalphin<br>Professor<br>Dept. of Computer Science<br>Towson State University<br>Baltimore, MD 21204<br>(301) 321-3701 | Degree: Ph.D., Mathematics, 1973<br>Specialty: Computer Science<br>Assigned: Electronics Systems Division |
| Mr. Darin S. DeForest<br>Research Associate<br>Dept. of Computer Science<br>Arizona State University<br>Tempe, AZ 85287<br>(602) 965-3664 | Degree: B.Sc., Computer Science, 1984<br>Specialty: Programming Language Design<br>Assigned: Rome Air Development Center |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. David H. DeHeer<br>Associate Professor<br>Dept. of Biology<br>Calvin College<br>3201 Burton Street, S.E.<br>Grand Rapids, MI 49506<br>(616) 957-6083 | Degree: Ph.D., Molecular Biology, 1972<br>Specialty: Molecular Biology<br>Assigned: Engineering & Services Center |
| Dr. Eustace L. Dereniak<br>Associate Professor<br>Dept. of Optical Science<br>University of Arizona<br>528 N. Martin<br>Tucson, AZ 85719<br>(602) 621-1019 | Degree: Ph.D., Optics, 1976<br>Specialty: Infrared Physics<br>Assigned: Arnold Engineering Development Center |
| Prof. Paul T. Dingman<br>Assistant Professor<br>Dept. of Electronics Eng. Tech.<br>Oregon Institute of Technology<br>3201 Campus Drive<br>Klamath Falls, OR 97601-8801<br>(503) 882-6890 | Degree: MSEE., Electrical Eng., 1974<br>Specialty: Digital, Microprocessors<br>Assigned: Rome Air Development Center |
| Dr. David A. Dolson<br>Assistant Professor<br>Dept. of Chemistry<br>Murray State University<br>Murray, KY 42071<br>(502) 762-4490 | Degree: Ph.D., Physical Chem., 1981<br>Specialty: Laser Spectroscopy<br>Assigned: Weapons Laboratory |
| Dr. Hugh K. Donaghy<br>Assistant Professor<br>Dept. of Computer Science<br>Rochester Inst. of Technology<br>1 Lomb-Memorial Drive<br>Rochester, NY 14623<br>(716) 475-2994 | Degree: Ph.D., Philosophy, 1972<br>Specialty: Natural Language Processing<br>Assigned: Rome Air Development Center |
| Dr. Stephen J. Dow<br>Assistant Professor<br>Dept. of Math and Statistics<br>Univ. of Alabama in Huntsville<br>Huntsville, AL 35899<br>(205) 895-6252 | Degree: Ph.D., Mathematics, 1982<br>Specialty: Discrete Mathematics<br>Assigned: Armament Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Derek Dunn-Rankin<br>Assistant Professor<br>Dept. of Mechanical Engineering<br>University of California<br>616 Engineering<br>Irvine, CA 92717<br>(714) 854-0460 | Degree: Ph.D., Mechanical Eng., 1985<br>Specialty: Laser Diagnostics (combustion)<br>Assigned: Aero Propulsion Laboratory |
| Dr. Deanna S. Durnford<br>Assistant Professor<br>Dept. of Agric. & Chem. Eng.<br>Colorado State University<br>Ft. Collins, CO 80523<br>(303) 491-5252 | Degree: Ph.D., Civil Eng., 1982<br>Specialty: Groundwater<br>Assigned: Engineering & Services Center |
| Dr. Suren N. Dwivedi<br>Associate Professor<br>Dept. of Mechanical Eng.<br>University of North Carolina<br>Charlotte, NC 28223<br>(704) 547-2303 | Degree: Ph.D., Engineering, 1976<br>Specialty: Material Processing<br>Assigned: Materials Laboratory |
| Dr. Wayne A. Eckerle<br>Associate Professor<br>Dept. of Mech. & Ind. Eng.<br>Clarkson University<br>Potsdam, NY 13676<br>(315) 268-2203 | Degree: Ph.D., Fluid Mech., 1985<br>Specialty: Experimental Fluid Mechanics<br>Assigned: Aero Propulsion Laboratory |
| Dr. J. Kevin Ford<br>Assistant Professor<br>Dept. of Psychology<br>Michigan State University<br>East Lansing, MI 48824<br>(517) 353-5006 | Degree: Ph.D., Philosophy, 1983<br>Specialty: Industrial/Organ. Psychology<br>Assigned: Human Resources Laboratory:<br>Training Systems |
| Prof. Michael E. Frantz<br>Assistant Professor<br>Dept. of Math and Physics<br>University of LaVerne<br>1950 Third Street<br>LaVerne, CA 91750<br>(714) 593-3511 | Degree: M.S., Mathematics, 1978<br>Specialty: Partial Differential Equations<br>Assigned: Air Force Geophysics Lab. |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Barry K. Fussell<br>Assistant Professor<br>Dept. of Mechanical Engineering<br>University of New Hampshire<br>Kingsbury Hall<br>Durham, NH 03824<br>(603) 862-1352 | Degree: Ph.D., Mechanical Eng., 1987<br>Specialty: Systems Modeling & Controls<br>Assigned: Materials Laboratory |
| Dr. Hugh. P. Garraway, III<br>Associate Professor<br>Dept. of Computer Science<br>Univ. of Southern Mississippi<br>Box 5106<br>Hattiesburg, MS 39406<br>(601) 266-4949 | Degree: Ph.D., Instruc. Tech., 1980<br>Specialty: Computer Based Learning<br>Assigned: Human Resources Laboratory: Training Systems |
| Dr. Christopher P. Godfrey<br>Assistant Professor<br>Dept. of Computer Science<br>Missouri Western State College<br>4525 Downs Drive<br>St. Joseph, MO 64507<br>(816) 271-4372 | Degree: Ph.D., Physics, 1982<br>Specialty: High Energy Astrophysics<br>Assigned: Air Force Geophysics Lab. |
| Dr. Barry P. Goettl<br>Assistant Professor<br>Dept. of Psychology<br>Clemson University<br>108 Brackett Hall<br>Clemson, SC 29634-1511<br>(803) 656-2831 | Degree: Ph.D., Psychology, 1987<br>Specialty: Engineering Psychology<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. Gerald W. Grams<br>Professor<br>School of Geophysical Sciences<br>Georgia Tech.<br>Atlanta, GA 30332<br>(404) 894-3628 | Degree: Ph.D., Meteorology, 1966<br>Specialty: Atmospheric Physics<br>Assigned: Avionics Laboratory |
| Dr. Edward K. Greenwald<br>Assistant Professor<br>Engineering Professional Dev.<br>Univ. of Wisconsin-Madison<br>432 N. Lake Street<br>Madison, WI 53706<br>(608) 262-0573 | Degree: Ph.D., Physics, 1967<br>Specialty: Electrical Engineering<br>Assigned: Engineering & Services Center |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Prof. William M. Grissom<br>Assistant Professor<br>Dept. of Physics<br>Morehouse College<br>830 Westview Dr., S.W.<br>Atlanta, GA 30314<br>(404) 681-2800 | Degree: M.S.E., Mechanical Eng., 1978<br>Specialty: Combustion Diagnostics<br>Assigned: Arnold Engineering Development Center |
| Dr. David A. Grossie<br>Assistant Professor<br>Dept. of Chemistry<br>Wright State University<br>Dayton, OH 45435<br>(513) 873-2210 | Degree: Ph.D., Chemistry, 1982<br>Specialty: X-ray Crystallography<br>Assigned: Materials Laboratory |
| Dr. Vijay K. Gupta<br>Professor<br>Dept. of Chemistry<br>Central State University<br>Wilberforce, OH 45384<br>(513) 376-6423 | Degree: Ph.D., Chemistry, 1969<br>Specialty: Physical Chemistry<br>Assigned: Materials Laboratory |
| Dr. Awatef Hamed<br>Dept. of Aerospace Eng.<br>University of Cincinnati<br>Mail Location 70<br>Cincinnati, OH 45221<br>(513) 475-5630 | Degree: Ph.D., Engineering, 1972<br>Specialty: Engineering<br>Assigned: Flight Dynamics Laboratory |
| Dr. Albert A. Heaney<br>Professor<br>Dept. of Electrical Eng.<br>California State University<br>Shaw & Cedar Avenues<br>Fresno, CA 93740-0094<br>(209) 294-4823 | Degree: Ph.D., Electrical Eng., 1972<br>Specialty: Computer Engineering<br>Assigned: Eastern Space Missile Center |
| Dr. David Hemmendinger<br>Assistant Professor<br>Dept. of Compt. Sci. & Eng.<br>Wright State University<br>Research Bldg.<br>317 Research Blvd.<br>Kettering, OH 45420<br>(513) 259-1345 | Degree: Ph.D., Philosophy, 1973<br>Specialty: Logic Programming<br>Assigned: Avionics Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Bennye S. Henderson<br>Associate Professor<br>Dept. of Biology<br>Jackson State University<br>1325 Lynch Street<br>Jackson, MS 39217<br>(601) 968-2586 | Degree: Ph.D., Physiology, 1979<br>Specialty: Physiology<br>Assigned: School of Aerospace Medicine |
| Dr. Darrell E.P. Hoy<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>Tennessee Technological Univ.<br>Box 5014<br>Cookeville, TN 38505<br>(615) 372-3732 | Degree: M.S.E., Mechanical Eng., 1985<br>Specialty: Ballistic Impact Shocks<br>Assigned: Arnold Engineering Development Center |
| Dr. Manuel A. Huerta<br>Professor<br>Dept. of Physics<br>University of Miami<br>P O Box 248046<br>Coral Gables, FL 33124<br>(305) 284-2323 | Degree: Ph.D., Physics, 1970<br>Specialty: Plasma Physics<br>Assigned: Armament Laboratory |
| Dr. Randolph B. Huff<br>Professor<br>Dept. of Chemistry<br>Presbyterian College<br>Clinton, SC 29325<br>(803) 833-2820 | Degree: Ph.D., Inorganic Chem., 1969<br>Specialty: Physical-Inorganic Chemistry<br>Assigned: Occupational and Environment Health Laboratory |
| Dr. Neil J. Hutzler<br>Associate Professor<br>Dept. of Civil Engineering<br>Michigan Tech. University<br>Houghton, MI 49931<br>(906) 487-2194 | Degree: Ph.D., Environmental Eng., 1978<br>Specialty: Environmental Engineering<br>Assigned: Engineering & Services Center |
| Dr. Douglas E. Jackson<br>Professor<br>Dept. of Math Sciences<br>Eastern New Mexico University<br>Portales, NM 88130<br>(505) 562-2367 | Degree: Ph.D., Mathematics, 1969<br>Specialty: Math/Statistical Information<br>Assigned: Human Resources Laboratory: Manpower & Personnel Division |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Oleg G. Jakubowicz<br>Assistant Professor<br>Dept. of Elect. & Compt. Eng.<br>State University of New York<br>238 Bell Hall<br>Buffalo, NY 14260<br>(716) 636-2406 | Degree: Ph.D., Physics, 1984<br>Specialty: Neural Nets<br>Assigned: Rome Air Development Center |
| Dr. Manjit S. Jawa<br>Professor<br>Dept. of Mathematics<br>Fayetteville State University<br>Fayetteville, NC 28301<br>(919) 486-1675 | Degree: Ph.D., Applied Math., 1967<br>Specialty: Applied Mathematics<br>Assigned: Arnold Engineering Development Center |
| Dr. David W. Jensen<br>Assistant Professor<br>Dept. of Aerospace Eng.<br>Pennsylvania State University<br>233N Hammond Bldg.<br>University Park, PA 16802<br>(814) 863-1077 | Degree: Ph.D., Structures Tech., 1986<br>Specialty: Advanced Composite Materials<br>Assigned: Astronautics Laboratory |
| Dr. Eric R. Johnson<br>Associate Professor<br>Dept. of Chemistry<br>Ball State University<br>Muncie, IN 47306<br>(317) 285-8078 | Degree: Ph.D., Biochemistry, 1974<br>Specialty: Protein Biochemistry<br>Assigned: School of Aerospace Medicine |
| Dr. William M. Jordan<br>Assistant Professor<br>Dept. of Mech. & Indus. Eng.<br>Lousiana Tech. University<br>P O Box 10348<br>Ruston, LA 71272<br>(318) 257-4304 | Degree: Ph.D., Intersiciplinary Eng., 1985<br>Specialty: Composite Materials<br>Assigned: Weapons Laboratory |
| Dr. Mohammad A. Karim<br>Assistant Professor<br>Dept. of Electrical Eng.<br>University of Dayton<br>KL-241D<br>Dayton, OH 45469<br>(513) 229-3611 | Degree: Ph.D., Electrical Eng., 1982<br>Specialty: Electro-Optics<br>Assigned: Avionics Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Arkady Kheyfets<br>Assistant Professor<br>Dept. of Mathematics<br>North Carolina State Univ.<br>Box 8205<br>Raleigh, NC 27695-8205<br>(919) 737-3265 | Degree: Ph.D., Physics, 1986<br>Specialty: Mathematical Physics<br>Assigned: Weapons Laboratory |
| Prof. Daisy W. Kimble<br>Assistant Professor<br>Dept. of Chemistry<br>Southern University<br>P O Box 11487<br>Baton Rouge, LA 70813<br>(504) 771-3734 | Degree: M.S., Analytical Chem., 1986<br>Specialty: Analytical Chemistry<br>Assigned: School of Aerospace Medicine |
| Dr. Yulian B. Kin<br>Associate Professor<br>Dept. of Engineering<br>Purdue University Calumet<br>Potter Building<br>Hammond, IN 46323<br>(219) 989-2684 | Degree: Ph.D., Fatigue Stress Analysis 1971<br>Specialty: Stress Analysis<br>Assigned: Flight Dynamics Laboratory |
| Dr. Samuel P. Kozaitis<br>Assistant Professor<br>Dept. of Electrical Eng.<br>Florida Institute of Tech.<br>Melbourne, FL 32901-6988<br>(305) 768-8000 | Degree: Ph.D., Electrical Eng, 1986<br>Specialty: Optics, Computer Architecture<br>Assigned: Rome Air Development Center |
| Dr. Janet U. Kozyra<br>Assistant Research Scientist<br>University of Michigan<br>Space Physics Research Lab.<br>2455 Hayward<br>Ann Arbor, MI 48109-2143<br>(313) 747-3550 | Degree: Ph.D., Atmospheric Sci., 1986<br>Specialty: Space Physics<br>Assigned: Air Force Geophysics Lab. |
| Dr. Charles E. Lance<br>Assistant Professor<br>Dept. of Psychology<br>University of Georgia<br>Athens, GA 30602<br>(404) 542-3053 | Degree: Ph.D., Psychology, 1985<br>Specialty: Industrial/Organizational Psy.<br>Assigned: Human Resources Laboratory: Manpower & Personnel Division |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Thomas L. Landers<br>Assistant Professor<br>Dept. of Industrial Engineering<br>University of Arkansas<br>4176 Bell Engineering Ctr.<br>Fayetteville, AR 72703<br>(501) 575-6042 | Degree: Ph.D., Industrial Eng., 1985<br>Specialty: Reliability & Maintainability<br>Assigned: Human Resources Laboratory:<br>Logistics & Human Factors Div. |
| Prof. Anastas Lazaridis<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>Widener University<br>Chester, PA 19013<br>(215) 499-4487 | Degree: Sc.D., Thermal Fluids, 1969<br>Specialty: Ablation, Solar Energy<br>Assigned: Armament Laboratory |
| Dr. L. James Lee<br>Associate Professor<br>Dept. of Chemical Eng.<br>The Ohio State University<br>140 W. 19th Avenue<br>Columbus, OH 43210<br>(614) 292-2408 | Degree: Ph.D., Chemical Eng., 1979<br>Specialty: Polymer & Composite Processing<br>Assigned: Materials Laboratory |
| Dr. Robert Y. Li<br>Assistant Professor<br>Dept. of Electrical Eng.<br>University of Nebraska<br>Lincoln, NE 68588<br>(402) 472-5892 | Degree: Ph.D., Electrical Eng., 1981<br>Specialty: Image Processing<br>Assigned: Avionics Laboratory |
| Dr. Irving Lipschitz<br>Associate Professor<br>Dept. of Chemistry<br>University of Lowell<br>1 University Lane<br>Lowell, MA 01854<br>(617) 452-5000 | Degree: Ph.D., Physical Chem., 1965<br>Specialty: Vibrational Spectroscopy<br>Assigned: Air Force Geophysics Lab. |
| Dr. Harold G. Longbotham<br>Visiting Assistant Professor<br>Dept. of Electrical Eng.<br>Univ. of Texas - San Antonio<br>San Antonio, TX 78285<br>(512) 691-5518 | Degree: Ph.D., Electrical Eng., 1985<br>Specialty: Nonlinear Digital Filtering<br>Assigned: School of Aerospace Medicine |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. David A. Ludwig<br>Assistant Professor<br>Dept. of Mathematics<br>Univ. of North Carolina<br>at Greensboro<br>Greensboro, NC 27412<br>(919) 334-5836 | Degree: Ph.D., Biostatistics, 1982<br>Specialty: Biostatistics, Exp. Design<br>Assigned: School of Aerospace Medicine |
| Dr. Douglas A. Mandra<br>Associate Professor<br>Dept. of Psychology<br>Francis Marion College<br>P O Box 7500<br>Florence, SC 29501<br>(803) 661-1378 | Degree: Ph.D., Psychology, 1974<br>Specialty: Experimental Psychology<br>Assigned: Human Resources Laboratory:<br>Operations Training Division |
| Dr. Robert E. Masingale, Sr.<br>Professor<br>Dept. of Chemistry<br>Jarvis Christian College<br>Hawkins, TX 75765<br>(214) 769-2174 | Degree: Ph.D., Organic Chemistry, 1968<br>Specialty: Organic & Analytical Chemistry<br>Assigned: Harry G. Armstrong Aerospace<br>Medical Research Laboratory |
| Dr. John P. McHugh<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>University of New Hampshire<br>133 Kingsbury<br>Durham, NH 03824<br>(603) 862-1899 | Degree: Ph.D., Applied Mechanics, 1986<br>Specialty: Fluid Mechanics<br>Assigned: Air Force Geophysics Lab. |
| Dr. Michael L. McKee<br>Associate Professor<br>Dept. of Chemistry<br>Auburn University<br>Auburn, AL 36849-5312<br>(205) 826-4043 | Degree: Ph.D., Chemical Physics, 1977<br>Specialty: Molecular Orbital Theory<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. Thomas T. Meek<br>Associate Professor<br>Dept. of Materials Sci. & Eng.<br>University of Tennessee<br>434 Dougherty Engineering Bldg.<br>Knoxville, TN 37966-2200<br>(615) 970-0940 | Degree: Ph.D., Ceramic Eng., 1977<br>Specialty: Ceramic Processing<br>Assigned: Materials Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Tammy J. Melton<br>Assistant Professor<br>Dept. of Chemistry<br>St. Norbert College<br>DePere, WI 54115<br>(414) 337-3206 | Degree: Ph.D., Inorganic Chem., 1986<br>Specialty: Inorganic Synthesis<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. Carolyn W. Meyers<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>Georgia Inst. of Technology<br>School of Mechanical Eng.<br>Atlanta, GA 30332<br>(404) 894-3264 | Degree: Ph.D., Physical Metallurgy, 1984<br>Specialty: Microstructure<br>Assigned: Engineering & Services Center |
| Dr. David W. Mikolaitis<br>Assistant Professor<br>Dept. of Engineering Sciences<br>University of Florida<br>231 Aero<br>Gainesville, FL 32611<br>(904) 392-0961 | Degree: Ph.D., Theoretical & Applied Mechanics, 1981<br>Specialty: Applied Math<br>Assigned: Armament Laboratory |
| Dr. Kwang S. Min<br>Professor<br>Dept. of Physics<br>East Texas State University<br>Commerce, TX 75428<br>(214) 885-5483 | Degree: Ph.D., Physics, 1962<br>Specialty: Signal Processing<br>Assigned: Armament Laboratory |
| Dr. Joseph J. Molitoris<br>Professor<br>Dept. of Physics<br>Muhlenberg College<br>Allentown, PA 18104<br>(215) 821-3413 | Degree: Ph.D., Physics, 1985<br>Specialty: Nuclear Physics<br>Assigned: Armament Laboratory |
| Mr. Augustus Morris<br>Instructor<br>Dept. of Manufacturing Eng.<br>Central State University<br>Wilberforce, OH 45384<br>(513) 376-6435 | Degree: B.S., Biomedical Eng., 1981<br>Specialty: Biomedical Engineering<br>Assigned: Flight Dynamics Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. William P. Mounfield<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>Louisiana State University<br>R2513-A CEBA Bldg.<br>Baton Rouge, LA 70803-6413<br>(504) 388-6488 | Degree: Ph.D., Mechanical Eng., 1985<br>Specialty: Automatic Controls<br>Assigned: Engineering & Services Center |
| Dr. Nanda L. Mukherjee<br>Associate Professor<br>Dept. of Chemical Eng.<br>Tuskegee University<br>Tuskegee, AL 36088<br>(205) 727-8050 | Degree: Ph.D., Chemical Eng., 1967<br>Specialty: Kinetics<br>Assigned: Flight Dynamics Laboratory |
| Dr. Richard S. Myers<br>Professor<br>Dept. of Physical Sciences<br>Delta State University<br>P O Box 3255<br>Cleveland, OH 38733<br>(601) 846-4482 | Degree: Ph.D., Physical Chem., 1968<br>Specialty: Experimental Physical Chem.<br>Assigned: Engineering & Services Center |
| Dr. Himanshoo V. Navangul<br>Professor<br>Dept. of Chemistry and<br>Physical Science<br>North Carolina Wesleyan College<br>Wesleyan Station<br>Rocky Mount, NC 27804<br>(919) 977-7171 | Degree: Ph.D., Physical Chem., 1967<br>Specialty: Molecular Spectroscopy<br>Assigned: Air Force Geophysics Lab. |
| Dr. Mark A. Norris<br>Assistant Professor<br>Dept. of Mechanics<br>Virginia Polytechnic Inst.<br>and State University<br>227 Norris Hall<br>Blacksburg, VA 24061<br>(703) 961-4576 | Degree: Ph.D., Eng. Mechanics, 1986<br>Specialty: Structural Dynamics & Controls<br>Assigned: Astronautics Laboratory |
| Dr. Mufit H. Ozden<br>Associate Professor<br>Dept. of Systems Analysis<br>Miami University<br>2303 Kreger Hall<br>Oxford, OH 45056<br>(513) 529-5937 | Degree: Ph.D. Eng. Systems, 1975<br>Specialty: Operations Research<br>Assigned: Human Resources Laboratory:<br>Logistics & Human Factors Div. |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Prof. Martin A. Patt<br>Associate Professor<br>Dept. of Electrical Eng.<br>University of Lowell<br>1 University Ave.<br>Lowell, MA 01854<br>(617) 452-5000 | Degree: M.S., Electrical Eng., 1964<br>Specialty: Computer Applications<br>Assigned: Air Force Geophysics Lab. |
| Dr. David G. Payne<br>Assistant Professor<br>Dept. of Psychology<br>SUNY Binghamton<br>Binghamton, NY 13901<br>(607) 777-4610 | Degree: Ph.D., Cognitive Psy., 1984<br>Specialty: Human Memory<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. William Z. Plachy<br>Professor<br>Dept. of Chemistry & Biochem.<br>San Francisco State University<br>San Francisco, CA 94132<br>(415) 338-1436 | Degree: Ph.D., Physical Chem., 1967<br>Specialty: Physical Chemistry<br>Assigned: School of Aerospace Medicine |
| Dr. Patricia L. Plummer<br>Professor<br>Dept. of Physics & Chemistry<br>Columbia Univ. of Missouri<br>Columbia, NC 65211<br>(314) 882-3053 | Degree: Ph.D., Chemical Physics, 1964<br>Specialty: Quantum Chemistry<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. Leonard E. Porter<br>Professor<br>Dept. of Physics & Astronomy<br>University of Montana<br>Missoula, MT 59812<br>(406) 243-6223 | Degree: Ph.D., Nuclear Physics, 1965<br>Specialty: Nuclear Physics<br>Assigned: Weapons Laboratory |
| Dr. Ramalingam Radhakrishnan<br>Assistant Professor<br>Dept. of Civil Engineering<br>Prairie View A&M University<br>Prairie View, TX 77084<br>(409) 857-2418 | Degree: Ph.D, Structure Eng., 1974<br>Specialty: Structures<br>Assigned: Engineering & Services Center |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Periasamy K. Rajan<br>Professor<br>Dept. of Electrical Eng.<br>Tennessee Tech. University<br>Box 5004<br>Cookeville, TN 38505<br>(615) 372-3308 | Degree: Ph.D., Electrical Eng., 1975<br>Specialty: Digital Signal Processing<br>Assigned: Avionics Laboratory |
| Dr. Panapakkam A. Ramamoorthy<br>Associate Professor<br>Dept. of Elect. & Computer Eng.<br>University of Cincinnati<br>M.L. #30<br>Cincinnati, OH 45220<br>(513) 475-4247 | Degree: Ph.D., Digital Signal Processing, 1977<br>Specialty: Optical Memory<br>Assigned: Avionics Laboratory |
| Dr. Dharam S. Rana<br>Associate Professor<br>Dept. of Management & Marketing<br>Jackson State University<br>1400 J.R. Lynch<br>Jackson, MS 39217<br>(601) 968-2534 | Degree: Ph.D., Statistics, 1976<br>Specialty: Quantitative Techniques<br>Assigned: Human Resources Laboratory: Manpower & Personnel Division |
| Dr. Sunita S. Rana<br>Instructor<br>Dept. of Computer Science<br>Jackson State University<br>1400 Lynch Street<br>Jackson, MS 39217<br>(601) 968-2105 | Degree: Ph.D., Biology, 1969<br>Specialty: Computer Science<br>Assigned: Human Resources Laboratory: Training Systems |
| Dr. Hal C. Reed<br>Associate Professor<br>Dept. of Biology<br>Oral Roberts University<br>7777 S. Lewis<br>Tulsa, OK 74171<br>(918) 495-6945 | Degree: Ph.D., Entomology, 1982<br>Specialty: Insect Behavior<br>Assigned: School of Aerospace Medicine |
| Dr. Michael D. Rice<br>Associate Professor<br>Dept. of Computer Science<br>George Mason University<br>4400 University Dr.<br>Fairfax, VA 22030<br>(703) 323-3884 | Degree: Ph.D., Mathematics, 1973<br>Specialty: Computer Science/Math<br>Assigned: Weapons Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Mateen M. Rizki<br>Assistant Professor<br>Dept. of Computer Science<br>Wright State University<br>410 Fawcett Hall<br>Dayton, OH 45435<br>(513) 873-2394 | Degree: Ph.D., Computer Science, 1985<br>Specialty: Modeling and Simulation<br>Assigned: Avionics Laboratory |
| Dr. Thomas R. Rogge<br>Professor<br>Dept. of Eng. Science & Math<br>Iowa State University<br>3015 Black Eng.<br>Ames, IA 50010<br>(515) 294-2956 | Degree: Ph.D., Applied Math, 1964<br>Specialty: Finite Element Analysis<br>Assigned: School of Aerospace Medicine |
| Dr. Joe M. Ross<br>Assistant Professor<br>Dept. of Chemistry<br>Central State University<br>Wilberforce, OH 45384<br>(513) 376-6214 | Degree: Ph.D., Molecular Bio., 1977<br>Specialty: Biochemistry of Macromolecules<br>Assigned: School of Aerospace Medicine |
| Dr. Joseph E. Saliba<br>Assistant Professor<br>Dept. of Civil & Engr. Mechanics<br>University of Dayton<br>300 College Park<br>Dayton, OH 45469<br>(513) 229-3847 | Degree: Ph.D., Solid Mechanics, 1983<br>Specialty: Engineering Mechanics<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. Dhiraj K. Sardar<br>Assistant Professor<br>Dept. of Physics<br>University of Texas<br>Div. of Earth & Physical Sci.<br>San Antonio, TX 78285-0663<br>(512) 691-5462 | Degree: Ph.D., Physics, 1980<br>Specialty: Materials Science & Lasers<br>Assigned: School of Aerospace Medicine |
| Prof. Sonia H. Sawtelle<br>Teaching Associate<br>Dept. of Education<br>Univ. of Texas - San Antonio<br>San Antonio, TX 78285<br>(512) 691-4412 | Degree: MS., Exercise Physiology, 1975<br>Specialty: Exercise Physiology<br>Assigned: School of Aerospace Medicine |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Paul O. Scheie<br>Professor<br>Dept. of Physics<br>Texas Lutheran College<br>1000 West Court<br>Seguin, TX 78155<br>(512) 379-4161 | Degree: Ph.D., Biophysics, 1965<br>Specialty: Electrophysiology<br>Assigned: School of Aerospace Medicine |
| Dr. James L. Schmutz<br>Professor<br>Dept. of Chemistry<br>Central Wesleyan College<br>1 Wesleyan Drive<br>Central, SC 29630<br>(803) 639-2453 | Degree: Ph.D., Chemistry, 1976<br>Specialty: Inorganic Polymers<br>Assigned: Frank J. Seiler Research Lab. |
| Dr. Jodye I. Selco<br>Assistant Professor<br>Dept. of Chemistry<br>University of Redlands<br>P O Box 3080<br>Redlands, CA 92373-0999<br>(714) 793-2121 | Degree: Ph.D., Chemical Physics, 1983<br>Specialty: Spectroscopy, Kinetics<br>Assigned: Astronautics Laboratory |
| Dr. Shawky E. Shamma<br>Professor<br>Dept. of Math/Statistics<br>University of West Florida<br>Pensacola, FL 32514<br>(904) 474-2281 | Degree: Ph.D., Applied Math, 1969<br>Specialty: Applied Mathematics<br>Assigned: Armament Laboratory |
| Dr. Rameshwar P. Sharma<br>Associate Professor<br>Dept. of Mechanical Engineering<br>Western Michigan University<br>2065 Kohrman Hall<br>Kalamazoo, MI 49008<br>(616) 383-1408 | Degree: Ph.D., Mechanical Eng., 1978<br>Specialty: Fluid Mechanics<br>Assigned: Astronautics Laboratory |
| Dr. Larry R. Sherman<br>Professor<br>Dept. of Chemistry<br>University of Akron<br>Akron, OH 44325-0001<br>(216) 375-7333 | Degree: Ph.D., Analytical Chem., 1969<br>Specialty: Organotin Chemistry<br>Assigned: Occupational and Environment Health Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. James A. Sherwood<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>University of New Hampshire<br>Kingsbury Hall<br>Durham, NH 03824<br>(603) 862-2624 | Degree: Ph.D., Aerospace Eng., 1987<br>Specialty: Solid Mechanics<br>Assigned: Flight Dynamics Laboratory |
| Dr. Sanford S. Singer<br>Professor<br>Dept. of Chemistry<br>University of Dayton<br>300 College Park<br>Dayton, OH 45469<br>(513) 229-2833 | Degree: Ph.D., Biological Chem., 1967<br>Specialty: Enzymology<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |
| Dr. Trilochan Singh<br>Professor<br>Dept. of Mechanical Eng.<br>Wayne State University<br>Detroit, MI 48202<br>(313) 577-3845 | Degree: Ph.D., Mechanical Eng., 1970<br>Specialty: Chemical Combustion<br>Assigned: Astronautics Laboratory |
| Dr. Jorge L. Sintes<br>Chairman<br>Dept. of Preventive Dentistry and Community Health<br>Meharry Medical College<br>1005 D.B. Todd Blvd.<br>Nashville, TN 37208<br>(615) 327-6185 | Degree: Ph.D., Nutrition, 1978<br>Specialty: Dentistry<br>Assigned: Wilford Hall Medical Center |
| Dr. Kenneth M. Sobel<br>Associate Professor<br>Dept. of Electrical Engineering<br>The City College of New York<br>138th St. & Convent Ave.<br>New York, NY 10031<br>(212) 690-4241 | Degree: Ph.D., Electrical Eng., 1980<br>Specialty: Eigenstructure<br>Assigned: Flight Dynamics Laboratory |
| Dr. Jonathan M. Spector<br>Assistant Professor<br>CSIS<br>Jacksonville State University<br>Pelham Road<br>Jacksonville, AL 36265<br>(205) 231-5718 | Degree: Ph.D., Philosophy, 1978<br>Specialty: Logic<br>Assigned: Human Resources Laboratory: Training Systems Division |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Gary R. Stevens<br>Assistant Professor<br>Dept. of Statistics<br>Oklahoma State University<br>301 MS<br>Stillwater, OK 74078<br>(405) 624-5684 | Degree: Ph.D., Statistics, 1986<br>Specialty: Stochastic Processes<br>Assigned: Occupational and Environment Health Laboratory |
| Dr. Patrick J. Sweeney<br>Asst. Dean of Engineering<br>University of Dayton<br>300 College Park, KL201<br>Dayton, OH 45469<br>(513) 229-2736 | Degree: Ph.D., Mechanical Eng., 1977<br>Specialty: Computer Modeling<br>Assigned: Flight Dynamics Laboratory |
| Dr. Michael Sydor<br>Professor<br>Dept. of Physics<br>University of Minnesota<br>Duluth, MN 55812<br>(218) 726-7205 | Degree: Ph.D., Physics, 1965<br>Specialty: Optics, Material Science<br>Assigned: Materials Laboratory |
| Dr. Douglas G. Talley<br>Assistant Professor<br>Dept. of Mechanical Eng.<br>University of Michigan<br>313 Automotive Lab<br>Ann Arbor, MI 48109-2121<br>(313) 936-0429 | Degree: Ph.D., Mechanical Eng., 1978<br>Specialty: Combustion<br>Assigned: Aero Propulsion Laboratory |
| Dr. David J. Townsend<br>Associate Professor<br>Dept. of Psychology<br>Montclair State College<br>Upper Montclair, NJ 07042<br>(201) 893-7222 | Degree: Ph.D., Cognitive Psy., 1982<br>Specialty: Cognitive Science<br>Assigned: Rome Air Development Center |
| Dr. Donald R. Ucci<br>Associate Professor<br>Dept. of Elect. & Computer Eng.<br>Illinois Inst. of Technology<br>3300 S. Federal<br>Chicago, IL 60616<br>(312) 567-3405 | Degree: Ph.D., Electrical Eng., 1986<br>Specialty: Adaptive Arrays<br>Assigned: Rome Air Development Center |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Ahmad D. Vakili<br>Associate Professor<br>Dept. of AE/ME<br>Univ. of Tennessee Space Inst.<br>Tullahoma, TN 37388<br>(615) 455-0631 | Degree: Ph.D., Aerospace Eng., 1978<br>Specialty: Unsteady Flows<br>Assigned: Arnold Engineering Development Center |
| Dr. Richard S. Valpey<br>Assistant Professor<br>Dept. of Chemistry<br>Wilberforce University<br>Wilberforce, OH 45384<br>(513) 376-2911 | Degree: Ph.D., Organic Chemistry, 1983<br>Specialty: Organic Synthesis<br>Assigned: Materials Laboratory |
| Dr. Peter J. Walsh<br>Professor<br>Dept. of Physics<br>Fairleigh Dickinson University<br>Teaneck, NJ 07666<br>(201) 692-2493 | Degree: Ph.D., Physics, 1960<br>Specialty: Superconductivity<br>Assigned: Rome Air Development Center |
| Dr. Kenneth L. Walter<br>Associate Professor<br>Dept. of Chemical Engineering<br>Prairie View A&M University<br>Prairie View, TX 77446<br>(409) 857-2827 | Degree: Ph.D., Chemical Eng., 1972<br>Specialty: Chemical Engineering Process<br>Assigned: Rome Air Development Center |
| Dr. Gwo-Ching Wang<br>Associate Professor<br>Dept. of Physics<br>Rensselaer Polytechnic Inst.<br>Troy, NY 12180-3590<br>(518) 276-8387 | Degree: Ph.D., Materials Science, 1978<br>Specialty: Surface Sciences<br>Assigned: Rome Air Development Center |
| Dr. Andrew P. Whipple<br>Associate Professor<br>Dept. of Biology<br>Taylor University<br>Upland, IN 46989<br>(317) 998-5333 | Degree: Ph.D., Biology, 1979<br>Specialty: Cell Biology<br>Assigned: Harry G. Armstrong Aerospace Medical Research Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Prof. Sharon T. Williams<br>Instructor<br>Dept. of Chemistry<br>Southern University<br>Baton Rouge, LA 70813-2074<br>(504) 771-3990 | Degree: M.S., Biochemistry, 1981<br>Specialty: General Chemistry<br>Assigned: School of Aerospace Medicine |
| Dr. Lawrence A. Witt<br>Assistant Professor<br>Dept. of Psychology<br>Western Illinois University<br>Macomb, IL 61455<br>(309) 298-1593 | Degree: Ph.D., Psychology, 1985<br>Specialty: Industrial/Organ. Psychology<br>Assigned: Human Resources Laboratory:<br>Operations Training Division |
| Dr. Frank A. Witzmann<br>Assistant Professor<br>Dept. of Biology<br>IUPUI - Columbus<br>4601 Central Avenue<br>Columbus, IN 47203<br>(812) 372-8266 | Degree: Ph.D., Biology, 1981<br>Specialty: Protein Analysis<br>Assigned: Harry G. Armstrong Aerospace<br>Medical Research Laboratory |
| Dr. William E. Wolfe<br>Associate Professor<br>Dept. of Civil Engineering<br>Ohio State University<br>2070 Neil Avenue<br>Columbus, OH 43210<br>(614) 292-0790 | Degree: Ph.D., Engineering, 1979<br>Specialty: Geotechnical Engineering<br>Assigned: Flight Dynamics Laboratory |
| Dr. John R. Wright<br>Professor<br>Dept. of Chem., Physical Sci.<br>Southeast Oklahoma State Univ.<br>Box 4181, Station A, SEOSU<br>Durant, OK 74701<br>(405) 924-0121 | Degree: Ph.D., Chemistry, 1971<br>Specialty: Biochemistry<br>Assigned: School of Aerospace Medicine |
| Prof. Wafa E. Yazigi<br>Instructor<br>Dept. of Mathematics<br>Columbia Basin College<br>2600 N. 20th<br>Pasco, WA 99301<br>(509) 547-0511 | Degree: M.S., Aeronautical Eng., 1986<br>Specialty: Solid Mechanics<br>Assigned: Armament Laboratory |

| NAME/ADDRESS | DEGREE, SPECIALTY, LABORATORY ASSIGNED |
|---|---|
| Dr. Lawrence F. Young<br>Associate Professor<br>Dept. of QA/IS, CBA<br>University of Cincinnati<br>ML 30<br>Cincinnati, OH 45220<br>(513) 475-7169 | Degree: D.Sc., Industrial Eng., 1978<br>Specialty: Industrial Engineering<br>Assigned: Human Resources Laboratory:<br>Logistics & Human Factors Div. |
| Dr. Robert K. Young<br>Professor<br>Dept. of Psychology<br>University of Texas<br>Mezes 330, Psychology Dept.<br>Austin, TX 78713<br>(512) 471-9228 | Degree: Ph.D., Exp. Psychology, 1954<br>Specialty: Experimental Psychology<br>Assigned: Human Resources Laboratory:<br>Manpower & Personnel Division |
| Dr. Juin S. Yu<br>Professor<br>Dept. of Mechanical Eng.<br>West Virginia Tech.<br>Montgomery, WV 25136<br>(304) 442-3248 | Degree: Ph.D., Mechanical Eng., 1964<br>Specialty: Thermofluid Transport<br>Assigned: Aero Propulsion Laboratory |

PARTICIPANT LABORATORY ASSIGNMENT

## C. PARTICIPANT LABORATORY ASSIGNMENT (Page 1)

### 1988 USAF/UES SUMMER FACULTY RESEARCH PROGRAM

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

1. Bryan Becker
2. Mingking Chyu
3. Jerry Clark
4. Derek Dunn-Rankin
5. Wayne Eckerle
6. David Mikolaitis
7. Douglas Talley
8. Juin Yu

ARMAMENT LABORATORY (AD)
(Eglin Air Force Base)

1. Ibrahim Ahmad
2. Stephen Dow
3. Manuel Huerta
4. Anastas Lazaridis
5. Kwang Min
6. Joseph Molitoris
7. Shawky Shamma
8. Wafa Yazigi

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

1. Praphulla Bajpai
2. Shankar Bale
3. Charles Covington
4. Barry Goettl
5. Robert Masingale
6. David Payne
7. Joseph Saliba
8. Sanford Singer
9. Andrew Whipple
10. Frank Witzmann

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

1. Eustace Dereniak
2. William Grissom
3. Darrell Hoy
4. Manjit Jawa
5. Ahmad Vakili

ASTRONAUTICS LABORATORY (AL)
Edwards Air Force Base)

1. Clarence Calder
2. Phillip Christiansen
3. Susan Collins
4. David Jensen
5. Mark Norris
6. Jodye Selco
7. Rameshwar Sharma
8. Trilochan Singh

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

1. Mike Burlakoff
2. Gerald Grams
3. David Hemmendinger
4. Mohammad Karim
5. Robert Li
6. Periasamy Rajan
7. Panapakkam Ramamoorthy
8. Mateen Rizki

EASTERN SPACE AND MISSILE CENTER (ESMC)
(Patrick Air Force Base)

1. Albert Heaney

## C. PARTICIPANT LABORATORY ASSIGNMENT (Page 2)

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

1. George Bratton
2. John Dalphin

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

1. Wayne Charlie
2. David DeHeer
3. Deanna Durnford
4. Edward Greenwald
5. Neil Hutzler
6. William Mounfield
7. Richard Myers
8. Ramalingam Radhakrishnan

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

1. Larry Byrd
2. Karen Chou
3. Awatef Hamed
4. Yulian Kin
5. Augustus Morris
6. Nanda Mukherjee
7. James Sherwood
8. Kenneth Sobel
9. Patrick Sweeney
10. William Wolfe

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

1. Dan Bruss
2. Charles Bump
3. Richard Carlin
4. Michael McKee
5. Tammy Melton
6. Patricia Plummer
7. James Schmutz

GEOPHYSICS LABORATORY (AFGL)
(Hanscom Air Force Base)

1. Lucia Babcock
2. Stephen Baker
3. Pradip Bakshi
4. Reuben Benumof
5. Donald Collins
6. Michael Frantz
7. Christopher Godfrey
8. Janet Kozyra
9. Irving Lipschitz
10. John McHugh
11. Himanshoo Navangul
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**GRADUATE STUDENT-RESEARCH PROGRAM**

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**FINAL REPORT**

# CERAMIC COMPOSITES FOR STUDYING BONE INGROWTH AND REMODELING


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Date: 30 September 88

Contract No: F49620-87-R-0004

# CERAMIC COMPOSITES FOR STUDYING BONE INGROWTH AND REMODELING

by

Praphulla K. Bajpai

## Abstract

Two different ceramics were used in developing ceramic and organic acid composites for repairing traumatized bone and (eventually) studying bone remodeling. Composites of tricalcium phosphate (TCP) and polyfunctional acids (keto-glutaric acid and malic acid) with and without calcium hydroxide were developed for conducting studies in rats. Among the tricalcium organic acid composites studied, TCP, malic acid, and calcium hydroxide composites had the best setting and hardness properties. These composites also maintained an alkaline pH (ideal for bone growth) in an aqueous environment. Plugs of previously set composites of aluminum calcium phosphorous oxide (ALCAP), malic acid, and calcium hydroxide, as well as powders of composites set immediately before implantation, were used for repairing traumatized left femurs and L-4 vertebrae in 12 rhesus monkeys. Radiographs and scanning electron micrographs of repaired sites showed that the composites implanted in the bone were compatible with the surrounding tissue. Blood chemistry data obtained at the time of physical examination and euthanasia of 4 monkeys implanted for 4 weeks with the composites indicated that the the ALCAP-organic acid composites did not cause any adverse effects in the implanted animals.

## Acknowledgements

I wish to thank the Air Force Biodynamics and Biomechanics Division of the Armstrong Aerospace Medical Research Laboratory at Wright Patterson Air Force Base for the sponsorship of this research. I also wish to thank the Universal Energy Systems for the sponsorship of this program.

Acknowledgements are also due to the Department of Biology, University of Dayton, Dayton, OH., for providing the facilities and equipment necessary for preparing the ceramics and composites needed for this investigation.

I want to thank Elizabeth Kavran (GSSP student) for fabricating tricalcium phosphate, tricalcium phosphate composites, testing the composites and analyzing the data obtained on blood chemistry during this study. Thanks are also due to Brigitta Lusser for preparing ALCAP composites and overall assistance in this project.

Specifically, I want to thank to Dr. James Cooper and the staff of AAMRL/VS for performing the surgeries, Dr. D. R. Mattie and the AAMRL/TH staff for assisting in the evaluation of the implanted composites, and the Chemistry Laboratory personnel for conducting the blood chemistries. I am indebted to E.S. Eveland for allowing me to share his time, laboratory facilities, and expertise. Finally, I want to thank Dr. Leon Kazarian for providing moral support, financial support for the project, and the opportunity to work at AAMRL/BBD.

## I. INTRODUCTION:

Besides being subject to maxillofacial and orthopaedic injuries, air crew personnel are especially prone to vertebral column injuries and, in particular, anterior wedge fractures ($T_4$-$T_7$ and $T_{11}$-$L_1$) during ejection from the aircraft (1). Recently, my laboratory developed both totally and partially resorbable ceramic composites for use in repair of traumatized bone. These include aluminum calcium phosphate (ALCAP), and tricalcium phosphate (TCP) composites of organic acids with and without 1,25 dihydrocholecalciferol (DHCC) or with and without calcium hydroxide (2). These ceramic-organic acid composites can fill the bone defect, set to hardness, and provide a scaffold for ingrowth of new bone (2).

Bone resorption and formation occurs in pockets in normal bone and, according to Hori and Takahashi (3), bone remodeling occurs by an "on-off" mechanism. It is obvious that unless the events within a particular bone are synchronized, interpretation of the remodeling process can become quite difficult. However, synthetic bone substitutes, such as ceramic composites, can provide a zero baseline for studying bone ingrowth and remodeling. This procedure, if successful, probably can validate the "on-off" mechanism and explain the data obtained from on-going and completed studies at BBD on the effects of prolonged vibration and mechanical stress on remodeling of bone. Use of synthetic bone substitutes to replace traumatized bone has already been approved for dental and maxillofacial surgeries and is in orthopaedic clinical trials.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The specific objectives of this investigation were to:

a. Synthesize tricalcium phosphate (TCP), make ceramic composites of TCP, polyfunctional carboxylic acids (ketoglutaric or malic acid) with or without calcium hydroxide and/or 1, 25 dihydrocholecalciferol (active form of vitamin-D), and test the characteristics of the composites.

b. Synthesize aluminum calcium phosphorous oxide particles, form composites with malic acid and calcium hydroxide, test for the characteristics of the composite pre and post implantation in monkeys, and observe the effects of its implantation in monkeys.

## III. MATERIALS AND METHODS:

a. Tricalcium Phosphate (TCP) Studies.

I. Preparation of TCP and its Composites.

The TCP used in this study was prepared by an aqueous precipitation and sulfate catalysis procedure (4). The theoretical calcium/phosphorous ratio of 1.5 for TCP was used in preparing the reaction solutions. After centrifugation, the precipitate was dried at 75$^{o}$C and sintered in alumina crucibles by heating at a rate of 9$^{o}$C per minute to 1150$^{o}$C in a microprocessor-controlled high temperature furnace (Thermolyne Inc., Dubuque, IO). Sintering was completed by soaking the precipitate at 1150$^{o}$C for one hour and then air quenching it to room temperature. Large pieces of the dried precipitate sludge were broken up before sintering to aid in the rapid air quenching of

the material.

The sintered precipitate was crushed and ground in a laboratory-scale alumina ball mill apparatus and screened to the desired -400 mesh (-37 um) on a Tyler Ro-Tap Automatic Siever. X-ray diffraction performed on several batches of TCP prepared for this study indicated that the sintered precipitate was pure beta tricalcium phosphate.

Composites of TCP were prepared by mixing 133.3 mg of TCP and 30.0 mg calcium hydroxide with 66.7 mg of either ketoglutaric acid or malic acid. For studying the effects of 1-25 dihydrocholecalciferol, 50 mg of DHCC was added to TCP and ketoglutaric or malic acid composites of TCP.

II. Setting and Hardness of TCP Composites.

Tricalcium phosphate (200 mg) or tricalcium phosphate composite powders (230 to 287.5mg) were placed in a deep well slide. The setting and hardening of TCP or TCP composite dry powders were initiated by adding and mixing in 60 ul of deionized water. Hardness of the composite was tested by means of pushing on the set composite by a steel probe. Triplicates of each powder were used for this study. A rating of 1 was assigned to TCP or TCP composite powders which did not set on addition of water. A rating of 5 was assigned to set composites which developed the consistency of gum on addition of water. If the composite, on addition of water attained a plaster-like hardness, it was given a rating of 10. The hardness of each sample was measured every 5 minutes for one hour and then at 24 hours.

III. Effect of TCP and TCP Composites on pH of Saline.

Tricalcium phosphate or TCP composite dry powders were placed in 8 x 4 x 2mm flexible rubber molds and allowed to set and harden for 24 hours after addition of 60 ul of deionized water. Each of the dried pellets of TCP or TCP composites was then placed into a 3.3cm Spectrapor standard dialysis bag. The ends of the dialysis bags containing the pellets were folded and tied with silk thread. Each bag was then placed into a 100 ml serum bottle containing 70ml sterile neutralized saline (pH 7.02). The serum bottles were then placed into a 37°C water bath oscillating at 75 cycles per minute. The pH of saline in each bottle was measured at intervals of 1, 24, and 48 hours. Triplicates of each pellet and saline controls were used for this study.

b. Aluminum Calcium Phosphorous Oxide (ALCAP) Studies.

I. Fabrication of ALCAP Particles and its Composites.

Aluminum calcium phosphorous oxide (ALCAP) ceramic capsules were fabricated by calcining a mixture of (50:34:16 by weight) aluminum oxide, calcium oxide, and phosphorous pentoxide powders (Fisher Scientific Co., Fairlawn, NJ) at 1315°C in a microprocessor regulated high temperature furnace (Thermolyne Inc., Dubuque, IO) for 12 hours. The calcined material was ground in a ball mill (U.S. Stoneware, Mahwah NJ) and sized using a Tyler Ro-Tap Automatic Siever to obtain particles particles of less than 38um. Ceramic cylinders (green shape) were prepared by compressing a mixture of one gram ceramic particles and 0.025g polyvinyl alcohol (PVA, 60um particles) in a 5/16

inch die at loads of 2275Kg in a French cell press (American Instrument Co., Silver Spring, MD). The green shape cylinders were then sintered at 1400°C for 36 hours to increase the density and mechanical strength of the ceramic. Sintered ceramic cylinders were then ground in a ball mill and sieved to obtain ALCAP ceramic particles of 45-60um.

Sintered ALCAP ceramic particles of 45-60um size were mixed with malic acid in a ratio of 2:1. The ceramic-malic acid mixture was then mixed with calcium hydroxide (15% by weight of the total composite). One gram plugs of the ceramic, malic acid, and calcium hydroxide composites were obtained by adding 200ul distilled water and compressing the composite in a tuberculin syringe to expel excess water. The plug was then extruded from the cut end of the syringe and allowed to harden. The extruded and hardened composites were then scraped with a scalpel blade to yield cylindrical plugs, each measuring 3.5 x 10mm.

II. Animal and Blood Studies.

Blood was collected from each monkey prior to surgery, after injection of the bone labelling dyes, and at the time of euthanasia. Blood collected from each monkey on each occasion was analysed for albumin, alkaline and acid phosphatase, aspartate aminotransferase, alanine aminotransferase, bilirubin (total), blood urea nitrogen (BUN), calcium, carbon dioxide, chlorine, creatine, glucose, globulin, phosphate, potassium, protein (total), sodium, uric acid, white blood cells (total counts), red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC, neutrophil, lymphocytes, monocytes, eosinophils, and

basophils.

Each of the twelve rhesus monkeys used in this study was anesthetized and prepared using established procedures for monkey surgeries at AAMRL/VS by Dr. Cooper and his staff. The left femur and L-4 vertebra were exposed by dissecting the muscles away from the bone with minimum trauma. Holes were drilled in the two bones by means of a trephine (3.5mm in diameter). A continuous jet of sterile saline on the trephine was used to keep the trephine cool during the drilling of the bones. The bone plugs removed from the vertebra and femur of each monkey were saved for histopathologic and histomorphometric examination. After removing the bone, each site was implanted with a sterile pre-set and hardened plug. The top of the bone wound was then troweled with freshly set sterile composite to insure continuity and a tight fit.

**TABLE 1**

**LABELING, IMPLANTATION, AND ANALYSIS.**

| L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 | L10 | |
|---|---|---|---|---|---|---|---|---|---|---|
| B | 0 | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 Mo |
| | I | | E | | | E | | | | E |

B = Baseline L = Labeling I = Implementation E = Euthanasia

III. Histomorphometry and Radiography.

Histomorphometric procedures developed at AAMRL/BBD allow precise

quantification of formation of new bone. Bone was labeled at the times indicated in Table 1 (5). The labeling sequence involved an alternating series of fluorochromes such as tetracyclines (L1), xylenol orange (L2), and dicarbomethyl fluoroscein or DCAF (L3). To show normal bone growth, initial labeling of bone was conducted two weeks prior to implantation and again at implantation.

Radiographs of all 12 monkeys were taken one day after recovery from surgery, while radiographs of 4 monkeys were taken again 4 weeks after implantation of the composites at the time of euthanasia.

c. Scanning Electron Micrography.

Bones and surrounding tissues retrieved from each animal were frozen in liquid nitrogen and processed for scanning electron micrography by procedures used at AAMRL/BBD. A semiautomatic computerized video plan was used to determine the area of each pore from the scanning electron photomicrographs of hardened composites of both TCP and ALCAP with organic acids and calcium hydroxide.

d. Statistics.

The data obtained was analyzed by means of analysis of variance and T tests at $P < 0.05$., using computer programs.

**IV. RESULTS AND DISCUSSION:**

a. Tricalcium Phosphate and its Composites.

I. Setting and Hardness of TCP and TCP Composites.

Tricalcium phosphate alone did not set to hardness on addition of

water (Figure 1). Since all composites hardened (maximum) in 20 minutes, only 20 minute data is shown in Figure 2. Composites of DHCC and TCP, on addition of water, set and hardened to maximum hardness after 20 minutes. However, they were brittle in nature and disintegrated on prolonged exposure to air. Composites of TCP, ketoglutaric acid and calcium hydroxide showed the fastest setting and hardening time. These composites attained maximum hardness within 15 minutes. In comparison, composites of TCP, malic acid, and calcium hydroxide showed a slower initial setting and hardening time. However, these composites also attained maximum hardness within 15 minutes (Figure 1). Inclusion of DHCC enhanced the hardening time of TCP, malic acid, and calcium hydroxide and decreased the hardening time of TCP, ketoglutaric acid, and calcium hydroxide composites. Scanning electron micrographs of hardened TCP composites showed that the differences in the distribution of pores and pore sizes among the different composites were not significant. Ten percent of the surface of TCP composites was occupied by pores. The data on setting and hardening properties of TCP composites suggest that addition of DHCC alone is not desirable. Since composites of TCP, ketoglutaric acid or malic acid, and calcium hydroxide with or without DHCC set and harden within 15 minutes, it is obvious that these can be used within a short period of time after mixing with water for repairing bone defects.

II. Effect of TCP and TCP Composites on pH of Saline.

The data obtained on suspending TCP or TCP composites in neutralized saline solution (pH 7.02) is shown in Figure 2. Since composites of TCP with either ketoglutaric acid or malic acid lowered the pH of

saline to 4.02-4.22, these composites, on implantation, are likely to cause inflammatory responses in the initial stages. However, previous studies using rabbits have shown that implantation of these composites in traumatized tibiae allowed normal bone ingrowth. Addition of calcium hydroxide to composites of TCP and organic acids rendered them alkaline (Figure 2). According to Binderman and coworkers, an alkaline pH environment encourages new bone growth (6). Addition of DHCC to TCP, ketoglutaric acid or malic acid and calcium hydroxide lowered the pH of the saline solution. This suggested that DHCC binds with calcium hydroxide and allows the polyfunctional acids to exhibit their acidity on suspension in neutralized saline (Figure 2). Although such changes are subject to neutralization by the buffers of the host, the in vitro data obtained in this investigation suggest that addition of DHCC to the TCP composites may not be desirable.

b. Aluminum Calcium Phosphorous Oxide (ALCAP) Studies.

I. Implantation of ALCAP Composites in Monkeys.

Previous studies conducted in rats showed that dry powders of ALCAP-malic acid composites acted as excellent hemostats on application to experimentally induced bone wounds. Thus, powdered composites of ALCAP, malic acid, and calcium hydroxide were used to fill the drilled defects in the bones of monkey # 1. However, profuse bleeding from the wound prevented the composite from setting and hardening. To overcome this problem, the composite was set under pressure in a tuberculin syringe and extruded from the cut end. This composite, which hardened within 5 minutes, was used successfully as a plug to fill the drilled defects. Dry powder and pastes of the

composites were then applied to the wound to insure continuity and tight fitting between the bone and the composite. In subsequent surgeries the bleeding was stopped by manually applying a gauze with pressure to the open wounds. On cessation of profuse bleeding, the drilled holes in the bone were implanted with the composite plug and pastes without any complications.

II. Blood Chemistry.

Statistical analysis of the blood chemistry parameters indicated that the differences in total bilirubin content of plasma obtained at the 3 different intervals were significant. However, differences in plasma total bilirubin level at the time of physical examination and post implantation were not significant. Although all the values obtained for plasma total bilirubin were within the normal range, the plasma total bilirubin levels of monkeys decreased significantly after injection of the bone labeling dyes. Thus, it appears that these dyes inhibit the normal breakdown of erythrocytes. Since differences in all other parameters measured in the blood obtained from the three different bleedings were not significant, it is logical to conclude that implantation of composites of ALCAP, organic acid, and calcium hydroxide in the bones of monkeys had no adverse effects on the monkeys.

III. Histomorphometry and Radiography.

The tissues obtained for histomorphometry have been processed and embedded in plastic for sectioning, staining, and analysis. Radiographs of ALCAP composites <u>in situ</u>, one day and 4 week post

implantation, showed that the composites did not induce inflammatory response and provided continuity for ingrowth of endogenous bone (Figures 3 and 4).

IV. Scanning Electron Micrographs.

Scanning electron photomicrographs of set and hardened composites prior to implantation confirmed the porosity of the hardened composite. Estimation of the number of pores on the surface of the hardened composites indicated that only 4% of the surface contained macropores. However, each of the macropores was more than 100um in diameter (Figure 5a). Pore size of 100-400um are required for bone ingrowth. Scanning electron photomicrographs of implantation sites, 4 weeks post implantation, showed apposition of the host tissue with the composites and ingrowth of the host tissue within the ALCAP composites (Figure 5b).

**V. CONCLUSION:**

The data obtained from tissue samples and blood chemistries indicated that ALCAP composites of malic acid and calcium hydroxide are biocompatible. The ALCAP composite is accepted by the host without inducing inflammatory reaction and provides continuity for ingrowth of bone. The successful use of ALCAP composites and the data obtained on TCP composites indicates that TCP composites of organic acids and calcium hydroxide can also be used effectively to repair bone and study ingrowth and remodeling of bone.

## VI. RECOMMENDATIONS:

Since ALCAP composites can effectively repair traumatized bone and TCP has already been approved by the FDA for use in dental and maxillofacial surgeries and is in clinical trials for orthopaedic use, evaluation of TCP composites must be conducted in animals (We are planning to complete the study under the mini grant program).

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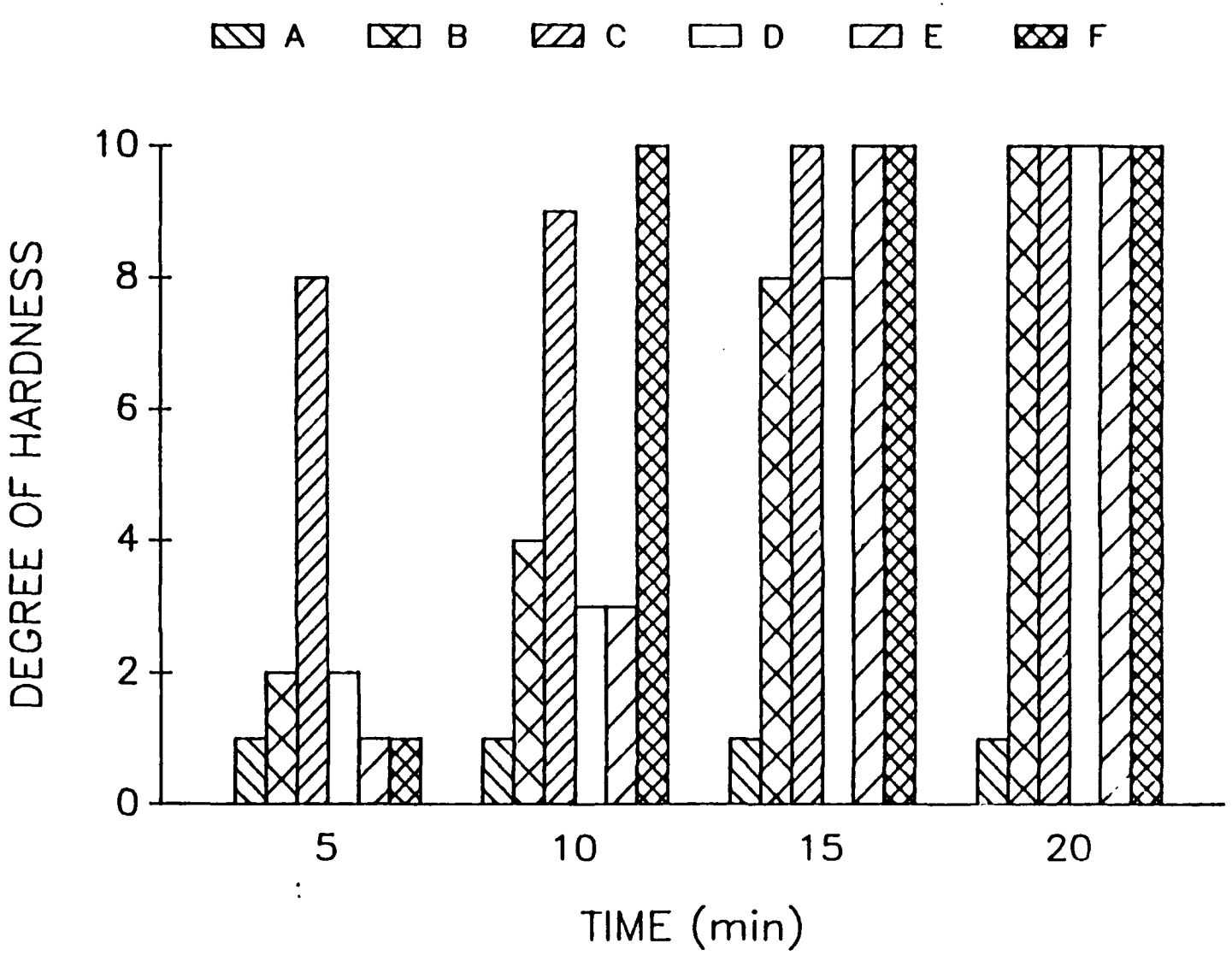



**Figure 1.** Setting hardness of Tricalcium phosphate (TCP) and its composites on addition of deionized water. (A) TCP, (B) TCP and Dihydrocholecalciferol (DHCC), (C) TCP, ketoglutaric acid, and calcium hydroxide, (D) TCP, ketoglutaric acid, calcium hydroxide and DHCC, (E) TCP, malic acid, and calcium hydroxide (F) TCP, malic acid, calcium hydroxide and DHCC.

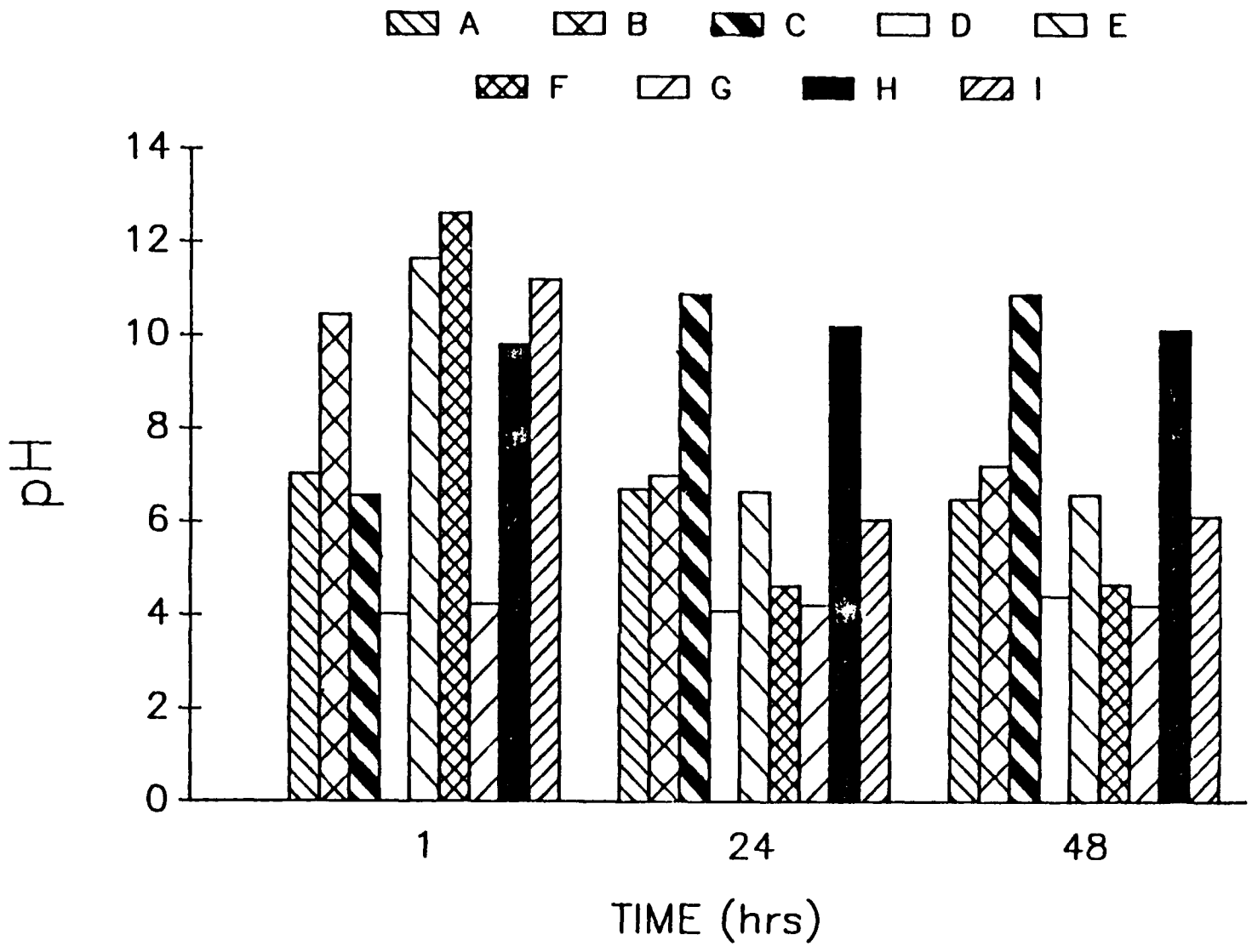



**Figure 2.** Effect of suspending Tricalcium phosphate (TCP) and its composites on the pH of neutralized saline (pH 7.02). (A) Saline, (B) TCP, (C) TCP and dihydrocholecalciferol (DHCC), (D) TCP and ketoglutaric acid, (E) TCP, ketoglutaric acid, and calcium hydroxide, (F) TCP, ketoglutaric acid, calcium hydroxide and DHCC, (G) TCP and malic acid, (H) TCP, malic acid and calcium hydroxide, (I) TCP, malic acid, calcium hydroxide and DHCC.

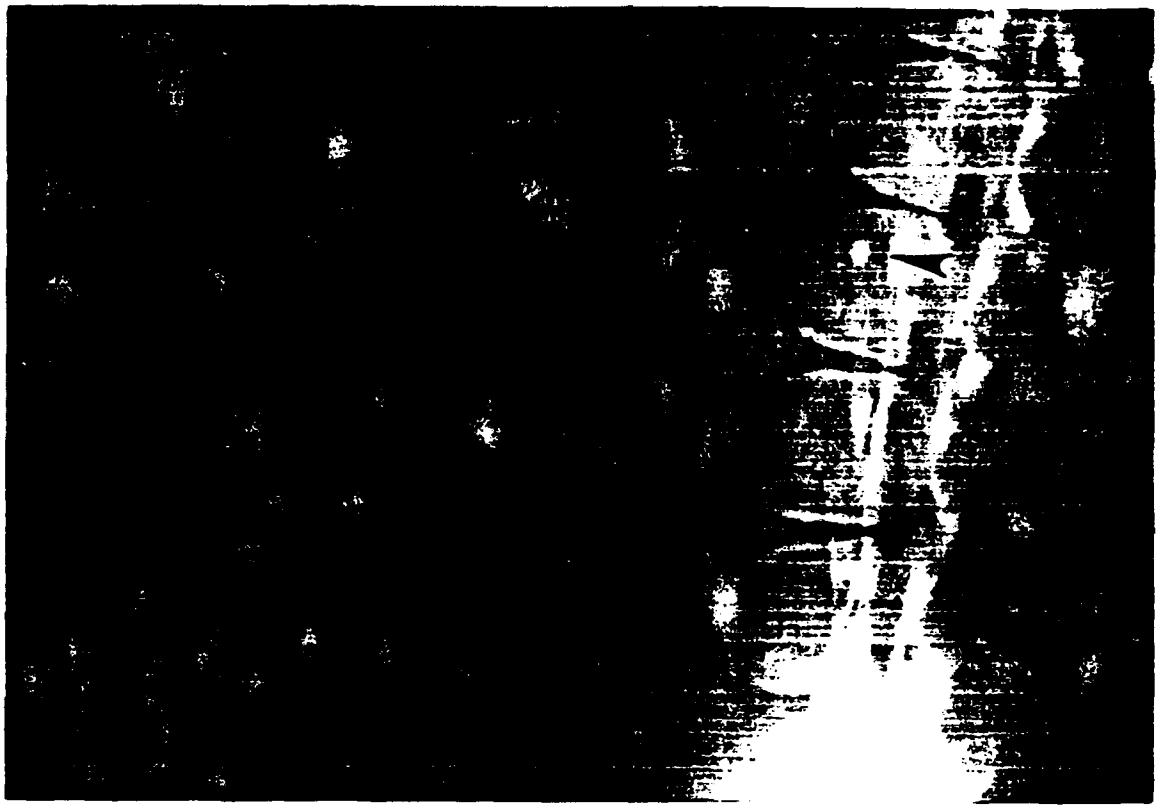


Figure 3. Radiographs of Rhesus monkey leg and spine showing ALCAP composite *in situ* in left femur and vertebra one day post implantation.

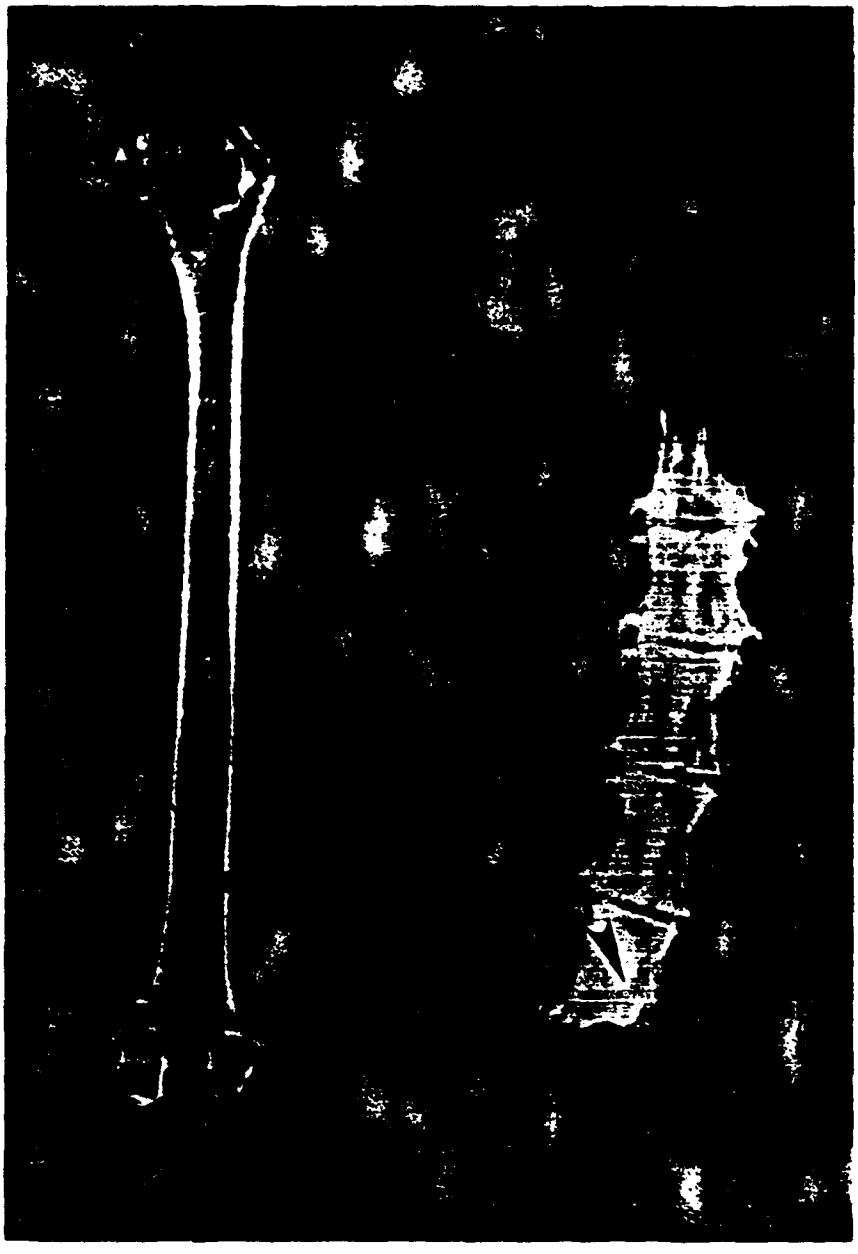
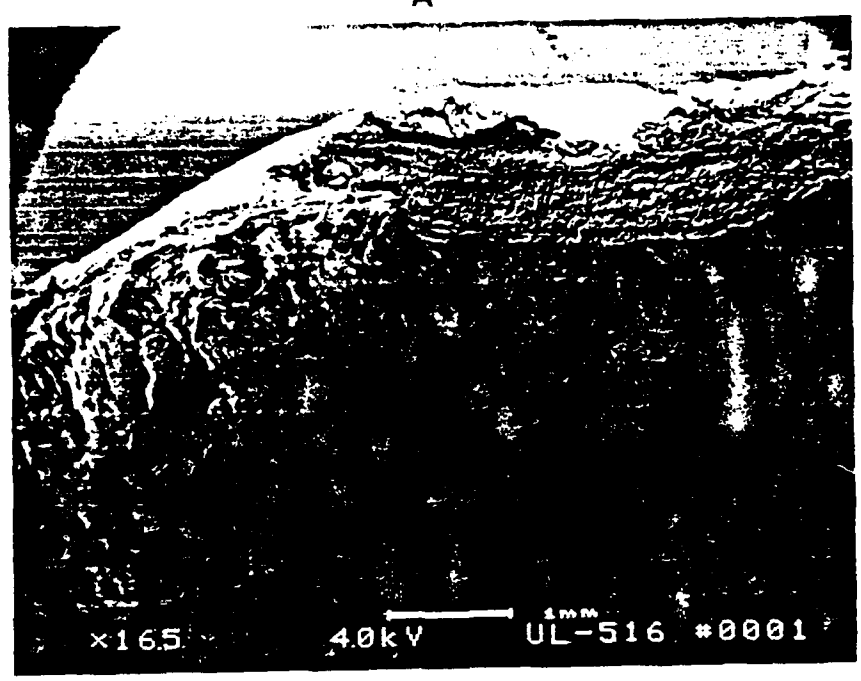


Figure 4. Radiographs of dissected femur and lower spine of Rhesus monkey showing the remnants of ALCAP composite in the last vertebra and bridging of the experimentally induced gap in the femur 4 weeks post implantation.




A




B

Figure 5. Scanning electron photomicrographs of hardened ALCAP composites. (a) Non-implanted composite showing two macropores (500 X). (b). Composite implanted for 4 weeks in the bone of a Rhesus monkey showing ingrowth and apposition of host tissue.

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FINAL REPORT

# IN VITRO CYTOTOXIC EFFECTS OF PERFLUORODECANOIC ACID ON HUMAN PERIPHERAL BLOOD LYMPHOCYTES


| | |
|---|---|
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| Date: | October 12, 1988 |
| Contract Number: | F49620-87-R-0004 |

# Invitro Cytotoxic Effects of Perfluorodecanoic Acid on Human Peripheral Blood Lymphocytes

By

Shankar S. Bale

## ABSTRACT

Cytotoxic effects of PFDA on human peripheral blood lymphocytes were studied. Unstimulated cells were exposed to 0, 2, 4, 6, 8, and 16 ug/ml of PFDA for 24 hours and analysed for cytotoxicity. PFDA at 6 ug/ml and below did not cause any significant change in cells compared to controls. Cells stimulated with mitogen were exposed to various concentrations of PFDA. Cells grown in RPMI medium supplemented with fetal bovine serum did not show any toxicity at the levels of PFDA used. However, the cells grown in RPMI medium supplemented with Nutriodoma-HU showed toxicity at all levels of PFDA. Cells were cultured in various media to determine the cell proliferation. RPMI medium supplemented with fetal bovine serum and Hana medium showed higher degree of cell proliferation compared to the cells grown in other media.

## ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and thanks to Dr. Sheldon A. London for providing me an opportunity to participate in the Summer Research Program and also for his encouragement and support.

Appreciation is also extended to Dr. Frank A. Witzman, another SFRP fellow in our laboratory for his help, encouragement and suggestions.

Finally, I wish to express thanks to the United States Air Force Systems Command, the Air Force Office of Scientific Research, and the Toxic Hazard Research Laboratory for sponsorship of this research.

## Introduction

Perfluorodecanoic acid is a perfluorocarboxylic acid that is readily obtainable in crystalline form. Perfluorocarboxylic acids have found wide use as lubricants, surfectants, and aqueous film forming foam fire extinguishants (Guenther and Vietor 1962, Shinoda and Nomura 1980).

Perfluorodecanoic acid (PFDA) causes acute toxicity in a variety of rodent species (Anderson et.al., 1981). Many perfluorinated compounds are chemically inert (Clark et al., 1973) and some perfluorinated compounds were retained in experimental animals for significant time periods following exposure (Clark et al., 1970). Perfluorinated compounds have been found in the serum of fluorochemical workers (Ubel et al., 1980).

Perfluorinated fattyacids caused toxicity in both invivo and invitro condiditions (Olson and Anderson 1983, Roger et al., 1982). The acute toxicity of PFDA includes hypophagia, thymic atrophy, delayed lethality, disruption of liver and testicular degeneration (Olson and Anderson 1983, Van Rafelghem et al., 1982). PFDA had no effect on L5178Y cells below 50 ug/ml, and dose above caused cell lysis (Roger et al., 1982). Flowcytometric analysis of L5178Y cells treated with PFDA showed an increase in the number of polyploid cells witn the increase in the levels of PFDA (Wigler and Shah 1986).

Numerous reports have appeared with respect to the toxic effects of PFDA, but assessment of its potential cytotoxicity appears to be incomplete. The present study was made to elucidate the cytotoxic effects of PFDA on Human Peripheral Blood Lymphocyte (HPBL) cells.

## APPROACH

Blood was collected by venipuncture from healthy individuals under aseptic conditions. Equal volumes of blood and separacel was mixed and centrifuged at 3000 rpm for thirty minutes. After centrifugation monocyte layer was decanted and washed with PBS-BSA twice and suspended in culture medium.

**Cell Proliferation Assay:**

For the cell proliferation assay, $1X10^6$/ml HPBL cells were placed in four different media (RMPI + FBS, RPMI + Nutridoma-HU, RPMI + Ventrex, and Hana) and 10 ul of phytohemaglutinin was added. One hundred microliters of cell suspension form each medium was placed in each microwell of the 96 well plate (8 wells/treatment). Cell controls and medium controls were prepared in similar manner without mitogen. The microwell plate was incubated at 37°C in a carbon dioxide incubator for five days. After five days, 10 ul of MTT (3-(4,5 dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) stock solution (5mg/mlPBS) was added to each well and incubated for four hours. The dark blue crystals were dissolved by adding acid isopropanol (150ul/well). The microplates were read on a Titertek Plate reader set at a wavelength of 560nm.

**Cytotoxicity Assay**

1. Non Proliferating Cells:

HPBL cells were separated from the blood and suspended in RPMI medium supplemented with Nutridoma-HU. One (1) ml of cell suspension ($1x10^6$ cells/ml) was placed in a multiwell plate (4 wells/treatment), and treated with 0, 2, 4, 6, 8, and 16 ug/ml of PFDA. Medium controls were set up in the similar manner with the exception of cells. After 24 hours of treatment, 100 ul of cell sus-

pension (8 wells/treatment) was placed in each well of the 96 well plate and assayed for cytotoxicity using MTT.

2. Proliferating Cells:

Pooled HPBL cells were split into half and suspended in RPMI supplemented with FBS and RPMI + Nutridoma-HU. One (1) ml of cell suspension (2x106 cells/ml) was placed in sterile tubes and 10 ul of phytohemaglutinin was added. The cells were treated with 0, 8, 12, 16, and 20 ug/ml of PFDA and incubated for 5 days. Medium controls were also prepared in the similar manner with the exception of cells. After the treatment period, 100 ul of cell suspension (8 wells/treatment) was placed in each well of the 96 micro well plate and assayed for cytotoxicity using MTT.

## RESULTS AND DISCUSSION

Mitogenic response of human peripheral blood lymphocytes is presented in Table 1. Mitogen stimulated cells showed both an increase in the number of formazan containing cells and an increased concentration of formazan compared to unstimulated cells in all the media used. In the quatitative assay, unstimulated cells showed a 580nm 0.191-0.283 while those cells stimulated with the mitogen showed an increased concentration of formazan, a 580nm 0.461-0.521 after five days of incubation.

In vitro assay of HPBL cells (unstimulated) treated with various concentrations of PFDA is presented in Table 2. Cells treated with 2, 4, and 6 ug/ml of PFDA did not differ significantly from controls. However, at 8 ug/ml and 16 ug/ml PFDA caused cell lysis and cell death. The effect of PFDA increased with the increase in level of PFDA.

Mitogen stimulated cells were treated with various concentrations of PFDA. Cells grown in medium supplemented with FBS did not show any toxicity in all the

concentrations of PFDA used (Table 3). The toxic effect of PFDA was evident in cells grown in medium supplemented with Nutriodoma-HU, PFDA induced cell lysis and death in all the concentrations used. The effect seems to be linear with the increase in levels of PFDA.

It is evident from the results that PFDA did not cause toxicity in the cells grown in FBS medium. However, toxicity was evident in cells grown in serum substitute medium. Roger et al. (1982) have indicated that PFDA failed to induce toxicity at concentrations lower than 100 ug/ml. Wigler and Shah (1986) observed an increase of polyploid cells in PFDA treated cells indicated that PFDA may block the $G_2$ stage of the cell cycle.

It has been suggested that PFDA alters membrane function by changing both oxidative status and fattyacid composition (Olson and Anderson 1983). The toxic effects of perfluorinated compounds could be diminished by lowering temperature and adding serum cell cultures. Serum albumin has been suggested to provide greater protection against PFDA toxicity (Levitt and Liss 1986). The mechanism by which albumin diminished toxicity of PFDA is unclear at present.

The effect of PFDA can be rapid in serum free medium or medium supplemented with serum substitute. Cell swelling and lysis were observed in concentrations of 8 ug/ml and higher. Such results suggest that PFDA disrupts the membrane, supporting the earlier findings of Olson and Anderson (1983). PFDA did not cause mutation and long term viability in cultured cells below their lethal dose (Roger et al., 1982, Levitt and Liss 1986).

The cytotoxic effects of PFDA described in this study clearly indicates a need for further investigation to determine the potential effects of PFDA on chromosomes, protein and DNA content in mammalian cells.

## RECOMMENDATION

Cell culture systems allow more accurate quantitation of toxicity and related reactions to chemicals than do animals. Cell culture systems can be used in place of animals for assay the toxicity of chemical pollutants. However, it is necessary to develop techniques that will provide accuracy of measuring toxicity which can be extrapolated to animals and humans.

Table 1. MTT assay of HPBL cells cultured in different media.

| Medium | Unstimulated Cells* (B) | Stimulated Cells* (A) | Proliferation Rate (A-B) |
|---|---|---|---|
| RPMI + FBS | 0.191 | 0.461 | 0.270 |
| RPMI + Nutridoma HU | 0.236 | 0.409 | 0.173 |
| RPMI + Ventrex | 0.283 | 0.521 | 0.238 |
| Hana | 0.202 | 0.508 | 0.306 |

* Absorbancy Values (Average of eight wells)

Table 2. MTT assay of unstimulated HPBL cells treated with various concentrations of PFDA.

| Concentration (ug/ml) | Time - 24 hours* |
|---|---|
| 0 | 0.365 |
| 2 | 0.313 |
| 4 | 0.233 |
| 6 | 0.238 |
| 8 | 0.164 |
| 16 | 0.070 |

*Absorbancy Values (Average of eight wells)

Table 3. MTT assay of mitogen stimulated HPBL cells treated with various concentrations of PFDA.

| Medium Supplement | Concentration (ug/ml) | Time - 120 hours* |
|---|---|---|
| FBS | 0 | 0.331 |
| | 8 | 0.392 |
| | 12 | 0.391 |
| | 16 | 0.448 |
| | 20 | 0.436 |
| Nutridoma-HU | 0 | 0.333 |
| | 8 | 0.193 |
| | 12 | 0.065 |
| | 16 | 0.016 |
| | 20 | 0.012 |

* Absorbancy Values (Average of eight wells)

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FINAL REPORT

# Auditory Modeling


| | |
|---|---|
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| USAF Researcher: | Timothy R. Anderson |
| Date: | 15 Aug 88 |
| Contract No: | F49620-86-R-0004 |

# Auditory Modeling

by

C. David Covington

and

Michael K. Ellis

## ABSTRACT

Several promising auditory models exist as reported in the literature, but they execute in different systems. We have ported two of three chosen auditory models to the Symbolics lisp machine. This affords the advantage of direct comparison of output and permits a more modular approach to the auditory modeling problem. In addition with all models residing in the powerful rapid-prototyping lisp environment, the researcher can then conveniently apply model output to either conventional pattern recognition algorithms or to more recently introduced simulated neural network systems. This report also discusses our efforts to develop a programming environment suitable to implement the more promising neural network approaches as means of modeling postprocessing by the brain on auditory periphery output in the human.

## 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 aspects of this program.

Our work found its first direction from an initial meeting with Dr. Thomas Moore and Timothy Anderson. Tim subsequently provided close contact and much needed direction for the duration of the project. Special thanks go also to Dr. Stephanie Seneff of MIT for providing the lisp source code to her auditory model and to Dr. Karen Payton of Johns Hopkins (temporarily at MIT) for providing the FORTRAN source code corresponding to her Ph.D. work. Special assistance also came from Dr. Jont B. Allen who took time out of his schedule to help us resolve computational precision difficulties encountered in porting Payton's model to lisp.

## 1. INTRODUCTION:

The Air Force studies how humans process sounds to protect hearing in high noise environments, to enhance audio quality in the cockpit, and to disrupt enemy communication. A variety of auditory models have been proposed and implemented, but these systems reside in different hardware and thus are not directly comparable. In addition, cochlear models are not readily available to researchers involved in speech processing, and the simulations created to date often do not coincide in the physical parameters estimated.

The inclusion of synchrony and active amplification phenomena gives cochlear modeling a new appeal as means to improved speech processing and represents acoustical transformations in the inner ear that cannot be adequately captured in simple filter bank models. In particular, speech recognition should benefit significantly from the analysis of both time proven and novel cochlear models.

This work serves to unify modeling efforts in an attempt to 1) provide an improved perspective of the underlying processes in the human speech perception system, 2) modularize existing simulation algorithms of individual processing steps in the middle and inner ear, and 3) create a platform providing appropriate input to both traditional pattern recognition algorithms and more novel approaches such as those available from the emerging body of simulated neural network techniques.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

This report outlines the reimplementation of three promising cochlear models on a Symbolics lisp machine. This approach provides the advantage of direct comparison of output with identical simulation input and serves to merge efforts in the field of cochlear modeling with improved commonality and portability. This also opens up the possibility of mixing the various modules in the chosen models in order to compare results at a lower level.

Our assignment as participants in the 1988 Summer Faculty Research Program (SFRP) was to accomplish as much meaningful work as possible toward the implementation of the three chosen auditory models listed below. We did successfully implement two of the systems, concentrating our efforts at the end of the project on delivering completed software packages rather than to introduce a new model. In addition Mike worked primarily on implementing programming environments for future experiments involving artificial neural network simulations on model output.

The following sections review work accomplished toward the reimplentation of the three chosen models, specifically those by Seneff (1988), Payton (1988), and Lyon (1988). Seneff's model focuses primarily on matching neural firing rate data without specifically attempting to model internal physical processing mechanisms. Payton's model builds on work initiated by Allen and Sondhi (1979) on cochlear mechanics and includes a separate synaptic transduction model. The model by Lyon relies heavily on active filtering, but no attempt was made to predict neural firing patterns. These models incorporate a variety of nonli-

near effects which cannot be reduced to simple harmonic analysis or linear prediction.

## III. SENEFF MODEL:

Dr. Seneff of MIT graciously provided prototype lisp source code for her auditory model. We successfully operated the system after some additional programming effort, primarily to uncomment some required sections of code and to activate the remote FPS array processor feature. Seneff's model operates as an attachment to the Speech and Phonetic Integrated Research Environment (SPIRE) system provided by MIT. The user activates this model by creating a new display in SPIRE by selecting it from the display menu.

This model does not provide physiologically significant parameters as intermediate variables, but rather concentrates on providing output data in a form suitable for speech recognition. The system represents the output in matrix form as a time sampled vector and displays the results of several forms on the Symbolics screen: critical band filter outputs, hair cell outputs, synchrony model outputs, and a synchrony spectrogram.

Since SPIRE represents a complete user interface with many inherent features we decided to make SPIRE the default display system for all models reimplemented on the Symbolics. We subsequently expended considerable effort to understand how to extend SPIRE to incorporate new features using internal SPIRE macro calls.

## IV. PAYTON MODEL:

After gaining a cursory understanding of how Payton's model functions, we obtained the FORTRAN source code and proceeded to translate the system to lisp. This proceeded fairly smoothly until we encountered major numerical accuracy problems in trying to use the array processor for basilar membrane displacement computations. Payton computed all parameters in her model using double precision floating point in FORTRAN. The FPS-100 array processor functions only in single precision arithmetic, so we reimplemented the entire system to perform all computation in lisp. This allowed us to run the system in double precision and permits us to port the system to our Texas Instruments Explorer lisp machines at the Unversity of Arkansas.

In a subsequent conversation with Jont Allen of Bell Labs, we learned that the critical computation is the accurate determination of the transform of q(x) in the denominator of the kernal expression for the basilar membrane model. Inaccuracies in the denominator dominate errors encountered in model output. Fortunately, this needs to be computed only once. The numerator expression must be determined at every time increment, which in our case was a maximum of five microseconds.

After obtaining satisfactory model performance, we created raster-line SPIRE displays to represent the model output graphically. Currently the system operates by creating SPIRE utterances consisting of synthetic acoustic patterns, the simplest being the impulse and sine waves. The lisp Payton model also processes standard SPIRE utterances

and thus interfaces well with existing Air Force databases.

The Payton model produces physically significant intermediate results and thus we created SPIRE displays corresponding to these forms. Currently the middle ear model and the basilar membrane model outputs can be displayed.

## V. LYON MODEL:

We gained an understanding of the Lyon model and hope to implement this system as part of the follow-on effort. Time did not permit us to perform satisfactorily on the first two systems and begin work on this third model as well. We do not anticipate that this model will require as much effort as the Payton model since it does not solve differential equations directly, and it makes no attempt to model synaptic behavior, only reporting membrane displacement as output. This system simply models the basilar membrane as a cascade of slightly underdamped lowpass filters.

## VI. NEURAL NETWORKS:

As part of our project we developed a simulated neural network programming environment as means of processing auditory model output in similar fashion to the human brain. Some networks recognize predetermined speech categories while others determine suitable recognition classes automatically. In any case, all networks accept training data in order to determine a set of numbers called weights associated with each network node. Thus neural networks learn slowly by repeated

example and also offer the advantage of parallel execution once the network weights have been determined.

In order to relate our results to established neural network techniques, we implemented six standard neural network prototypes: the Hopfield net, the Hamming net, the Carpenter/Grossberg classifier, the single layer perceptron, the multi-layer perceptron, and Kohonen's self-organizing feature map. This permited us to experience first-hand some of the problems identified by other researchers with particular networks. For example, the multi-layer perceptron requires large initial weights in order to adapt satisfactorily, and individual node decision boundaries gravitate toward the origin when the decision region is offset from the origin.

We additionally spent a great deal of time probing into the inner workings of P3, a generalized neural network simulation language written in lisp by Zipser and Rabin of UCSD. While P3 is robust in its graphic user interface, we found it to become burdensome for larger networks and ran into problems with errors in the history recorder code which caused us to abandon this approach.

We ultimately modified our own multi-layer perceptron code to accept SPIRE utterances as input data and used a (50 20 50) size identity-mapped network to produce slightly noisy but clearly identifiable speech at the output after some 300,000 training passes. The (50 20 50) notation implies 50 input nodes, 50 output nodes, and 20 intermediate hidden nodes. Intentity-mapping provides means of automatically identifying significant underlying features by examining the reaction

of the small number of intermediate nodes when standard speech classes are applied to the network.

## VII. RECOMMENDATIONS:

a. Porting a variety of successful auditory models to common software affords a tremendous capability in terms of deciding how best to represent the human auditory process and to further utilyze the best model configuration to obtain a more accurate perspective into the speech perception process. This also provides a basis to compare component modules in various systems to gauge accuracy in representing the biological system.

b. The speed with which the various auditory models execute is important. Both models implemented execute slowly enough that the user will not request the computation and wait, but rather an overnight batch job approach must be taken and results saved for future manipulation and display. The tradeoffs depend on available computational resources and available memory to store results online for an entire library of utterances.

Payton's model will not run fast enough in pure lisp to operate even in batch mode. The solution includes either 1) using the original double precision FORTRAN on the faster MicroVax and moving data across machines or 2) renewing the effort to utilyze the array processor to compute membrane displacement now that we know how to perform this computation in single precision.

c. The models as they stand represent a great start but are far from complete. Much work remains to be done, but the results obtained at this writing are encouraging that the work is meaningful and tractable. We hope to finish the third model in the follow-on effort.

d. The neural network simulation language P3 while very powerful and flexible, represents overkill for the task at hand and still incorporates a number of bugs, particularly in the history recorder. We recommend the use of our own code already delivered for reasons of efficiency, simplicity, and extensibility.

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FINAL REPORT

# Cognitive Demands of Tracking Strategies as Assessed by the Optimum-Maximum Procedure


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| Date: | August 22, 1988 |
| Contract No: | F49620-87-R-0004 |

# Cognitive Demands of Tracking Strategies as Assessed by the Optimum-Maximum Procedure

by

Barry P. Goettl

## ABSTRACT

Twenty subjects performed two compensatory first order tracking tasks concurrently while using either a double impulse strategy or a continuous strategy. The cognitive load of either task was increased by changing the control dynamics to second order. Attention allocation was manipulated using Navon's (1985) optimum-maximum procedure. Results indicated that subjects using the continuous strategy were more adversely effected by increased cognitive demands than subjects using the impulse strategy. This result suggests that the continuous strategy draws more heavily from central processing resources than does the impulse strategy. Also, subjects showed only limited trade-off between the two tasks. This finding has major implications for a multiple resources theory of attention. However, several problems with the optimum-maximum procedure were identified and discussed.

## ACKNOWLEDGEMENTS

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsoring this program. In addition, I would like to express my appreciation to Universal Energy Systems for efficient organization and execution of the program and for making my visit uneventful.

This has been a rewarding and stimulating experience. I would like to thank Dr. Michael Vidulich, Gary Reid, and Capt. Martin L. Fracker for allowing me this opportunity to temporarily leave the academic environment. Theoretical discussions with Dr. Michael Vidulich were particularly enriching. That a project of this magnitude could be finished in a 10-week period is testimony to the organization and efficiency of the Workload Laboratory and the dedication of laboratory personnel. Special thanks are given to Phil Masline, for his laboratory supervision, Jim Berlin, for his programing efficiency and flexibility, and Sharon Dorsey, for her dedication to data collection.

## I. INTRODUCTION:

When controlling a dynamic system that has high order control dynamics, such as a high performance aircraft, the human operator can use different strategies of control. One strategy, referred to in this report as the double impulse strategy, emphasizes low position error. Deviations from the desired position are reduced rapidly by large, jerky control inputs. The strategy is exemplified by the task of landing an aircraft where the pilot's primary objective is to maintain the proper glide path. The bumpy ride often experienced during landings arises from the rapid, non-linear control movements of the double impulse strategy.

A second strategy, referred to here as the continuous or smooth ride strategy, emphasizes minimal velocity error. Control inputs are small, linear, and subtle. The continuous strategy is typical of smooth level flight where passenger comfort is more critical to performance than is maintaining the proper glide path. Deviations from the command flight path are corrected gradually and smoothly to insure passenger comfort.

Many cockpit design decisions such as crew size, display format, and task assignment are based on estimations of pilot workload. Factors such as the number of tasks performed and the display configuration of those tasks influence cognitive workload. If strategy also influences

cognitive workload, then it too must be considered in making design decisions. Questions addressed by this research effort include whether different tracking strategies have different cognitive demands and what those differences are.

The goal of the Workload and Ergonomics Laboratory of the Human Engineering division (HEG) of Armstrong Aerospace Medical Research Laboratory (AAMRL) is to investigate pilot workload demands. The Workload and Ergonomics Laboratory has developed several subjective and performance based measures of workload and has researched several different subjective, performance, and physiological measures of workload.

My past research interests have been in the area of multiple resources theory of attention and its applications. I have also been interested in tracking strategies. Research I have done on tracking strategy attempted to assess the cognitive demands of the two strategies described above by combining a tracking task, using one of the two strategies, with various cognitive tasks. This research suggested that the different strategies placed different demands on the subject. This research as well as my research in the area of multiple resources theory, contributed to my selection by HEG for this project.

II. OBJECTIVES OF THE RESEARCH EFFORT:

One objective was to continue previous research on tracking strategies. Evidence suggests that the two

strategies differ in cognitive demands. For example, Young and Miery (1965) argue that the impulse strategy is less cognitively demanding than the continuous strategy. Young and Miery make the observation that humans tend to adopt the impulse strategy when tracking systems with higher order dynamics. Hess (1979) demonstrates that the impulse strategy is a means of reduced the mathematical computations required in tracking second order systems.

However, the impulse strategy has not always been found to be less cognitively demanding than the continuous strategy. Goettl and Wickens (1984) had subjects perform a second order tracking task using the impulse strategy on some trials and the continuous strategy on other trials. Various memory tasks were performed along with the tracking task to tap the type and amount the attentional resources demanded by each strategy. They predicted that the continuous strategy would demand more resources than the impulse strategy and would therefore interfere more with the memory tasks than would the impulse strategy. Performance on the memory tasks did decline when the continuous strategy was used, but this decline was accompanied by an improvement in tracking performance. Thus, strategy seemed to influence attention allocation rather than processing efficiency. That is, when using the continuous strategy subjects devoted more attention to the tracking task than when using the impulse strategy.

It was of interest to further investigate the cognitive demands of strategy in light of the conflicting findings discussed above. Do strategies really differ in demands? If the continuous tracking strategy draws more heavily from central processing resources than the impulse strategy, then when the central processing demands of a concurrent task are increased, the continuous strategy will result in greater decrements than will the impulse strategy. The first objective of this project was to investigate the processing demands of tracking strategies using this logic.

The second objective resulted from discussions with my focal point, Dr. Michael Vidulich. He has become interested in recent criticisms of dual task methodology (Navon, 1984, 1985). Navon argues that the procedure of variable resource allocation, commonly used in dual task experiments, imposes demand characteristics on the subject. The data from such experiments, according to Navon, reflect the subjects' willingness to follow instructions rather than the amount of resources consumed by the tasks.

The method of variable resource allocation for assessing cognitive demands is based on Wickens (1980; 1984) multiple resources theory of attention. Wickens theorizes that there are several different kinds of processing resources. For example, central processing resources, used for processing and holding stimuli in memory, are distinguished from response resources required for selecting

and executing responses.

The amount and type of resources demanded by a task can be determined by pairing the task in question with various other tasks. The performance changes on one task, resulting from changes on the concurrent task, indicate the resource demands of the first task. If two concurrent tasks demand different resources, they will be time-shared easily. However, if the two tasks require similar resources, then they will compete for those limited resources. The result is a reduction in performance. Moreover, when similar resources are demanded by both tasks and the difficulty of one task increases, resources from the other task can be recruited to maintain performance on the more difficult task. This trade-off between similar resources underlies the logic of the variable resource allocation method. With this procedure, a task of primary interest (Task A) is performed with several loading tasks (Tasks B & C). Allocation policy is manipulated by instructing subjects to allocate attention to the two tasks in specific ratios. Sometimes the task of interest is emphasized (e.g. 70% to task A and 30% to task B), sometimes the loading task is emphasized (e.g. 30% to task A and 70% to task B). If the two tasks share common resources, then performance will trade-off; one task will improve while the other declines. However, if the tasks do not share common resources, then performance will not trade-off. Note that with this

procedure a level of effort is specified for both tasks.

Navon proposes an alternative method which he calls the Optimum-Maximum procedure. With this procedure the amount of effort is specified for only one task (optimized task); the other task must be performed as well as possible (maximized task). The amount of effort specified for the optimized task varies over trials. Performance on the maximized task is assumed to reflect the resources available when the optimized task is performed at the specified level. For example, if a high level of effort is specified for the optimized task, then fewer resources will be available for the maximized task and performance will be poor. Navon argues that this method reduces demand characteristics since performance levels are not specified for both tasks.

Using the optimum-maximum procedure with two concurrent verbal tasks, Navon (1985) showed that subjects do not trade-off. This result is problematic for multiple resources theory since this theory predicts that two verbal tasks should trade-off. One potential explanation for the failure to find a trade-off is that Navon's verbal tasks were discrete rather than continuous. Discrete tasks may allow for attention switching which could reduce trade-off effects. Thus, the second objective of the present research is to provide a more critical test of Navon's criticisms by employing the optimum-maximum procedure on two continuous tracking tasks.

III. APPROACH:

Both objectives were served by a single approach; the following experiment was conducted. Subjects performed two tracking tasks concurrently. One task was oriented vertically and performed with the left hand, the other was oriented horizontally and performed by the right hand. Half of the subjects used the impulse strategy and half used the continuous strategy. Cognitive difficulty of the two tasks was manipulated to determine which strategy was more demanding. In addition, attention allocation was manipulated using Navon's optimum-maximum procedure to address the issue of resource trade-off.

The subject's task was to keep two cursors, one moving horizontally and one moving vertically, as close as possible to a red cross in the center of a display. Two Measurement Systems 541 Joysticks, interfaced with a Zenith 158 computer via a Systems Research Laboratory Labpack board, were used to control the cursors.

Tracking strategy was manipulated between groups. One group, consisting of 7 males and three females performed tracking tasks with the impulse strategy. The other group, 6 males and 4 females, used the continuous strategy. Subjects in the impulse group were instructed to reduce errors as rapidly as possible by making large, rapid stick deflections. Subjects in the continuous group were instructed to minimize errors but also to "give the cursor a

smooth ride" by applying small smooth control stick movements.

The tracking task dynamics were manipulated to assess the cognitive demands of the strategies. Increasing the control dynamics from 1st to 2nd order is assumed to increase the central processing load of the tracking task. Since the continuous tracking strategy is assumed to demand more central processing resources than the impulse strategy, subjects using the continuous strategy should be more effected by the increased load than impulse subjects.

To address the criticisms of Navon, the optimum-maximum procedure was used. On all experimental trials, Subjects were asked to optimize either the horizontal task or the vertical task while maximizing the other task. The following four optimization levels were used: (1) X - 1 s.d. (the mean minus 1 standard deviation), (2) X, (3) X + 1 s.d., and (4) single task performance. Across trials, subjects optimized the vertical task at all four levels then the horizontal task at all four levels (half the subjects received the opposite assignment).

IV. RESULTS:

The first step in the analysis was to verify that subjects actually used the proper strategies. Since the double impulse strategy entails large, rapid, and non-linear control stick movements, average control stick velocity is predicted to be higher for the impulse group than for the

continuous group. Control stick velocities were collected for all dual task trials and submitted to a 2x3x2 ANOVA (Strategy x Dual Task Configuration x Tracking Hand). The mean velocity for the impulse strategy group was much larger than that for the continuous strategy group: .198 versus .Ø62 for the impulse and continuous groups respectively. This difference was statistically significant ($F(1,18)=45.72$, $p<.Ø1$) and is consistent with the conclusion that subjects used the proper strategies.

The second step was to evaluate the cognitive demands of the two strategies. To address this question, performance measures, Root Mean Square (RMS) tracking error, on the 1st order-maximized tasks were analyzed. Cognitive load is assessed by observing the change in performance on a first order tracking task as the other task changes from first to second order. Because subjects were instructed to maintain specific performance levels on the optimized task, cognitive load is more readily assessed by performance on the maximized tasks. Since the continuous strategy is assumed to draw more heavily from the central processing resources than does the impulse strategy, the continuous tracking group should show a larger load effect than the impulse group.

RMS error scores on the maximized tasks are shown in Table 1. These data were submitted to a 2 (Strategy) x 2 (Load) x 2 (Task) Mixed design ANOVA. The analysis revealed

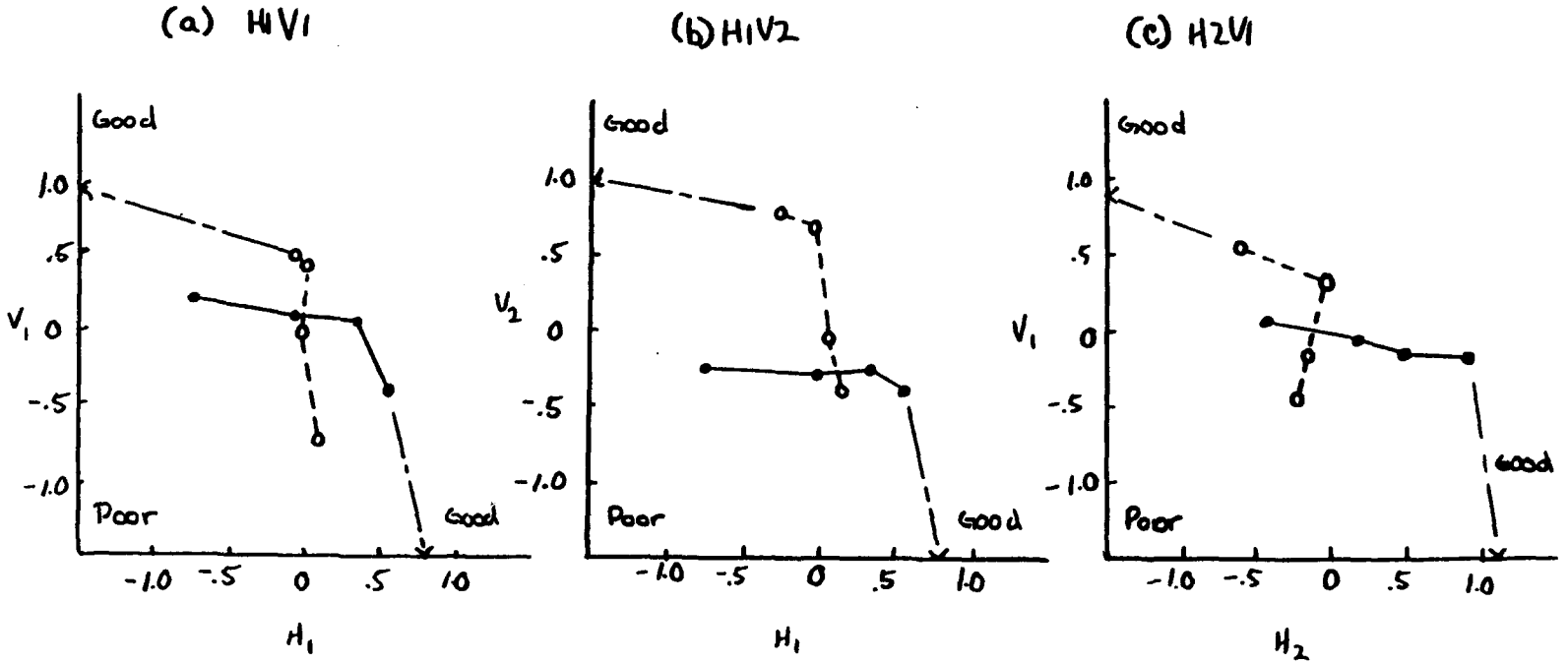
| Strategy | | Impulse | | Continuous | |
|---|---|---|---|---|---|
| Task | | H1 | V1 | H1 | V1 |
| Concurrent Task | 1st | .122 | .143 | .159 | .168 |
| Control Dynamics | 2nd | .121 | .150 | .168 | .195 |
| | Difference | .001 | -.007 | -.009 | -.027 |

Table 1. Mean RMS tracking error scores on first order tasks as a function of control dynamics on the concurrent task.

a statistically significant Load x Strategy interaction ($F(1,18)=4.53$, $p<.05$). Upon closer analysis, the continuous group showed a significant simple main effect of Load ($F(1,9)=7.63$, $p<.05$), while the impulse group did not ($F(1,9)=2.79$, $p>.10$). Thus, the continuous tracking group showed a larger effect of load than did the impulse group. This finding is consistent with the conclusion that the continuous strategy places greater demands on central processing resources does the impulse strategy. That the effect only occurs with maximized data and not optimized data suggests that subjects were complying with instructions to maintain the optimized task at the specified levels as the dynamics of the concurrent task were manipulated.

The second objective of the study was to determine if resources trade-offs could be observed using Navon's optimum-maximum procedure. This was the next step in the analysis. Performance data are plotted in Figure 1. This figure presents normalized RMS tracking error for the vertical task (y-axis) as a function of normalized RMS error for the horizontal task (x-axis) for the three dual task configurations: (a) H1V1, (b) H1V2, and (c) H2V1. Solid points connected by a solid line indicates trials in which the horizontal task was optimized, while open circles with dashed lines indicate trials in which the vertical task was optimized. Single task performance scores are indicated on the x and y axes.

Figure 1. Normalized RMS tracking errors for vertical tasks plotted as a function of horizontal tasks. Scores were normalized using the formula: (Mean - Score)/Standard deviation. Positive values indicate good performance



First, performance scores for the optimized task were submitted to 2 x 2 x 4 MANOVAs (Strategy x Task x Optimization Level): a separate MANOVA for each dual task configuration. All three MANOVAs revealed a significant main effect of Optimization Level as estimated by Wilke's criterion F-test: $F(3,16)=23.42$, $p<.01$ for H1V1, $F(3,16)=45.75$, $p<.01$ for H1V2, and $F(3,16)=24.91$, $p<.01$ for H2V1. These analyses indicate that optimization instructions influenced performance on the optimized task. As expected, Subjects performed best when optimizing at the single task level, and worst when optimizing below the mean. Once it has been established that performance on the optimized task changes according to instructions, trade-off effects are tested by testing for changes on the maximized task.

Performance scores on the maximized tasks were submitted to 2 x 2 x 4 MANOVAs as described above. A main effect of Level was found only for H1V1 and H2V1 dual task configurations: $F(3,16)=4.79$, $p<.05$, and $F(3,16)=5.44$, $p<.01$, respectively. The third dual task configuration, H1V2, did not show a Level main effect, however, it did show a marginally significant Level x Strategy interaction ($F(3,16)=3.02$, $p=.06$). Thus, there does appear to be some performance trade-offs, although the size of the trade-off is not very impressive, nor consistent. Analysis of orthogonal contrasts revealed that the trade-offs occur only

at extreme levels of optimization. For example, H2V1 showed a significant contrast between optimizing at the single task level and the average of the remaining three levels ($F(1,18)=18.07$, $p<.01$).

Thus, for the most part, the data seem to support Navon's predictions, except at extreme levels of optimization. The weak trade-off effect was surprising since it seems to contradict multiple resources predictions which have dominated the literature for years. However, several issues remain and will be discussed in the following section.

## V. RECOMMENDATIONS:

This project was successful in accomplishing its major objectives. Differences in the cognitive demands of tracking strategies were obtained and trade-off issues were investigated. In spite of these accomplishments, this project represents a starting point. Serving as a preliminary investigation of several issues, many questions remain. The major accomplishments are described below along with recommendations for follow-up research.

The primary objective was to demonstrate differences in the cognitive demands of different tracking strategies. This was accomplished by having human subjects perform two concurrent tracking tasks with either strategy as attention allocation, and task difficulty were manipulated. As predicted, the continuous strategy seemed to draw more

heavily from central processing resources. Subjects using this strategy showed larger decrements resulting from increased demands for central processing resources than did subjects using the impulse strategy.

It is recommended that future research be directed at demonstrating load effects for the impulse strategy. One potential criticism of the present research is that, because subjects could not track as well with the continuous strategy, the larger decrements may have been due to the greater difficulty associated with this strategy rather than demands for central processing resources. Since the impulse strategy is assumed to demand response related resources, confidence in the present results would be increased by demonstrating decrements with the impulse strategy resulting from increased demands for response resources.

The second objective was to address criticisms of the variable allocation procedure in dual task experiments. A procedure suggested by Navon was used in an attempt to demonstrate trade-off effects between two concurrent tracking tasks. Only limited trade-off effects were obtained. Assuming it can be replicated, this result is an important contribution to research on human attention and challenges a major theory of attention.

Future research on this topic would be best served by replication of the present findings and a close evaluation of three issues that remain unanswered. First, a multiple

resource prediction can be given for the present results if it is assumed that subjects recruit general resources to deal with the demands of optimization. These additional resources, perhaps subjectively experienced as increased effort, may be used to maintain performance on the maximized task. Second, although subjects did not show strong trade-offs, they were unable to perform the maximized task at single task levels. In other words, there were performance costs associated with dual task performance. These costs can be interpreted as either resource competition or outcome conflicts. Third, the present experiment uncovered several potential problems with the optimum-maximum procedure.

The optimum-maximum procedure requires on-line feedback displays and thus adds the additional task of self-monitoring performance. The procedure assumes that the additional task of monitoring is equally demanding in all situations. Such an assumption may not be warranted. Variable demands to monitor one's performance may be confounded with optimization instructions. All these issues are best addressed through experimentation and empirical data.

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# FINAL REPORT

# EVALUATION OF AN EXTRACTION PROCEDURE FOR THE ANALYSIS OF SERUM STEROIDS


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|---|---|
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| Date: | 19 September 1988 |
| Contract No: | F49620-88-C-0053 |

ABSTRACT

Evaluation of an Extraction Procedure

for the Analysis of Serum Steroids

by

Dr. Robert E. Masingale

and

Deborah J. Mitchell

ABSTRACT

Two methods are described for the screening, extraction and confirmation of the free and conjugated steroids in rat serum. It was demonstrated that a liquid-solid extraction technique combined with a three-solvent extraction system allows for more expedient sample preparation of the steroids. These methods were evaluated by screening for the presence of the following from sera: (1) androstenedione, (2) corticosterone, (3) estradiol, and (4) progesterone. Gas chromatographic/mass spectrometric analyses of the derivatized products were performed to evaluate the two procedures.

## ACKNOWLEDGEMENTS

The authors (REM and DJM) gratefully acknowledge the research support of the Naval Medical Research Institute Toxicology Detachment, specifically LT Linda Lininger, Major John Latendresse, and Jose A. Rivera. We thank the Air Force Office of Scientific Research for sponsorship of the summer research program and Universal Energy Systems for their administrative and financial support.

Our experiences were rewarding because of new procedures and developments to which we were exposed. LT Lininger and Maj. Latendresse provided us with support, encouragement, and a truly enjoyable working atmosphere.

## I. INTRODUCTION

Dr. Robert Masingale's research areas have included the isolation and study of lipids and the LCAT reaction in the formation of cholesterol esters. He has also undertaken the organic synthesis of amino and azido substituted nucleosides of certain nucleic acid bases with isosteric replacement of carbon to be used as possible metabolic antagonists. More recently, Dr. Masingale has studied hazardous waste disposal as it relates to the complete chemical destruction of chemotherapeutic wastes as opposed to incineration.

Ms. Deborah Mitchell's research has included the development and evaluation of new synthetic methods for preparing soluble mono and di-metallated phthalonitriles, the precursor to the desired phthalocyanine catalyst. It was the goal of this research to adjust the solubility of these compounds to a desirable level for catalytic application and to gain an understanding of the exact position of the substituents on the aromatic rings. Her experience as a research associate in organic synthesis at the Argonne National Laboratory contributed to her assignment to the Naval Medical Research Institute Toxicology Detachment.

The Toxicology Detachment of the Naval Medical Research Institute at Wright-Patterson Air Force Base is particularly concerned with the effect of organophosphate-based hydraulic fluids on endocrinologic and reproductive dysfunction in exposed rats. Recent toxicologic evaluations have shown that exposure to the hydraulic fluid MIL-H-19457B (Latendresse, et al, unpublished data), a phosphorylated petroleum distillate, resulted in testicular

degeneration, adrenal, and ovarian changes in rats. In addition, testing of the replacement fluid MIL-H-19457C resulted in similar adrenal and ovarian lesions. Steroid hormone production occurs in cells of both the adrenal gland and ovary and are affected by these hydraulic fluids. Therefore, a sensitive means of determining steroid concentration levels would be of great use in the screening, confirmation and quantitation of sera from exposed rats.

Steroids and steroid conjugates may be extracted from sera by appropriate solvents or absorbent columns. These methods have provided the sensitivity and speed required for measurement of physiological concentrations of steroid hormones in small amounts of sample. Therefore it was the purpose of this summer research project to develop the methodology for isolation and quantitation of four steroid hormones from rat serum.

## II. OBJECTIVES OF THE RESEARCH EFFORT

Recent toxicologic evaluations of two triaryl phosphate-based Navy hydraulic fluids have identified adrenocortical, ovarian and testicular lesions in exposed rats. The adrenal gland and ovarian lesions were observed in rats exposed to MIL-H-19457C which is primarily p-tertiary butyl phenyl phosphate, a specific member of the triaryl phosphates. Furthermore, these studies demonstrated altered reproductive cycles in female rats exposed to this hydraulic fluid. These observations strongly suggest that some phosphate-based hydraulic fluids may cause endocrinologic and reproductive dysfunction in exposed rats.

Additional investigations, most recently by the Naval Medical Research Institute Toxicology Detachment and another laboratory in 1986, have identified similar endocrinologic changes in rats and mice exposed to tricresyl phosphate (TCP), another specific member of the triaryl phosphate family of compounds. Parallel studies have shown severely depressed reproductive capacity caused by TCP's in male and female rats and mice.

Abnormal steroid hormone levels appear to be a common factor among affected adrenal and ovarian cells in rats exposed to MIL-H-19457C and TCP. Preliminary investigations (Latendresse, unpublished data) have shown increased serum estradiol concentrations with MIL-H-19457C and TCP exposed test groups compared with controls. The lesions observed in selected steroid producing cells are elevated serum estradiol levels and reproductive dysfunction. It has been proposed that these biological effects may have resulted from altered steroid metabolism.

In recent years, gas chromatographic and mass spectrometric (GC/MS) techniques have been used to assay steroids in body fluids. The advantage of this methodology over Radio Immunoassay (RIA) is the determination of profiles of steroids and their metabolites from a single sample. These profiles should assist in the elucidation of specific steroid producing pathways affected by these toxins.

The purpose of the overall investigation is to assess steroid metabolism of affected cells by examining metabolic steroid profiles in rats exposed to MIL-H-19457C and tricresyl phosphate.

To obtain this evidence free and conjugated steroids were extracted from sera of MIL-H-19457C and TCP exposed rats. Sep-pak $C_{18}$ absorbent cartridges were used for the separation and isolation of steroid conjugates.

The conjugates were hydrolyzed enzymatically using Sigma Type-I sulphatase and beta-glucuronidase, further purified by Sep-pak $C_{18}$ cartridges, and eluted with the appropriate solvent system. An alternative procedure used in these investigations utilized hydrochloric acid to hydrolyze steroid conjugates.

Our assignment as participants in the Summer Faculty/Graduate Student Research Program was to develop methodology to isolate the steroids androstenedione, corticosterone, estradiol and progesterone from single samples of rat serum. The steroids were derivatized using the reagents hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS). The derivatized steroids were assayed using gas chromatographic and mass spectrometric analysis. These samples were compared with authenic steroid standards found in the National Bureau of Standards Mass Spectral Library.

## III. MATERIALS AND METHODS

### EXTRACTION

Solvents were of ACS reagent grade and distilled before use. Pyridine was redistilled from potassium hydroxide. The fraction collected between $114^{o}C$-$115^{o}C$ was then stored in a brown bottle. Materials included Sep-pak $C_{18}$ cartridges obtained from Waters Associates, Milford, Massachusetts. The

authentic standards androstenedione, corticosterone, estradiol and progesterone, and the enzymes beta-glucuronidase (type H-1 from Helix pomatia) and sulphatase were all purchased from the Sigma Chemical Company. The derivatization reagents Sigma Sil-A and methoxamine hydrochloride were also purchased from Sigma. A Lab-Line shaker incubation bath, Thermolyne Dri-Bath (Sybron), Buchi Rotavapor, Mettler balance, 50 ul pipettes, a 100 ml round-bottom flask and 10 ml extraction tubes were used for sample preparation. All other glassware was disposable.

## DERIVATIZATION

To initiate the study, the Sigma steroids were derivatized to provide gas chromatographic and mass spectral reference standards for analysis.

### SILYL DERIVATIVES

ESTRADIOL. Dissolve 1.0 mg of the steroid in 1 ml redistilled pyridine and add 200 ul Sigma Sil-A. Vortex one minute. Let stand 20 minutes at room temperature, centrifuge and analyze by GC/MS. (Pierce, 1968)

ANDROSTENEDIONE. Dissolve 1.0 mg of the steroid in 1 ml redistilled pyridine and add 2.0 mg methoxime HCl. Heat at 60$^{o}$C for 2 hrs and allow to stand at room temperature; examine by GC/MS.

PROGESTERONE. Same procedure as above is followed.

METHOXIME AND SILYL DERIVATIVES

CORTICOSTERONE. Dissolve 1.0 mg of steroid in 1.0 ml of redistilled pyridine and add 2.0 mg of methoxylamine HCl. Heat at 60°C for 2 hours. Allow to stand for 2 hours at room temperature; then add 250 ul of Sigma Sil-A (excess) to the mixture. Vortex and let stand again for 30 minutes. Centrifuge to remove the precipitate. Analyze by GC/MS. (Hara, 1966)

INSTRUMENTAL ANALYSIS

Analyses were performed on a Hewlett Packard 5895A Gas Chromatographic Chemstation. The column used was 25m x 0.25mm ID fused silica capillary column coated with 0.2 um film thickness of polydiphenyldimethylsiloxane. A flame ionization detector was used with helium as the carrier gas with 1 ml/min flowrate.

A Finnigan 5100 Gas Chromatograph/Mass Spectrometer Data System was employed for analysis in the electron impact mode. The column used was 25m x 0.25mm ID bonded phase FSOT capillary column (#952525 Alltech and Associates), coated with 0.2 um film thickness polydiphenyldimethylsiloxane. 1.0 ul splitless injections were made with helium as the carrier gas. The injector temperature was set at 200°C, transfer line at 280°C and mass analyzer 80°C. The temperature program employed for rapid analyses was 50-280°C with a 20°C per minute ramp. As it became evident that estradiol and androstenedione were nearly co-eluting, more elaborate oven ramp programs were tested to improve

resolution of these steroids. A temperature program of 80°C-240°C at 20°C/minute, 240-280°C at 3°C/minute, and 280° for 5 minutes resolved the derivatized estradiol, androstenedione, progesterone and corticosterone recovered from a spiked serum sample. Normally, a 1:2 dilution of spiked samples was prepared for injection.

## IV. RESULTS

The extraction procedures employed for these experiments were adapted from methods reported by Covey, et al.(1988), Shackleton(1980), and Sjovall(1965).

### LIQUID-SOLID (Sep-pak) EXTRACTION

A. 500 ul of rat serum was added to 5 ml of absolute alcohol at 60°C. The solution was vortexed, centrifuged and the supernatant separated. The residue as washed with an additional 5 ml of absolute ethanol. The alcohol extracts were combined and evaporated under vacuum at 45°C. The residue was reconstituted in 5 ml of deionized water and passed through a prepared Sep-pak $C_{18}$ cartridge, washed with 10 ml of water, then eluted with 5 ml 100% methanol. The methanol was evaporated and the residue reconstituted in 10 ml deionized water. It was then treated with 1 ml of 1:1 beta-glucuronidase/sulfatase enzymes and incubated at 37°C. After 48 hours, the enzyme hydrolyzate was passed through a Sep-pak $C_{18}$ cartridge and eluted with 5 ml of methanol. The methanol was evaporated and the residue reconstituted in 1 ml of pyridine. The methoxime and silyl derivatives were

prepared as described previously. (Dray and Weliky, 1970; Pierce, 1968). No steroids were found upon the GC/MS analysis of these samples.

B. A second experiment was carried out exactly as in A, except that the serum sample was spiked with the Sigma standards androstenedione, corticosterone, estradiol and progesterone. These steroids were observed in the GC/MS analysis of the derivatized product.

C. 1 ml rat serum was diluted to 10 ml with 1.7 M HCl and incubated at $100^{o}$ for 3 hrs. The reaction was cooled to room temperature, and spiked with 1 mg of each steroid. To this, 20 ml acetonitrile, 8 ml n-hexane and 2 ml methylene chloride were added. The solution was shaken on a vortex for five minutes and centrifuged. The three layers were separated by pipette. The acetonitrile layer was evaporated, the residue reconstituted in 2 ml of a 1:1 isopropyl/methanol mixture and passed through a Sep-pak $C_{18}$ cartridge. The first 5 ml of the 100% methanol eluant was evaporated and the residue derivatized with methoxime hydrochloride and Sigma-Sil A. Androstenedione, estradiol and progesterone were found upon analysis.

LIQUID-LIQUID (Three Solvent) EXTRACTION

A. 500 ul of rat serum was diluted to 10 ml with deionized water, and the pH adjusted to 0.8 with concentrated $H_2SO_4$. This mixture was stirred overnight at room temperature. The serum sample was then spiked with 1 mg each of androstenedione, progesterone, corticosterone, and estradiol. 20 ml of acetonitrile, 8 ml n-hexane and 2 ml methylene chloride were added. The

solution was shaken vigorously for 5 minutes with a mechanical shaker, then centrifuged at 1500 rpm for 2 minutes. The solution separated into three layers. The top n-hexane layer and the middle acetonitrile layer were separated, and each evaporated under vacuum. Both residues were dissolved in 1 ml of pyridine, and attempts to prepare the methoxime and silyl derivatives were made. The n-hexane residue contained no steroids on instrumental analysis. Estradiol, androstenedione, and progesterone were found by GC/MS in the acetonitrile layer.

B. 1 ml rat serum was added to 10 ml of absolute alcohol at 60°, centrifuged and the supernatant separated. The residue was extracted once with 10 ml absolute alcohol at 60°C. The extracts were combined and evaporated under vacuum. The residue was reconstituted in 10 ml of 1.7 M HCl, and incubated at 100° for 3 hrs. After cooling to room temperature, the sample was spiked with 1 mg each of androstenedione, progesterone, estradiol and corticosterone. 20 ml acetonitrile, 8 ml n-hexane and 2 ml methylene chloride were added. The solution was shaken vigorously for five minutes, and the acetonitrile layer separated, evaporated and reconstituted in 1 ml pyridine. The pyridine solution was treated with methoxime hydrochloride and Sigma Sil-A for derivatization. GC-MS detected all four derivatized steroids.

## V. CONCLUSIONS AND RECOMMENDATIONS

Liquid-liquid and liquid-solid procedures for extracting steroids from rat sera are described. Gas chromatography and gas chromatography with mass spectrometry were employed for screening and confirmation. These methods

proved successful in the analysis of sera samples spiked with the steroid hormones. However, project efforts to detect physiological concentrations of these steroids have been unsuccessful. At the sample size tested, it appears the instrumentation discussed here may not have had the sensitivity required for evaluation of these hormones in rats. The literature indicates that circulating steroids are in picogram per milliliter concentrations as determined by Radio Immunoassay (Overpeck, 1978). The limited volume of serum obtainable from a rat also contributed to the sensitivity problem. However, it may be possible to pool serum samples and concentrate them to obtain the 1-5 parts per million threshold required by the Finnigan 5100 GC/MS. It appears, therefore, that the procedures  d in this study will require additional refinements to reach appropriate detection thresholds.

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# 1988 USAF-UES FACULTY RESEARCH PROGRAM/
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FINAL REPORT


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| Date: | August 19, 1988 |
| Contract No. | F49620-87-R-0004 |

# Performance in a Visual Monitoring Task with Serial and Simultaneous Display Formats

by

David G. Payne

## ABSTRACT

Thirteen adults monitored either four or eight sets of three-digit numbers that appeared on a computer monitor. These stimulus items were labelled with an uppercase letter A - H and each stimulus was paired with a unique response key. The stimulus values were periodically incremented or decremented and the subject's task was to respond whenever a value exceeded a prespecified limit. In the simultaneous condition, all four (or eight) number-letter combinations were presented concurrently in spatially distinct locations. In the serial condition, each item was presented individually in the same central location .8 seconds and was then replaced by the next item in sequence. Results showed that subjects responded more quickly in the serial condition than in the simultaneous condition. Although subjects made more errors in the serial condition, these error rates did not increase across trials, even though the reaction times improved. These results indicate that the rapid serial presentation format has the potential for yielding better performance in visual supervisory monitoring tasks than does the conventional (i.e., spatially distributed) display format.

## Acknowledgements

I wish to thank the Air Force Systems Command, the Air Force of Scientific Research and the Human Engineering Division of the Armstrong Aerospace Medical Laboratories for supporting this research. I would also like to thank the staff of Universal Energy Systems, Inc. for the concern and assistance they provided to me in all administrative and directional aspects of this program. In particular, Drs. Birt, Christensen, and Morgan of Universal Energy Systems made a special effort to ensure that my participation in the program was an educational experience and I thank them for their efforts.

A number of people working in the Design Effectiveness Technology Laboratory at Wright-Patterson contributed to making the summer research program a success. Lt. Kristen Morton effectively handled a number of logistic matters that made the summer program run very smoothly. Ms. Rebecca Donovan provided a great deal of help in aiding with the day-to-day operation of the laboratory. Drs. Lew Hahn, Sarah Osgood and Dieter Zirkler provided a very pleasant and stimulating environment during my stay in Dayton. Dr. Kenneth R. Boff contributed much of his time and knowledge to this research, and provided a number of insights that added to this work. Finally, I would like to thank collectively all of the members of the Design Effectiveness Laboratory for making my summer research experience a rewarding and enjoyable one.

## I. INTRODUCTION

The disciplines of human engineering and human factors are concerned with the design and development of equipment and systems that are compatible with the skills and limitations of the human operator. One of the goals of researchers in these areas is to improve the manner in which system information is presented to the operator of the system. The Design Effectiveness Technology (DEf Tech) Laboratory within the Armstrong Aerospace Medical Research Laboratories (AAMRL) at Wright Patterson Air Force Base is currently examining a new visual display technology that may improve the presentation of information in the "glass cockpit" (i.e., CRT displayed information). This work has the potential for dramatically improving the efficiency with which information may be transferred to crew station personnel.

My background and training are in cognitive psychology and human factors psychology. My research interests are in the areas of human memory and attentional processes and person-machine interaction. Recently, my graduate students and I have been investigating a number of issues concerning the memorial and attentional demands imposed on operators as they monitor visual displays in multitask environments. My background in human information processing, along with my ongoing visual display research, contributed to my assignment to the DEf Tech Laboratory.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

Research conducted in the DEf Tech Laboratory has compared standard visual display technology with a new display technology that offers the promise of increasing the efficiency of information presentation in person-machine systems. This new display technology involves rapidly presenting individual frames of information in the same location on a computer monitor. Previous studies by reading researchers have documented that a similar rapid serial visual presentation (RSVP) technique can significantly improve reading rates for prose passages. (see Potter, 1984 for a review of the RSVP literature).

Whereas the RSVP technique requires the reader to integrate information across successive display frames, the procedure being studied in the Def Tech Laboratory involves the presentation of discrete pieces of information, each of which constitutes a meaningful item. In an early study Matin, Boff, and Donovan (1987) showed that this rapid serial presentation format has the potential for efficiently communicating such individual pieces of information. Based on these findings, Matin et al. termed this serial presentation format "RAP COM", for rapid communication.

The RAP COM display format can be compared to the traditional spatial display format in which different pieces of information are presented in spatially distinct locations. In a series of experiments Matin, Boff, and their colleagues have compared the RAP COM display

technology with the more standard spatial display format using a variety of experimental tasks (e.g., Matin, Boff, & Donovan, 1987; Matin & Boff, 1988; Osgood, Boff, & Donovan, 1988). The results of these initial experiments have indicated that, at least with tasks that require subjects to process short lists of items, the RAP COM format is more efficient than the spatial format. For example, Matin et al. (1987) compared a RAP COM condition with a Spatial condition using a task that required subjects to report the identity of three digits presented on a single trial. The presentation durations that were required to produce 90% accuracy were significantly lower in the RAP COM condition than in the spatial condition. In a second study Osgood, Boff, and Donovan (1988) presented subjects with three sets of digit pairs in either a RAP COM or a spatial format. The subject's task was to respond whenever the number pairings did not correspond to an odd-even ordering. Here again, RAP COM produced a higher information transfer rate than did the spatial display.

These and other studies demonstrate that observers can extract information very quickly from rapidly presented visual displays. One possible reason for the advantage of RAP COM displays over spatial displays is that there is no need for eye movements (or saccades) when the RAP COM display is used. It has been demonstrated in reading research that although a saccade and the ensuing fixation typically last approximately 250 msec, almost all of the information processing that occurs during a fixation occurs during the first 50 msec. (see Rayner & Pollatsek, 1988, for a review of research on eye movements in reading). By removing the need for the relatively

slow eye movements that are required to view spatially distributed displays, the RAP COM procedure affords higher information transfer rates than can be achieved with spatial displays.

One potential limitation of the extant RAP COM studies is that each study employed a discrete trial task in which a small series of items was presented in either a spatial or a RAP COM format, the subject responded to those items and then some time later the next set of items appeared. This leaves unanswered the question of whether the advantage of RAP COM over spatial displays would obtain when the task required monitoring a continuous stream of information. This latter question is of considerable importance for (at least) three reasons. First, in many operational settings the tasks being performed are ongoing supervisory monitoring tasks (e.g., *checking* air speed, navigational information). Second, a basic research issue concerning the cognitive processes required to extract information from a RAP COM display is whether the person is responding to the items at a relatively low, sensory level or whether the information is processed more fully. This is obviously an important consideration if one wishes to use the RAP COM display in more complex decision making tasks. Finally, it would be useful to know the rate at which subjects learn to perform various tasks using spatial vs. RAP COM displays. The work that has been done to date has not addressed issues concerning the learning curves associated with these display formats. These questions motivated the present research.

## III. METHOD

Subjects. The subjects were 13 paid participants--11 males, 2 females and 11 right hand dominant and 1 left hand dominant. None of the subjects had participated in any other studies that used RAP COM displays.

Design. The design used was a 2 (Display Type: RAP COM vs. Spatial) x 2 (Number of Indicators: 4 vs. 8) x 2 (Day: 1, 2) x 3 (Trial Block: 1, 2, 3) within-subjects design. Subjects participated in two sessions over a two day period. Within each session subjects performed three blocks of 4 trials each, with each block consisting of one of each of the four display conditions (RAP COM/Spatial x 2/4 Indicators).

Equipment. An IBM XT micro-computer with an IBM enhanced color monitor was used to present stimuli and record responses and response latencies. Two response boxes were interfaced with the microcomputer. Each box had four keys arranged in a diamond shape with the letters A through D on Box 1 and E through H on Box 2 (see Figure 1). A Marsona model 1200 white noise generator was used to mask extraneous noises in the experimental area.

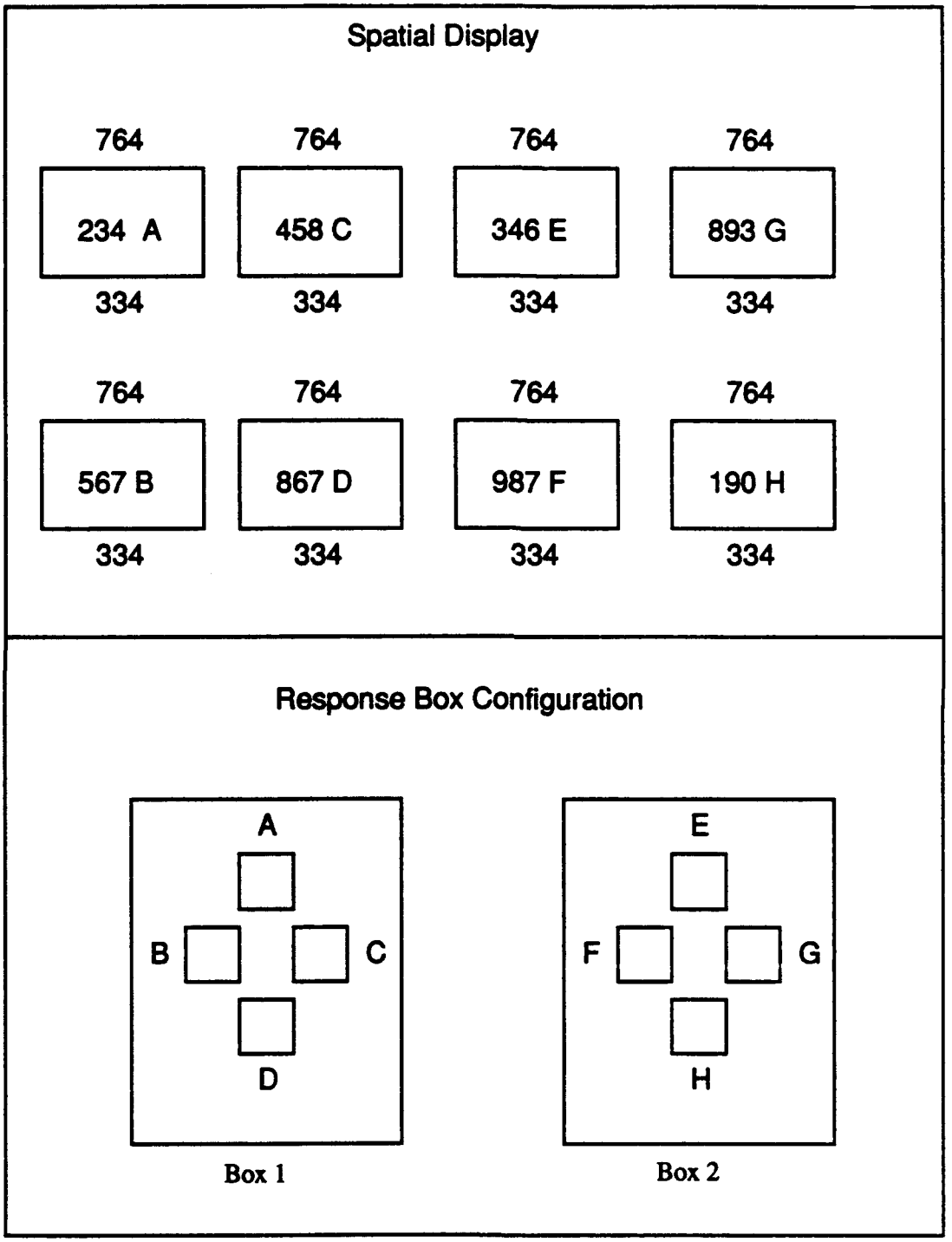



Figure 1. Upper Panel: Example of Eight Indicator Spatial Display
Lower Panel: Response Box Configuration

Task. Subjects performed a visual monitoring task that required them to monitor either 4 or 8 sets of three digit number-letter combinations. These values periodically increased/decreased in a monotonic fashion. The subjects were instructed to respond whenever a numeric value went out of bounds, i.e., it either exceeded the upper bound value of 764 or went below the lower bound value of 334. These boundaries remained the same for all trials and all display conditions. When an indicator went beyond its boundary, subjects responded by pressing the appropriate response key.

The Spatial display condition consisted of presenting eight rectangular shaped boxes that remained on the screen constantly during the trial. Above and below each box were printed the upper and lower boundaries (i.e., 764 and 334, respectively). Appearing inside each box was the stimulus to be monitored, namely, a three digit numeral plus a letter (A through D or A through H) that corresponded to a response key. In the four indicator condition only the four leftmost boxes contained number/letter combinations; however, all eight boxes were displayed.

The RAP COM display condition was identical to the spatial condition, with one major exception. In the RAP COM condition, only a single rectangle appeared centered on the screen with the boundary values presented above and below. Each of the 4 (or 8) indicators was displayed within the the rectangle for .8 seconds followed immediately by the next indicator in alphabetic order. The 4 (or 8) indicators were presented in a continuously cycling fashion (i.e., A, B,

C, D, A, B, C, D, A, etc.). The two display conditions (RAP COM and Spatial) were therefore identical in all regards other than the presentation format.

At the beginning of each trial the 4 or 8 indicator values started near the midpoint (549) between the upper and lower bounds. Each indicator value was incremented or decremented in a monotonic manner, with the direction of change (increase/decrease) being determined randomly. The algorithm used to change indicator values was a time-dependent sampling procedure in which the probability of incrementing/decrementing each indicator increased as the time since the last indicator change increased. The maximum interval between indicator updates was 7 seconds. The absolute value of each indicator change was randomly sampled from the range 0-110. Subjects were instructed to press the key corresponding to an indicator as soon as the indicator crossed the upper/lower boundary. When a subject made a correct response, or if the next numeral to be displayed went above 999 or below 100, the indicator was reset to a value near the midpoint between the upper and lower bounds. The direction of change for each indicator was randomly determined each time an indicator was reset. Auditory feedback was given following correct responses and a .2 second, 247 hz tone for incorrrect responses (i.e., responding before an indicator value had crossed its' boundary. Each trial lasted three minutes, during which time subjects were continually monitoring and responding/resetting the displays.

Procedure. On the first day, subjects were given practice using the response boxes, practice performing the four different monitoring conditions, and then they performed 12 critical trials. Practice with the response boxes consisted of responding to sets of 50 single letter stimuli presented on the computer monitor at a rate of one letter per second. For the first two sets, subjects used their right hand with Box 2 (letters E through H), the second two sets with their left hand using Box 1 (letters A through D) and the final two sets using both hands and both boxes (letters A through H). The goal of this practice was to train subjects to respond without looking at the response boxes.

Subjects were next shown the two different types of displays with explanations of the visual monitoring task. After this demonstration, subjects were given two minutes of practice on each of the display types and number of displays--RAP COM/Spatial and four vs. eight displays. After the practice sessions subjects performed 12 critical trials that alternated between type and number of displays across three blocks of four trials.

On Day 2 (the following day) subjects were presented 100 single letter stimuli (A through H) for the purpose of practicing responses with both response boxes using both hands. This was identical to the last practice session on Day 1. They then performed 12 more critical trials starting with the alternate display type that they had used on the previous day. The order of display type/number of indicators was counterbalanced between subjects.

## IV. RESULTS.

The two primary performance measures employed in this experiment were reaction time and error rates. Reaction time was defined as the time interval between when an indicator crossed its' boundary and when the subject made a response to that indicator. An error was defined as responding to an indicator that had not yet crossed its' boundary. We also recorded misses, which were defined as not responding to an indicator before it reached 100 or 999. These events occurred very infrequently with the modal number of misses per trial being zero.

Presented in Figure 2 are the mean reaction times for the four conditions across each of the three blocks on Day 1 and Day 2. There are several important points to note in regard to these data. First, as expected, subjects performance levels improved across the six blocks of training. Also as expected, subjects responded more quickly in the four indicator conditions than in the eight indicator conditions, and this result was obtained with both the spatial and RAP COM conditions.

Of greater interest is the comparisons between the RAP COM and spatial conditions. As is evident in Figure 2, subjects responded much more rapidly to the RAP COM displays than to the spatial displays. Furthermore, the improvement in reaction times across blocks was

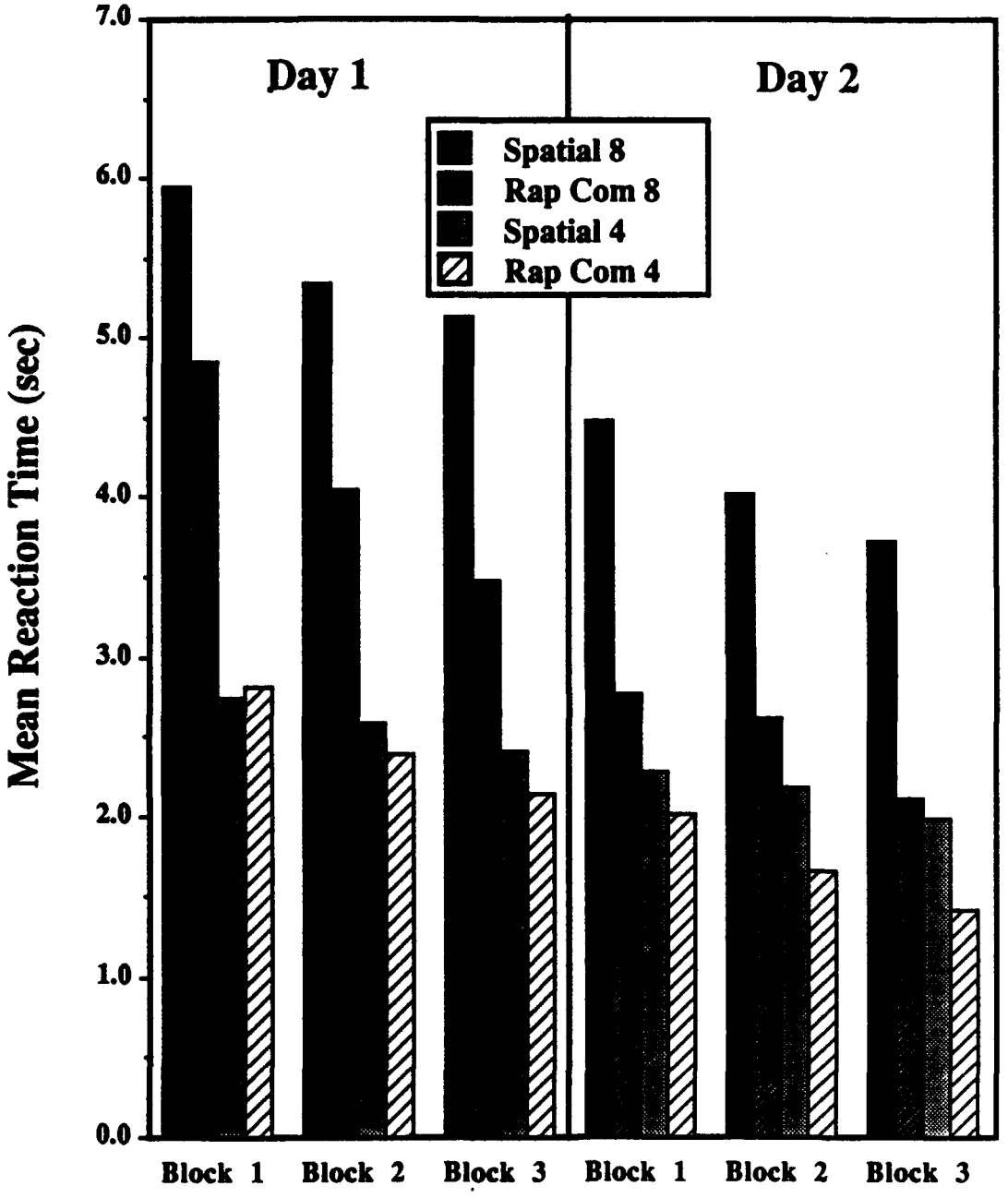



**Figure 2. Mean Reaction Time**

much greater with the RAP COM displays than with the spatial displays. Perhaps the strongest indication of this is the fact that by the end of Day 2 subjects were responding as quickly to 8 RAP COM displays as they were to 4 spatial displays.

Overall, then, the reaction time data suggest that the RAP COM display format is a much more efficient format than is the conventional spatial display. Before accepting this conclusion, however, we must examine the error rates obtained with these four conditons. It is possible that the speed advantage observed with the RAP COM displays is obtained at the expense of a high error rate. If the reaction time advantage obtained with RAP COM is due simply to a speed-accuracy tradeoff, then we would expect to find higher error rates obtained whenever there was a speed advantage (e.g., the improvement in RT observed across blocks would be accompanied by an increase in error rates.)

The mean percentage of total responses that were errors (averaged across Day 1 and Day 2) are presented in Figure 3. (Error rates are collapsed across Days because there were too few errors to allow a meaningful analysis for the effect of Days). There are two important points to be gleaned from Figure 3. First, the error rates were higher with the RAP COM displays than with the spatial displays. Second, while the RAP COM conditions lead to a higher error rate, the RT advantage obtained with the RAP COM displays is not due simply to a speed-accuracy tradeoff: The error rates in all four conditions are relatively constant across trial blocks. Hence the advantages in RT

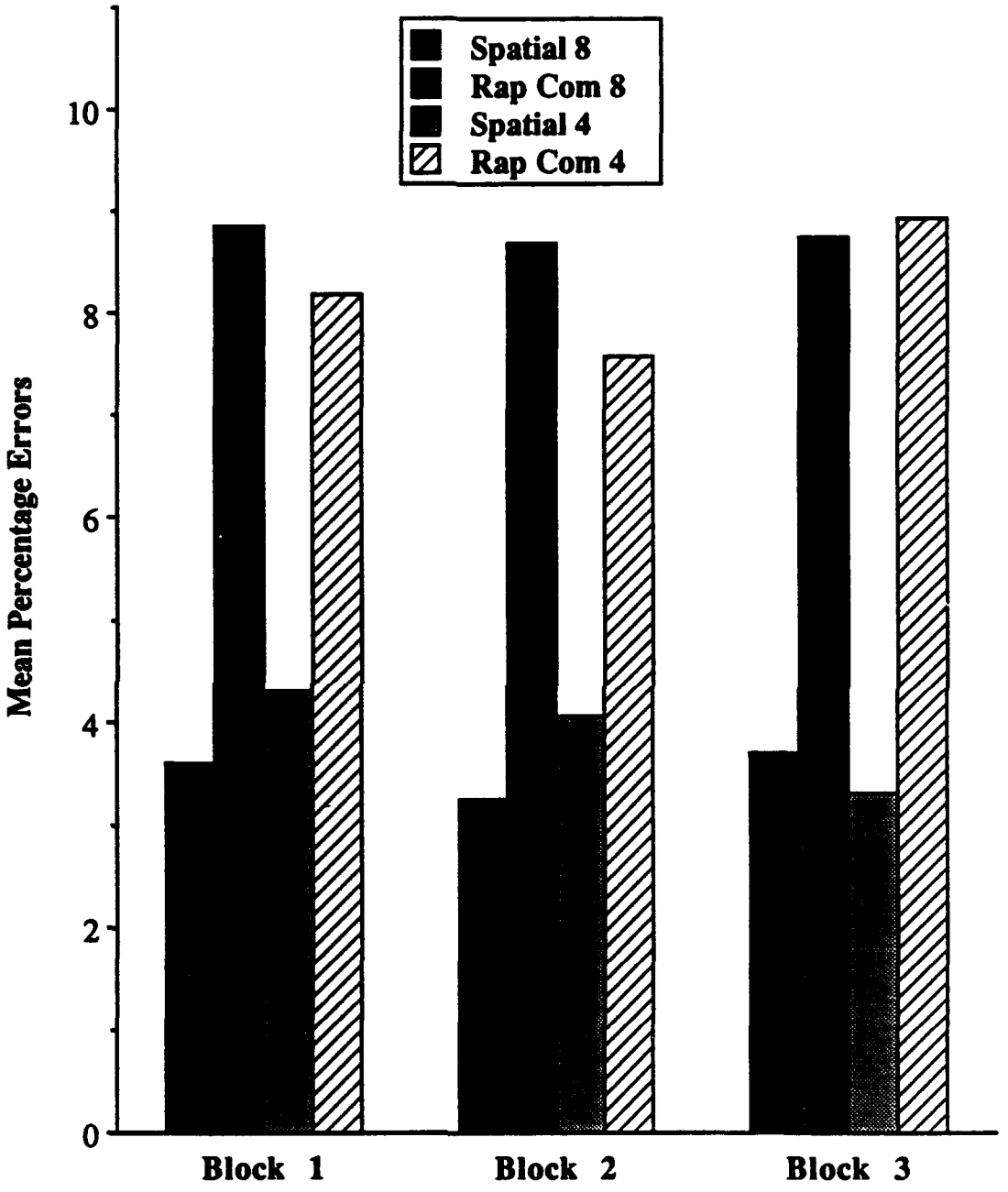



**Figure 3. Mean Error Rate (averaged across Days 1 & 2)**

that were obtained as subjects became more familiar with the task were not accompanied by an increase in error rates.

## V. RECOMMENDATIONS

The results of this experiment are consistent with the previous RAP COM studies showing RAP COM to be a very effective display technology. The present work extends these prior studies by employing a continuous, rather than a discrete task. Taken together, these studies clearly establish the viability of RAP COM as an alternative means of presenting visual information to crew station members.

One potential problem with the RAP COM technique that was identified in this study is the higher error rates obtained with RAP COM than with the Spatial displays. It appears that this differential error rate is attributable to the difficulty subjects' had in establishing the stimulus-response mapping with the RAP COM displays. The question of whether this problem is inherent to the RAP COM technique or whether it is attributable to the specific stimulus-response mappings employed in this experiment cannot be determined unambiguously based on the results of this study. Several observations, however, suggest that the high error rates obtained with RAP COM are due to the response box configuration.

First, as indicated in Figure 1, the response boxes employed in this study had a somewhat unusual arrangement of the response keys. These keys did not correspond to a clockwise pattern, nor did they correspond to a simple linear pattern. It is probable that this

arrangement is less than optimal. Second, the arrangement of the letters on the response box is inconsistent with the layout of a standard QWERTY-style keyboard, and this may have caused some confusion for subjects. Finally, a number of subjects reported that on the RAP COM trials they knew which indicator had crossed its boundary, but they had trouble selecting the appropriate response key.

Based on this analysis, it is clear that additional research is necessary to identify the locus of the response selection problem observed with the RAP COM displays. It would be useful to replicate the present experiment, this time using response boxes with more compatible response key configurations. An alternative approach would be to use the additive factors method pioneered by Sternberg to attempt to experimentally determine the nature and extent of differences in the processes underlying performance in RAP COM vs. Spatial tasks. It may well be that there are a number of fundamental differences in the cognitive processes engaged by these two tasks that go beyond simply differences in eye movements. Finally, it is advisable to examine the types of stimulus coding features (e.g., spatial, color) that could be implemented within a RAP COM display. That is, by switching from a Spatial display to a RAP COM display, the information transfer rates may be improved, but this improvement comes at the cost of losing one stimulus dimension (spatial location) that operators may use to decrease memory load. This issue will become more important as the RAP COM research program moves from studies employing laboratory tasks to

investigations that are closer to an operational setting (e.g., simulator research).

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Final Report

# A Nonlinear Lumped Parameter Model For The Seated Humans


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Date: 29 July 1988

Contract No: F49620-87-R-0004

# A NONLINEAR LUMPED PARAMETER MODEL

# FOR THE SEATED HUMANS

By

Joseph E. Saliba

## ABSTRACT

A brief review of both continuous and lumped-parameter models describing the structural response of the human body due to the acceleration environment associated with seat ejection is first presented. The need for a nonlinear lumped-parameter model to remedy the inadequacy of reproducing laboratory experiments was then examined. Four different tasks were then shown. The first task was to insure that the tests conducted in the laboratory were yielding meaningful output, otherwise the model developed would be meaningless. Then a nonlinear lumped-parameter model that best predicts the behavior of the human subject was developed. The values of the lumped-parameter models were then obtained by using a least-squares fitting technique. This process was performed and validated on one single laboratory test.

Finally, a more realistic and comprehensive validation plan to ensure the effectiveness of this approach in addition to a sensitivity study on the shape, duration and magnitude of the input acceleration was recommended.

## ACKNOWLEDGMENT

I would like to respectfully thank the Air Force Systems Command, the Air Force Office of Scientific Research, and Universal Energy Systems for providing me with the opportunity to spend a very stimulating and enriching ten weeks at the Harry G. Armstrong Aerospace Medical Research Laboratory , Wright-Patterson AFB, Ohio. I would like to acknowledge the Laboratory, and in particular the Biomechanical Protection Branch for its hospitality and help in overcoming many technical roadblocks.

I would like also to thank Mr. James W. Brinkley for his support, encouragement and guidance during this project. Finally, I would like to thank Lt. Randy Mays, Mr. Steve Mosher, and in particular, Mr. Jonathan Henderson for their invaluable help and collaboration in accomplishing this project.

## I. INTRODUCTION

Investigations of aircraft escape systems and spinal injuries sustained from seat ejections have involved a wide spectrum of analytical and experimental studies. The analytical efforts have concentrated primarily on the development, dynamic response solution, and validation of several discrete parameter and continuum models subjected to specified acceleration-time inputs. The experimental work has entailed measurement of constitutive and inertial properties of anatomical components and determinations and comparisons of biomechanical responses of human volunteers, cadavers, animals, and dummies to controlled impacts. Injury criteria and human tolerance thresholds formulated from these studies and accident statistics provide a basis for design and analysis of escape systems.

In describing the structural response of the human body due to the acceleration environment associated with seat ejection, one can represent the physical laws that are applicable in terms of rather complex equations. The obvious most popular simplification is the linear discretization which in turn leads to simpler equations for the idealized problem. This linearization is, in many instances, inadequate to describe or reproduce experimental studies done under very controlled environments.

A. Theoretical models, both continuous and lumped-parameter, with varying degrees of complexity have been formulated in efforts to predict responses to specified inputs. The lumped-parameter models usually have been single or multidegree-of-freedom systems with linear, passive elements, whereas the human body is neither a passive nor a linear system. Carmichael[1] (1968) states, "the body is a nonlinear system with respect to the equations of motion". Wittmann

and Phillips[2] (1969) conclude, "existing nonlinearities are sufficient to be of concern, and that appropriate measures to determine the effect of these nonlinearities should be taken." Perng[3] (1970) states, "The most striking feature of the mechanical behavior of biological tissues, the stress strain relation, is remarkably nonlinear." Markoff and Steidel[4] (1970) state, "It is known that joint stiffness increases with deformation." Belytschko, et al.[5], (1972) refer to, but neglect nonlinearities, stating, "because of the difficulties of characterizing a nonlinear orthotropic composite material." When representing nonlinear elements of the body, the assumption of linear springs and dashpots may be too great a simplification for some inputs.

## II. OBJECTIVES

To remedy this inadequacy of reproducing laboratory experiments, a nonlinear discrete parameter model will be examined. To accomplish this, four different tasks need to be investigated.

- Checking of the experimental data
- Selecting a model
- Fine-tuning the model
- Validating the model

The first task is to insure that the test conducted in the laboratory on the volunteer subjects are yielding meaningful output, otherwise the reproduction or calibration of the model is meaningless. The second task is to find a possible model that best predicts the behavior of the human subject during seat ejection. The third step involves investigating experimental means of fine-tuning the model by proposing additional experimental studies that best

determine the mechanical properties which enter the constitutive relations of the nonlinear model. The final step is to calibrate or validate the model, by simply attempting to reproduce as many as possible tests on volunteer subjects, making sure that the analytical output does match the experimental one.

## III. CHECKING OF THE EXPERIMENTAL DATA

The first task was to familiarize myself with test plan and the results of the "vertical impact of humans and anthropomorphic manikins" test conducted at the Biomechanical Protection Branch at Wright-Patterson Air Force Base. This entailed familiarization with the test procedure, fixtures used, and checking locations of load cells, accelerometer and other devices. Next a careful study of the equilibrium equations where all the force and moment equations were checked. This is done to insure that the tests conducted in the laboratory on the volunteer subjects are yielding meaningful output. These results will be used later for calibration of a nonlinear model that will be developed.

## IV. SELECTING A MODEL

There seems to be an orderly progression towards the development of realistic spiral models. The two parallel approaches are discrete lumped-parameters and continuum models.

The first spiral lumped-parameter model was proposed by Latham[6] (1957), which consisted of a double-mass spring-coupled system subjected to a base excitation. In 1962, Payne[7] proposed a dynamic response index (DRI) in his single degree-of-freedom model which is still in use today. Many more models have been proposed since then,

extending the degree-of-freedom to include other parts of the human body other than the spine.

Different types of spinal models have been proposed with less success. These models assume the spine to be a continuum and were initiated by Hess and Lombard[8] (1958). The spine is represented by a straight homogenous elastic rod, free at one end and subjected to a prescribed acceleration at the other end. Non-linear models have also been attempted, again with little success.

It has been demonstrated that the lumped-parameter models represent a more viable approach and a more realistic injury model can be deduced from them. Thus, a discrete model will be used.

## V. REFINEMENT OF THE MODEL

Figure 1 represents our attempt to model the spine.

If we call $U(t) = Z_2(t) - Z_1(t)$ (Eq. 1)

then $U'(t) = (Z'_2(t) - Z'_1(t))$ (Eq. 2)

where the $U' = \frac{dU}{dt}$ means the first derivative with respect to time

and $U''(t) = (Z_2''(t) - Z_1''(t))$ (Eq. 3)

From the free-body-diagram of figure 1, Newton's second law gives the following equations of motion in the form:

$$M\,(U''(t)) + F_{(spring)} + F_{(dashpot)} = F(t) \quad \text{(Eq. 4)}$$

where $F_{spring} = K_1\,(U(t)) + K_3\,(U(t))^3$ (Eq. 5)

$$F_{dashpot} = C_1\,(U'(t)) + C_3\,(U'(t))^3 \quad \text{(Eq. 6)}$$

so $M\,U''(t) + K_1U(t) + K_3\,[U(t)]^3 + C_1\,[U'(t)] + C_3\,[U'(t)]^3 = F(t)$ (Eq. 7)

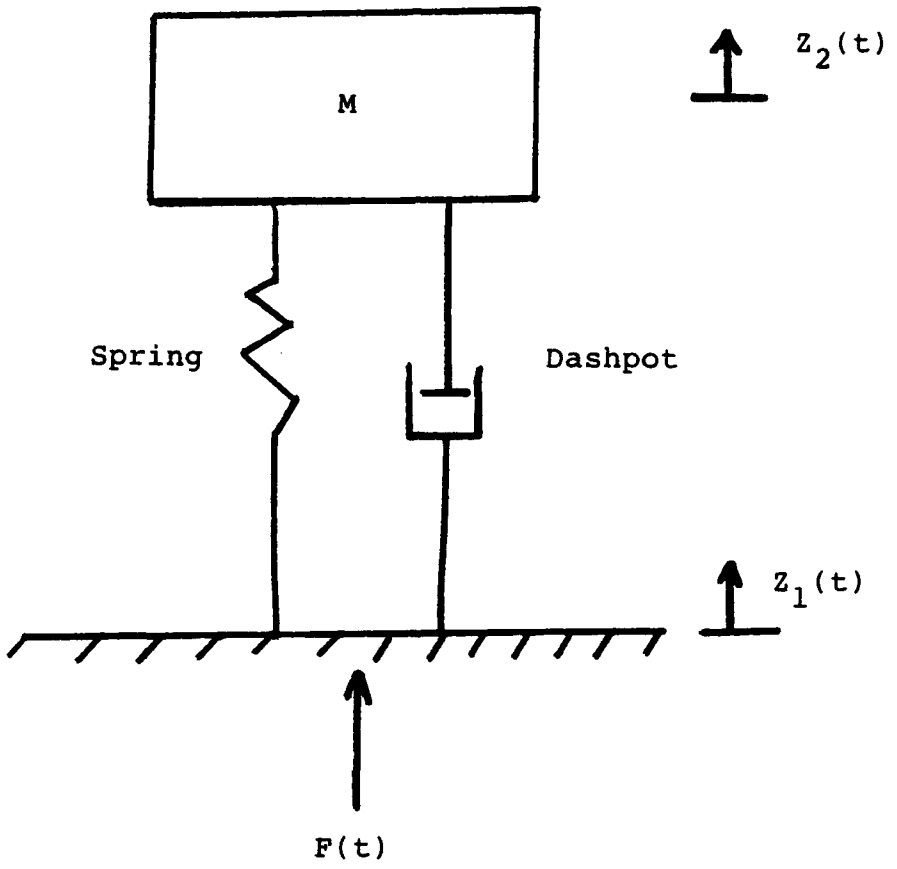



Figure 1

Since we know $U'' = \frac{d}{dt}\frac{dU}{dt}$ we can then integrate in function of time

to get $\int_{t_1}^{t_2} U(t)''\,dt = \frac{dU(t)}{dt}\Big|_{t_1}^{t_2} = U'(t)\Big|_{t_1}^{t_2} = U'(t_2) - U'(t_1)$ (Eq. 8)

or $U'(t_2) = U'(t_1) + \int_{t_1}^{t_2} U''(t)\,dt$ (Eq. 9)

but $U'(t) = \frac{du}{dt} \Rightarrow U'(t)\,dt = du$ (Eq. 10)

integrating in function of time both sides, we then get

$$U(t_2) = U(t_1) + \int_{t_1}^{t_2} U'(t)\,dt \qquad \text{(Eq. 11)}$$

the integral in the above equations can be simply estimated by any numerical quadrature formula such as the Trapezoidal rule

$$\int_a^b f(x)\,dx \approx S = \frac{1}{2} * (b-a)*[f(a) + f(b)] + \text{error} \qquad \text{(Eq. 12)}$$

or by another method like Simpson's rule.

The error term for the trapezoidal rule is

$$\text{error} = -\frac{f''(\eta)(b-a)^3}{12} \qquad \eta \in (a,b) \qquad \text{(Eq. 13)}$$

It is difficult to estimate the error term, especially since our value, for example, of the acceleration is given only in tabular form. In other words, we do not know what the actual equation for the acceleration is, hence, we cannot compute the second derivative of the acceleration to approximate the error. To remedy this, one can use the idea of the extrapolation to the limit in conjunction with the composite Trapezoidal rule. Such a method is commonly referred to as the Romberg integration

method. The best possible estimate of the integral can be found by simply looking at the table of ratios generated by this method.

Once the relative velocity and displacement are computed, the best values of the spring and dashpot constant can be found using the idea of the least squares method.

Since we do not know the values of $K_1$, $K_3$, $C_1$ and $C_3$ exactly, or which satisfy the equality of equation (Eq. 7), an error will be introduced for every time value. The fundamental idea behind the least squares method, is to minimize the sum of the squares of these errors.

If we call

$$\phi_1(t) = U(t)$$

$$\phi_2(t) = (U(t))^3$$

$$\phi_3(t) = U'(t)$$

$$\phi_4(t) = (U'(t))^3$$

and call N the total number of point given and

$$f_m = F(t_m) - m\ U''(t_m)$$

where we can then write the square of the individual error as:

$$E(K_1,K_3,C_1,C_3) = \sum_{m=1}^{N} [f_m - G(t_m;\ K_1,K_3,C_1,C_3)]^2$$

where $G(t_m,K_1,K_3,C_1,C_3) = K_1\phi_1(t_m) + K_3\phi_2(t_m) + C_1\phi_3(t_m) + C_3\phi_4(t_m)$

To minimize the above error function, it is necessary that the gradient of E vanish, i.e.;

$$\frac{\partial E}{\partial K_1} = \frac{\partial E}{\partial K_3} = \frac{\partial E}{\partial C_1} = \frac{\partial E}{\partial C_3} = 0$$

$$\frac{\partial E}{\partial K_1} = -2\sum_{m=1}^{N} [f_m - G(t_m;K_1,K_3,C_1,C_3)]\ \phi_1(t_m) = 0$$

$$\frac{\partial E}{\partial K_3} = -2\sum_{m=1}^{N} [f_m - G(t_m;K_1,K_3,C_1,C_3)]\ \phi_2(t_m) = 0$$

$$\frac{\partial E}{\partial C_1} = -2\sum_{m=1}^{N} [f_m - G(t_m;K_1,K_3,C_1,C_3)]\ \phi_3(t_m) = 0$$

$$\frac{\partial E}{\partial C_3} = -2\sum_{m=1}^{N} [f_m - G(t_m;K_1,K_3,C_1,C_3)]\ \phi_4(t_m) = 0$$

the error vector

$$e = [e_1, e_2, \ldots e_N]$$

with

$$e_m = f_m - G(t_m;\ K_1,K_3,C_1,C_3)$$

are normal orthogonal to the following vector

$$\theta_i = [\phi_i(t_1),\ \phi_i(t_2) \quad \phi_i(t_N)]^T \quad i = 1,2,3,4$$

since

$$-2\ e^T\ \theta_i = 0 \quad i = 1,2,3,4$$

calling the vector

$$f = [f_1, f_2, \ldots f_N]^T$$

the normal equations can be rewritten in the form:

$$\sum_{j=1}^{4} b_j\ \theta_j^T\ \theta_i = f^T\theta_i \quad i = 1,2,3,4$$

where

$b_1 = K_1$

$b_2 = K_3$

$b_3 = C_1$

$b_4 = C_3$

Solving these normal equations will then yield the four constants $K_1$, $K_3$, $C_1$, $C_3$, which will minimize the square of the error introduced by approximating the behavior by this discrete lumped-parameter model.

## VI. VALIDATION OF THE MODEL

The model was validated using the VIHAM STUDY TEST: 1390 SUBJECT: B-1 weight 163.0 NOM G=8-0 CELL: X. The chest minus seat acceleration time profile is shown in figure 2. Because of the deviation from zero, the above figure was shifted upward and then integrated to get the velocity, and integrated once again, to obtain the displacement. Then the data was passed to the least-square program and the best possible fit was obtained. The force profile used was the sum of the three load cells of the seat. (See Figure 3). After the four parameters were obtained, the actual total force on the seat was then plotted against the calculated one with the results showing in Figure 4.

## VII. RECOMMENDATION

Due to the nature of the project and its complexity, I was able to validate this nonlinear model on one single test alone. A more realistic and comprehensive validation plan should be proposed and implemented to ensure the effectiveness of this

Figure 2

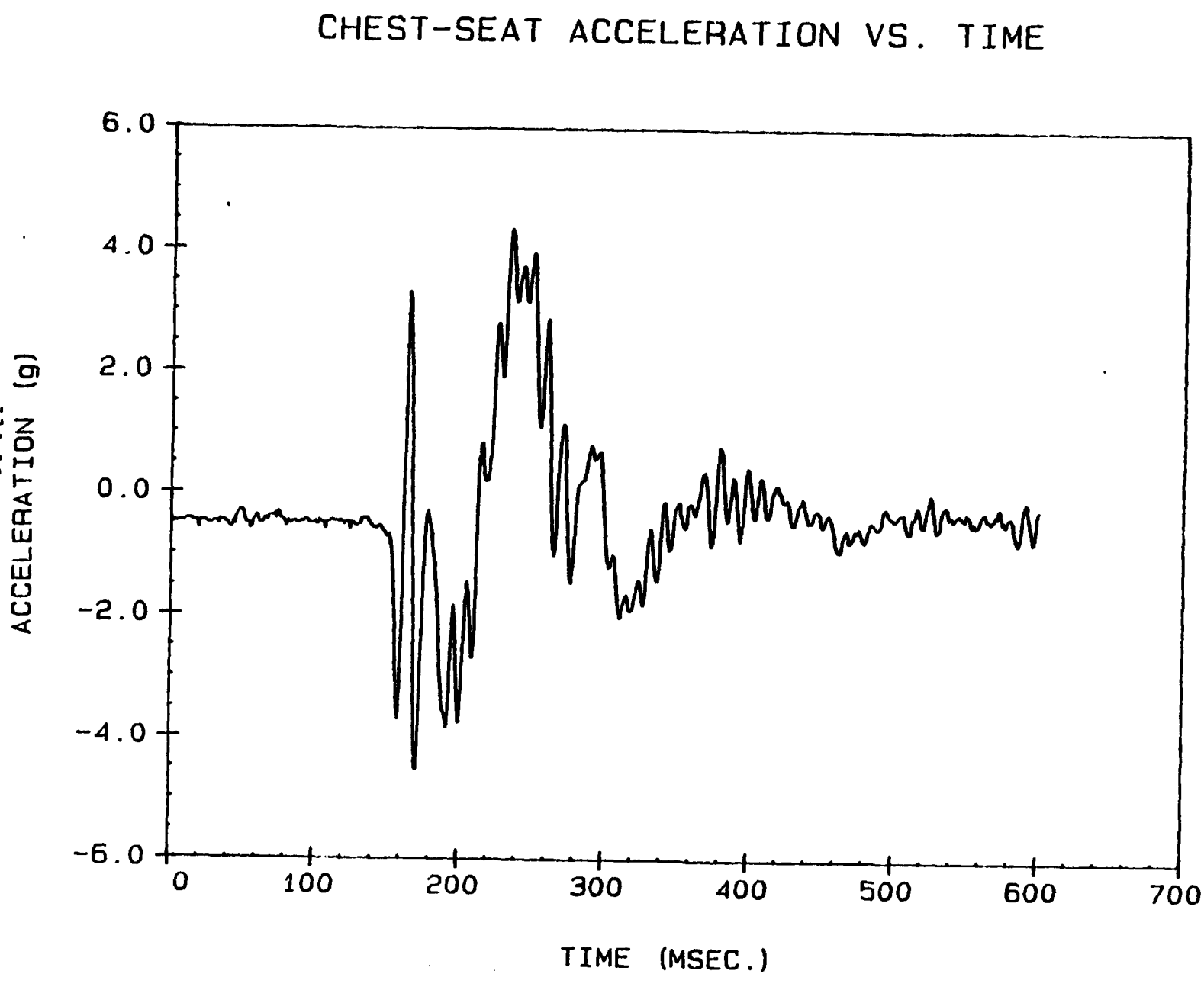

CHEST-SEAT ACCELERATION VS. TIME
ACCELERATION (g)
114-13
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
0
100
200
300
400
500
600
700
TIME (MSEC.)

Figure 3

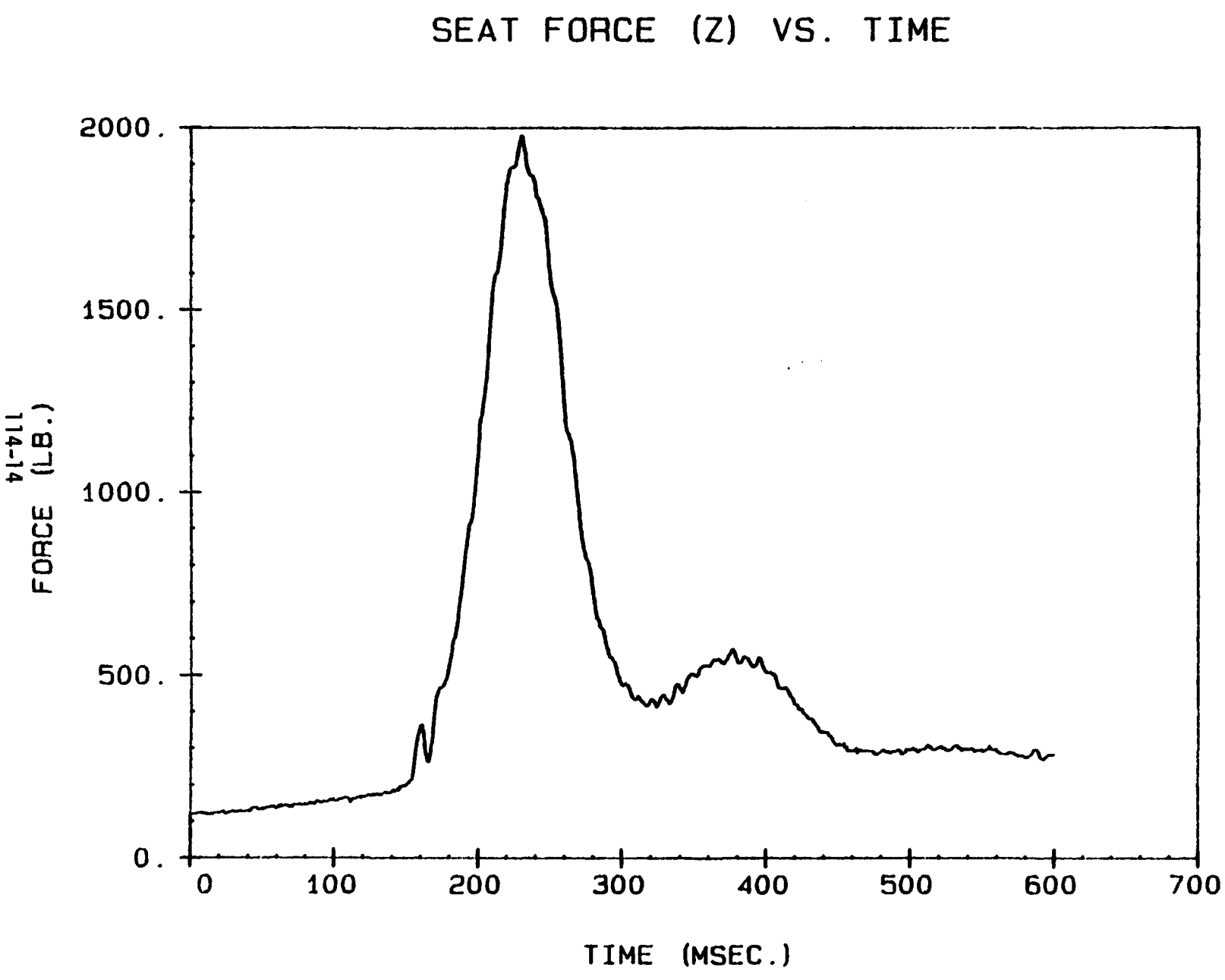
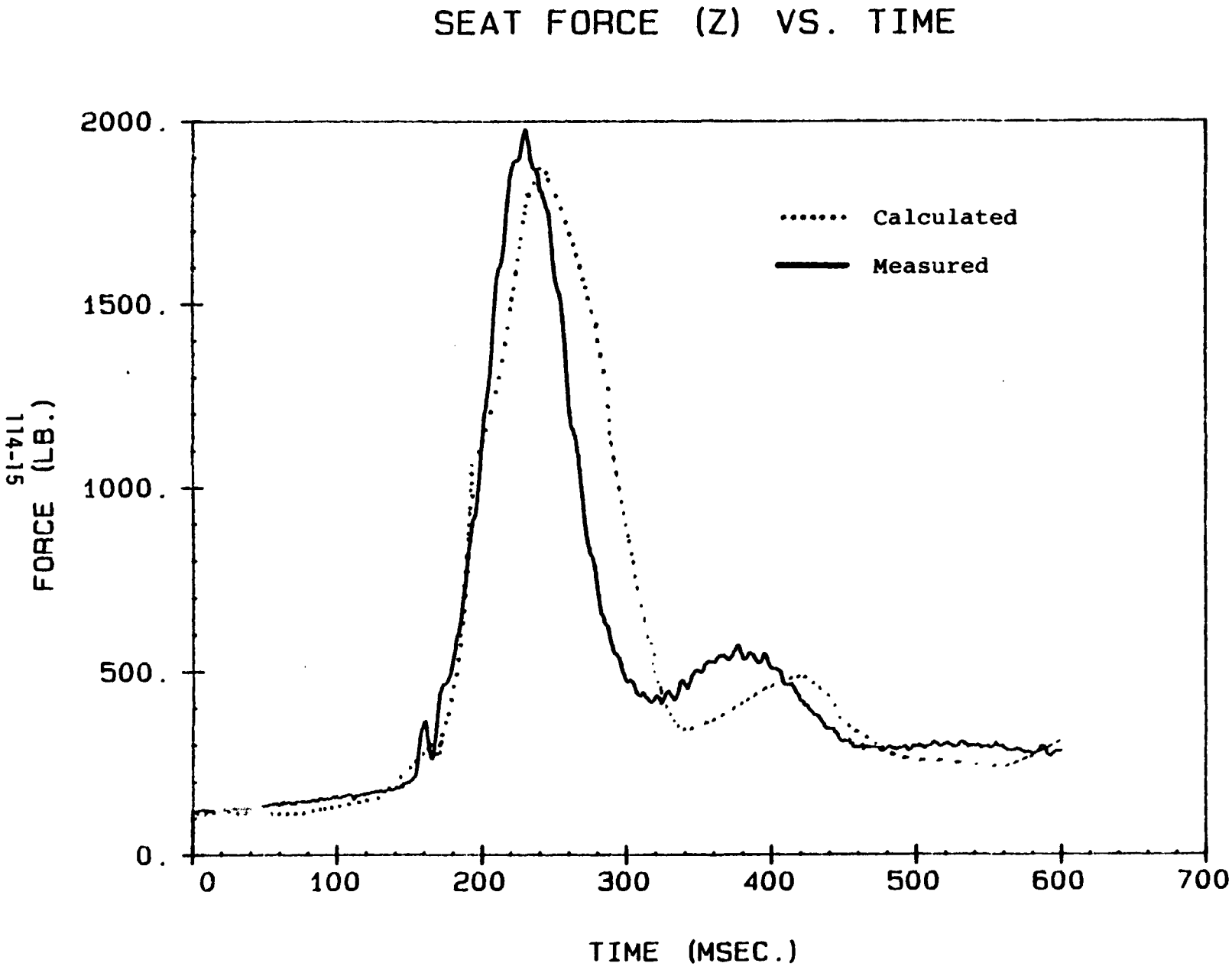

SEAT FORCE (Z) VS. TIME
2000.
1500.
1000.
500.
0.
FORCE (LB.)
0
100
200
300
400
500
600
700
TIME (MSEC.)

Figure 4


SEAT FORCE (Z) VS. TIME
2000.
1500.
1000.
500.
0.
FORCE (LB.)
114-15
Calculated
Measured
0
100
200
300
400
500
600
700
TIME (MSEC.)

nonlinear lumped-parameter model. This plan should include a wide spectrum of excitation magnitudes (4g to 10g) and also different excitation durations and shapes.

Although this model was developed for the Z direction, the methodology is the same for a similar model in the other two directions. Similar validation plans can be used to determine the effectiveness in the X and Y direction.

The same method can also be used on future spiral models of impact which will also include the abdominal musculature and interaction of ribs with the thoracic vertebrate. This will mean that more lumped masses should be included, as well as, more spring and viscous damping forces.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/ UNDERGRADUATE STUDENT SUMMER SUPPORT PROGRAM

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FINAL REPORT

# IN-VITRO MODELING OF PERFLUORO-N-DECANOATE EFFECTS ON ENZYMES OF FATTY ACID METABOLISM


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Date: 22 July 1988

Contract Number: F49620-87-R-0004

# IN-VITRO MODELING OF PERFLUORO-N-DECANOATE EFFECTS ON ENZYMES OF FATTY ACID METABOLISM

by

Sanford S. Singer

## ABSTRACT

Acyl-SCoA synthetase, acyl-SCoA oxidase & carnitine acetyltransferase were studied in our efforts to model, in vitro, the basis for effects of perfluoro-n-decanoate(PFDA) on fatty acid metabolism. We found that: **(1)** Acyl-SCoA synthetase used palmitate, oleate, laurate & decanoate as substrates to similar extents. However, it converted PFDA to PFDA-SCoA very slowly. PFDA inhibited acyl-SCoA formation from the fatty acids. The inhibitions appeared to be competitive. Palmitoyl-SCoA formation was inhibited most & decanoyl-SCoA formation was inhibited least. Palmitoyl-SCoA formation was inhibited up to 30% when the [PFDA]/[palmitate] was 4. **(2)** Acyl-SCoA oxidase, used palmitoyl-SCoA, lauroyl-SCoA & decanoyl-SCoA as substrates. It preferred the smaller acyl-SCoAs. Inhibition of oxidation of the C-10 and C-12 acyl-SCoAS by PFDA was more extensive than that of palmitoyl-SCoA. PFDA inhibition of decanoyl-SCoA & palmitoyl-SCoA oxidation was examined in depth & found to be competitive, with $K_I$s of $593 \pm 150$ μM & $76 \pm 6.0$ μM. **(3)** Carnitine acetyltransferase used acetyl-SCoA as its best substrate. Butryryl-SCoA, hexanoyl-SCoA, and octanoyl-SCoA were less effective sybstrates than acetyl-SCoA. Transfer of all acyl groups to carnitine was inhibited to a similar extent by PFDA. The $K_I$s were $111 \pm 15$ μM & $76.0 \pm 28$ μM.with the C-2 and C-8 acyl-SCoAs. Inhibition was competitive with acetyl-SCoA & noncompetitive with octanoyl-SCoA. Our examination of the PFDA effects on the enzymes gave useful information that may provide connections between isofunctional enzymes of rat liver and in-vivo effects of PFDA on lipid metabolism in the rat. Study of inhibition by PFDA-SCoA was tabled , as it was unavailable during the SFRP.

**ACKNOWLEDGEMENT**

I wish to thank the Air Force Systems Command and the Air Force Office for Scientific Research for sponsoring my efforts in the Summer Faculty Research Program for 1988. Universal Energy Systems is to be commended for their administrative leadership in the program.

My research effort at AAMRL/TH was exceptionally rewarding. I am grateful to Dr. Melvin Anderson for giving me this research opportunity, and to Marilyn George for invaluable aid in the background, planning, procurement, and logistics aspects of my efforts. I also wish to thank all of the many researchers at AAMRL/TH for their constant, day to day aid. Without their inputs this research effort would not have succeeded. I specially wish to thank Dr. David Mattie, Dr. Steve Godin, and Mr. Nick Del Raso for the many enlightening conversations we had and for their help and encouragement. Also I wish to express my gratitude to all of the Sgts who helped me out and cheerfully tolerated my myriad of questions, particularly, Bob Burgess, Ron Gaither, Joe Maslanka, and Dale Voisard.

## I. INTRODUCTION

**A. RELATIONSHIP TO EFFORTS AT AAMRL/TH** Ten-carbon, perfluorodecanoic acid(PFDA) was recently found to be toxic. In recent years, a number of related fluorinated chemicals, used in a variety of places including foam fire extinguishers and vascular fluid replacements, have also been shown to be toxic(see **IB**). Air Force personnel may be exposed to such chemicals. Consequently, evaluation of the extent of and basis for their toxicity is important.

The Biochemistry Branch of AAMRL/TH is actively engaged in evaluation of the toxicology of PFDA and related chemicals and in building toxicologic models for the processes. Whole animal studies have been conducted with rats and other species in which the pathology, variation in species mortality and alterations of energy and lipid metabolism have been determined. These studies suggest a central role for the liver in the toxicity. It is presently unclear what the basis for the responses to PFDA is. However, it is clear that these responses must involve enzymes of lipid metabolism.

Understanding of the enzymatic basis for the changes of lipid metabolism is likely to be obtained from examination of the enzymology of purified enzymes involved in the process. In addition, appropriate utilization of in-vitro enzymology should add a new dimension to screening of chemicals deemed likely to operate like PFDA. The advantages of such studies over whole animal studies include simplicity and amenability to experimental manipulation, lack of need for large numbers of animals on a routine basis, cost advantages and diminished researcher risk due to the smaller quantities of toxicants and reagents utilized for evaluation processes. The use of in-vitro enzymology is complicated by limitation of the exactitude of interpretation of the relationship to the in-vivo situation where the information base relating to in-vivo concentrations of metabolic products is incomplete and the needs for specialized equipment and personnel with specific training in enzyme purification and enzymologic technique.

My enzymologic research interests and long experience in purification, characterization and manipulation of enzymes suits me to carry out such efforts. This fits with the AAMRL/TH move to use of in-vitro technology, in addition to whole animal studies, for toxicologic estimations.

**B. TECHNICAL BACKGROUND:** Perfluorocarboxylic acids and related compounds have many industrial uses(1-3), including corrosion inhibitors, hydraulic fluids, wetting agents, foam fire extinguishers, coatings that impart water and oil resistance to paper and fabrics. Such uses have been predicated upon their supposed inertness and lack of toxicity. Recent study(4-6) has shown that perfluorinated carboxylic acids of chain length exceeding 8 carbons are toxic. Perfluoro-n-decanoic acid(PFDA), $CF_3(CF_2)_8COOH$, has been shown to be one of the more toxic of these compounds in rats and other species(5,7).

Most toxicologic research on PFDA has been carried out with Fisher 344 rats. There, a single i.p. dose of 41 mg PFDA kills 50% of injected animals. Toxic manifestations of PFDA(5,6) are similar to those of 2,3,7,8-tetrachloro-p-dioxin. These include acute anorexia, extensive weight loss, testis and thymus atrophy, bone marrow depression, excessive liver size and disruption of hepatic architecture. Both toxic agents also alter cellular lipid levels in liver, relating particularly to early alterations of fatty acid levels(8,9). Although the mechanism of the alterations is unclear, it appears likely to include alterations of lipid metabolism.

To this end, it is already clear(10) that PFDA leads to peroxisome proloferation and large elevations of apparent acyl-SCoA oxidase activity due to those organelles. Such enzyme elevations could alter production and disposition of cellular fatty acids, as the enzyme will not use short chain acyl-SCoA substrates(11). Disruptions of hepatic fatty acid metabolism could disturb cell function, leading to the toxicity observed, by altering the composition and properties of cellular membranes and/or altering the availability of lipids for energy production, hormone production and other cell processes.

PFDA itself could have disruptive effects on lipid metabolism, by interacting with various enzymes involved. These interactions could cause differential activation or inactivation of the enzymes. Certainly, it has been shown that PFDA is retained at high concentrations in hepatocytes for 30 days after initial insult with a 50 mg/kg dose(9). Also, PFDA appears to be converted to a polar metabolite(9). This molecule may also contribute to PFDA action. Or, alternatively, it may be responsible for "late effects" of PFDA, exemplified by the bimodal response of acyl-SCoA oxidase described in a recent report(10).

More information is required to explain the basis for the lipotransformations resultant from PFDA administration. It would seem possible to model the basis for the effects of PFDA and its polar metabolite, using purified enzymes. Such modeling could enable us to ascertain why observed alterations of fatty acid metabolism ooccurred. It would also provide insight into metabolites to which PFDA could be converted.

## II. SUMMARY OF PROPOSED RESEARCH GOALS:

In recent years PFDA has been shown to be very toxic to rats and other animals. Its toxic effects are similar to those of the dioxins. Prominant among these are large alterations of liver lipid levels and composition. The toxic mechanism is unknown. However, it appears likely to include altered lipid metabolism resulting from interactions of PFDA and/or its metabolites with lipid-metabolizing enzymes, which change their catalytic abilities.

The proposed effort will begin by examining three commercially available enzymes of fatty acid metabolism likely to be related to observed effects of PFDA. These are nonrat acyl-SCoA synthetase, acyl-SCoA oxidase, and carnitine acetyltransferase. We will adapt standard assays for the enzymes to use at AAMRL/TH and examine the ability of PFDA to inhibit or activate the enzymes. Several rat liver enzymes, unavailable commercially, appear even more relevant to study. These include enzymes isofunctional to the ones just mentioned, acyl-SACP synthetase, acyl-SCoA dehydrogenase and acyl-SCoA hydrolase. Depending upon the rate of completion and the consequences of our studies with the commercially available enzymes, we hope to have time to attempt to isolate one relevant rat enzyme, adapt its assay to use at AAMRL/TH and examine interactions with PFDA.

The studies proposed are expected to serve as the basis for longer term interactions with AAMRL/TH. It is hoped that they will: **(a)** Inform us about the ability of PFDA to interact with the individual enzymes and the effects of the interactions **(b)** Provide data relevant to understanding the basis for some aspects of PFDA action. **(c)** Lead us to develop the facility for assay and purification of the relevant rat liver enzymes at AAMRL/THS.

## III. STUDY OF ACYL-SCOA SYNTHETASE FROM PSEUDOMONAS

### A. MATERIALS AND METHODS:

Enzyme And Supplies(all from Sigma Chemical Company)

The acyl-ScoA synthetase enzyme will be used to model whether PFDA affects the conversion of fatty acids to their respective coenzyme A derivatives and whether PFDA is converted to PFDA-SCoA. Oleic, palmitic, lauric and decanoic acids will be tested. The enzyme assay is basically that described by Shimizu et al(12), as modified by Sigma. The supplies necessary are: Acyl-SCoA Synthetase(A-2777) ,Pyruvate Kinase -LDH (40-7), Myokinase(M-3003), Coenzyme A(C-3019) NADH(N-8129), Disodium EDTA(ED-255), Triton X-100(X-100), Oleic Acid(O-3879), Palmitic Acid (P-5917), Lauric Acid(L-4250), Decanoic Acid (C-1875), Phosphoenolpyruvate(P-7002), Disodium ATP(A-2383), Trizma Base(T-1503), $MgCl_2$(M-9272). Assay is based upon reading 340 nm decrease due to NADH oxidation as a consequence of:

$$\text{Fatty Acid} + \text{ATP} + \text{HSCoA} \xrightarrow{\text{Synthetase}} \text{Acyl-SCoA} + \text{AMP} + \text{PPi}$$

$$\text{ATP} + \text{AMP} \xrightarrow{\text{Myokinase}} 2\text{ADP}$$

$$2\text{ADP} + 2\text{PEP} \xrightarrow{\text{Pyruvate Kinase}} 2\text{ ATP} + 2\text{ Pyruvate}$$

$$2\text{ Pyruvate} + 2\text{ NADH} + 2\text{H+} \xrightarrow{\text{Lactate Dehydrog.}} 2\text{ Lactate} + 2\text{ NAD+}$$

Enzyme Assay:

Usually, each 1.5-mL reaction mixture contains 226 µmol tris, 3.83 µmol EDTA, 20.4 µmol $MgCl_2$, 370 nmol ATP, 1,009 nmol PEP, 128 nmol coenzyme A, 383 nmol dithiothreotol, 963 nmol NADH, 110 nmol palmiitate, 0.2% Triton X-100, 1.5% glycerol, 3% propylene glycol, 1.5 U LDH, 1.5 U PK

pyruvate kinase and 1.77 units myokinase. Variations of procedure in kinetic and other studies can be found in USAF R & D Notebook 63907. **Enzyme activity in units per mL enzyme solution is obtained from:**

$$U/mL = [(\Delta A_{340}\ \text{Test} - \Delta A_{340}\ \text{Blank}) \times \text{vol rxn mix}]/[12.4 \times \text{vol enz} \times \text{min}]$$

**B. RESULTS**

1. The Acyl-SCoA Synthetase Assay With Palmitate as Substrate The method designed for use at AAMRL/THS modified the procedure of Shimazu et al **(12)**described by Sigma. We first examined the reproducibility of the Sigma assay. Half-sized reaction mixtures were used to minimize costs.In all our studies the reproducibility of the method was excellent, as duplicates differed by only 2-3%. The assay exhibited zero order kinetics with 73 µM palmitate(halving fatty acid diminished observed activity only 4-5%) and 83.5 µM coenzyme A(halving coenzyme had no effect)with up to 12 µg enzyme per reaction mixture. The reaction rate was always constant for 12 minutes.

2. PFDA as Substrate for Acyl-CoA Synthetase Here, 73 µM PFDA,730 µM PFDA and 73 µM palmitate were compared. The data(Fig. 1), indicated that PFDA was a poor substrate. Four experiments showed that 73 and 730 µM PFDA supported formation of only $4.55 \pm 2.6$ % and $7.30 \pm 4.60$ % as much acyl-SCoA as did 73 µM palmitate. The low activity was not due to inhibition of the coupled enzymes used to visualize the reaction (e.g., 73 µM PFDA, Section 3 below, had almost no effect on conversion of 73 µM palmitate to palmitoyl-SCoA in mixed assays).

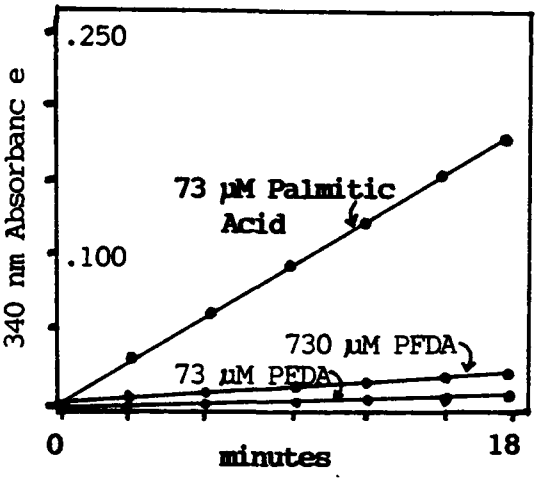



Fig.1 Palmitate & PFDA as Acyl-SCoA Synthetase Substrates

3. Inhibition of Acyl-SCoA Synthetase When it is Nearly Saturated with Palmitate. Here, we first examined the effects of 73 or 730 µM PFDA on formation of palmitoyl-SCoA with 73 µM palmitate substrate. As shown(Fig. 2A), the lower concentration of PFDA effected a small inhibition of palmitoyl-SCoA

formation. Subtraction of the small amount of PFDA-SCoA made from 73 μM PFDA, indicated that the inhibition was 9.1%. Use of 730 μM PFDA(Fig. 2B) caused 54.8% inhibition of palmitoyl-SCoA formation. Examination of the effect of 73 μM PFDA on palmitoyl-SCoA formation with 37 μM palmtate(Fig. 2C) showed 21% inhibition of palmitoyl-SCoA formation. The data suggested competitive inhibition of palmitoyl-SCoA formation. Additional study of this inhibition will be described later in this report.

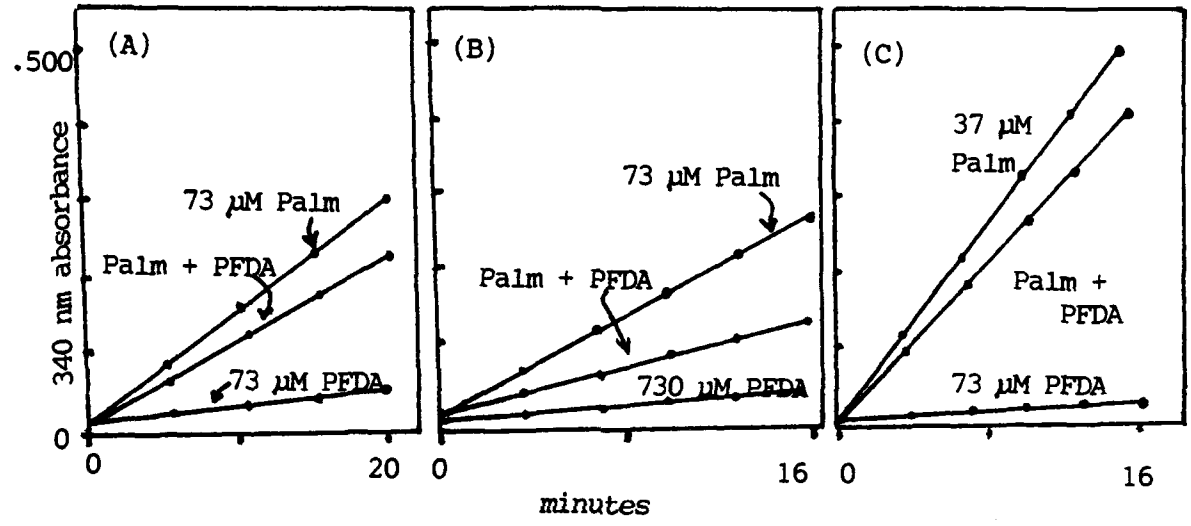



Fig. 2. The Effect of PFDA on Palmitoyl-SCoA Formation
The abbreviation Palm is used to indicate palmitic acid.

4. Comparison of PFDA Effects on Formation of Palmitoyl-,Oleoyl-, Lauroyl- and Decanoyl-SCoA. We first compared the ability of 73 μM palmitate, oleate, laurate and deca to support acyl-SCoA formation. We found that the relative activities e four substrates were 100%, 94.1±3.91%, 69.5±2.4% and 76.4±5.8% for oleate, palmitate, laurate and decanoate, respectively(5 experiments). Next, we tested the ability of 730 μM PFDA to inhibit conversion of each fatty acid(at 73 μM) to acyl-SCoA. We found(e.g., Fig. 3) that the production of the shorter-chain acyl-SCoAs was inhibited more weakly than that of palmitate or oleate. In 5 experiments % inhibitions were 59.5±2.0, 52.4±3.8, 43.4±6.4, and 32.0±9.6 for palmitate, oleate, laurate, and decanoate, respectively.

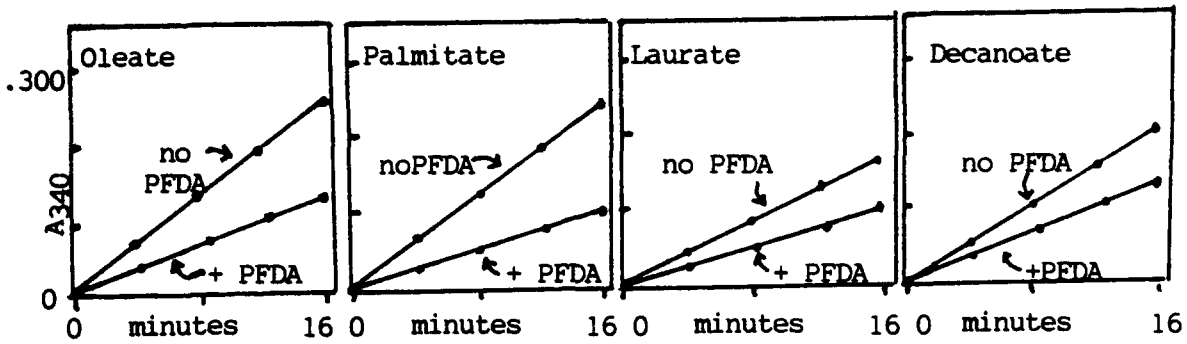



Fig. 3 The Effect of 730 μM PFDA on Formation of Acyl-SCoAs from 73 μM Oleate, Palmitate, Laurate or Decanoate.

5. Examination of Inhibition of Palmitoyl SCoA Formation by PFDA

We first examined the effect of 73 μM PFDA on palmitoyl-SCoA formation with differing amounts of palmitate. Three experiments showed(e.g. Fig. 4) that inhibition increased from 5-10% with 73 uM palmitate([PFDA]/[palmitate] = 1) to 30% or more with 19 uM palmitate. It was $24.9 \pm 5.5\%$ and $31.8 \pm 4.0\%$ with 24 and 19 μM palmitate([PFDA]/[palmitate] = 3 and 4, respectively). These data suggested that PFDA was a competitive inhibitor of acyl-SCoA synthetase. We could not carry out more extensive kinetic experiments that would have allowed us to definitively state that the inhibition was competitive and to ascertain $K_I$ for several reasons. First, were methodologic difficulties relating to the complexity of the experiment Secondly, at palmitate concentrations below 19 μM in assays, the enzyme inactivated spontaneously. Therefore, such studies will have to await future studies.

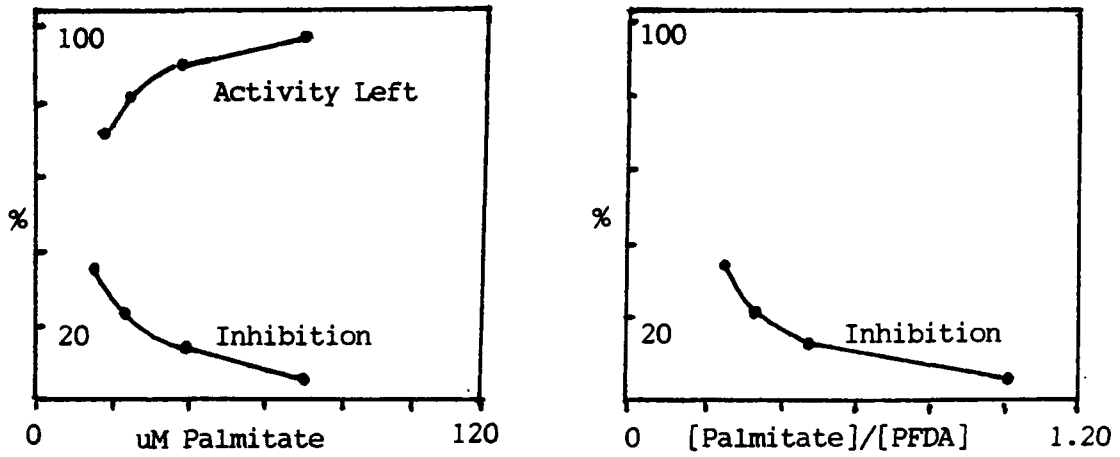



Fig. 4 The Effect of 73 μM PFDA on Acyl-SCoA Formation, using Several Different Amounts of Palmitate as Substrate.

**C. DISCUSSION-** Study of all the enzymes is interrelated, so discussion of our results will appear after the data of Sections IV -VI, as Section VII.

## IV. STUDY OF ACYL-SCoA OXIDASE FROM CANDIDA

### A. MATERIALS AND METHODS

Enzyme and Supplies(all from Sigma) The study will model whether PFDA affects acyl-SCoA oxidation in a way that could help explain observed alterations of fatty acid metabolism after PFDA. Palmitoyl-, lauroyl- and decanoyl-SCoA will be tested. The assay is as after Shimazu et al**(13)**, as modified by Sigma. Supplies are Acyl-SCoA Oxidase(A-2903), Palmitoyl-SCoA (P-9276), Lauroyl-SCoA(P-9012), Decanoyl-SCoA(D-5269),4-Aminoantipyrine(A-4482) Phenol (P-4161), Peroxidase(P-8375), $KH_2PO_4$(P-0662), $K_2HPO_4$(P-3786). Assay is based on pink dye production, quantitated by increased 500 nm absorbance.

$$\text{Acyl-SCoA} + \text{E-FAD} \xrightarrow{\text{Acyl-SCoA Oxidase}} \Delta^2\text{-Enoyl-SCoA} + \text{E-FADH}_2$$

$$\text{E-FADH}_2 + O_2 \xrightarrow{\text{Acyl-SCoA Oxidase}} \text{E-FAD} + H_2O_2$$

$$\text{Phenol} + \text{4-Aminoantipyine} \xrightarrow[2H_2O_2 \quad 4H_2O]{\text{Peroxidase}} \text{Pink Dye}$$

The Enzyme Assay: Usually, each 1.5-mL reaction mixture contains 62.5 µmol K phosphate, 0.792 µmol antipyrine, 11.0 µmol phenol, 5.00 U peroxidase, 0.218 µmol palmitoyl-SCoA, 0.036% Triton X-100, 7% propylene glycol and 3.3% glycerol. Procedural variations can be found in USAF R & D Notebook 62621. **Enzyme activity in units per mL enzyme solution can be obtained from:**

$$U/mL = (\text{Delta } A_{500} \times \text{vol rxn mix})/(6.39 \times \text{vol enz} \times \text{min})$$

### B. RESULTS

1. Examination of Acyl-SCoA Oxidase Assay This used facets of the method of Shimazu et al**(13)** and that provided by Sigma. Our routine assay used twice the palmitoyl-SCoA suggested. It was very reproducible, with duplicates differing by only 5-8%. The enzyme was nearly saturated with palmitoyl-SCoA (halving it changed enzyme activity only 14-19%). Kinetics were essentially zero order kinetics with up to 7 µg enzyme per reaction mixture. Reaction proceeded at a constant rate for 4 minutes. Comparison of 146 µM palmitoyl-, lauroyl- and decanoyl-SCoA as substrates showed the shorter-chain

acyl-SCoAs to be the better substrates.The relative activities for 146 μM palmitoyl-SCoA, lauroyl-SCoA and decanoyl-SCoA were 59.6±3.5%, 87.7±1.0% and 100% in 5 experiments.

2. PFDA Inhibition of Acyl-SCoA Oxidation with Near-Saturated Palmitoyl-SCoA or Other Acyl-SCoAs at Equal Concentrations Here we tested PFDA inhibition of oxidation of 146 μM lauroyl-SCoA, palmitoyl-SCoA or decanoyl-SCoA. The experiment used 1,520 μM PFDA because our first study, with 730 μM PFDA and and palmitoyl-SCoA, showed little, if any, inhibition of the enzyme activity. We found that with 1,520 μM PFDA --10.4 times the acyl-SCoA in reaction mixtures-- palmitoyl-SCoA oxidation was inhibited slightly. However,the oxidation of the short-chain acyl-SCoAs was affected much more substantially. In 4 experiments, the inhibitions observed were 20.8±3.5%, 81.7±3.3% and 82.9±2.5% for palmitoyl-SCoA, lauroyl-SCoA and decanoyl-SCoA, respectively. Fig. 5 depicts an inhibition experiment.

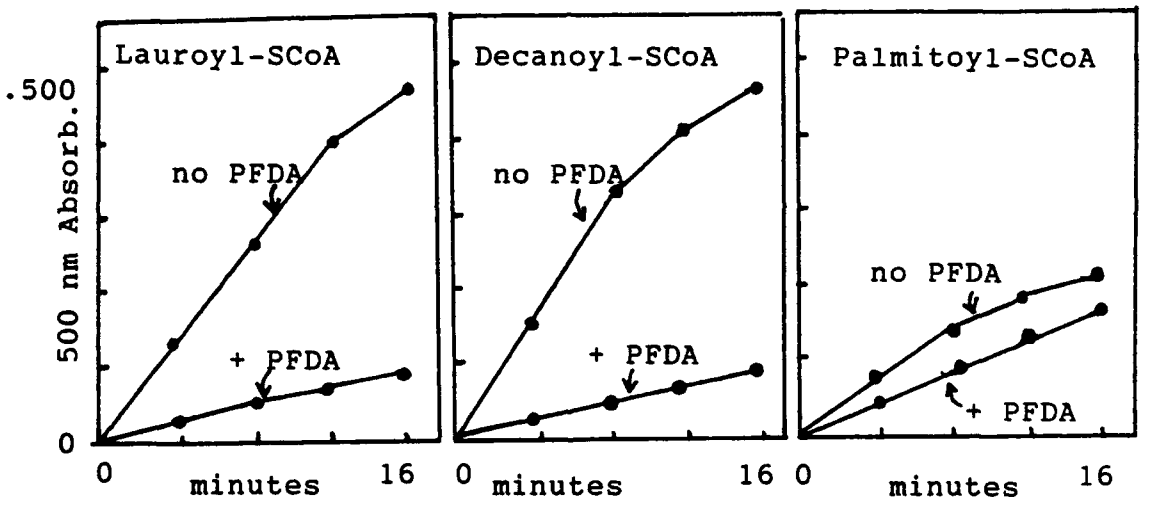



Fig. 5. Effect of 1,520 μM PFDA on Oxidation of 146 μM Lauroyl-SCoA, Decanoyl-SCoA or Palmitoyl-SCoA by Acyl-SCoA Oxidase.

3. Effects on Inhibition of Oxidation of 146 μM Palmitoyl-SCoA or 73 μM Decanoyl-SCoA of Varying PFDA Concentration The experiments just described supported palmitoyl-SCoA oxidation as less sensitive to PFDA inhibition than that of lauroyl-SCoA or decanoyl-SCoA. We next examined the effect(Fig. 6) of varying PFDA levels, to obtain more detailed information. We used 73 μM decanoyl-SCoA and 143 μM palmitoyl-SCoA for similar product formation. We planned to use the data that would be obtained to compare

PFDA effects on the oxidations and choice of [PFDA] to use to determine whether the inhibitions were competitive or noncompetitive. As shown, oxidation of palmitoyl-SCoA was much less sensitive to PFDA than that of decanoyl-SCoA. The data suggested that 50% inhibition of decanoyl-SCoA and palmitoyl-SCoA oxidation would have required 150 μM PFDA and 550 μM PFDA, respectively.

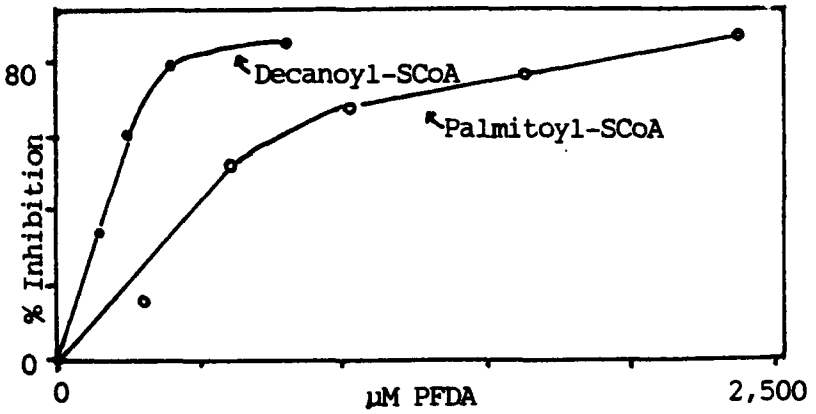



Fig. 6 Inhibition of Oxidation of 146 μM Palmitoyl-SCoA or 73 μM Decanoyl-SCoA by Various Amounts of PFDA.

4. The Nature of PFDA Inhibition of Oxidation of Palmitoyl-SCoA or Lauroyl-SCoA. We used 138 and 304 μM PFDA, concentrations expected(Fig. 6)to give 25-30% inhibition with the highest acyl-SCoA levels tested. Double reciprocal plots, Fig. 7, showed uninhibited Kms of 50.7±3.0 μM(2 exper)for decanoyl-SCoA and 53.8±11 μM (3 exper) for palmitoyl-SCoA. The inhibitions were competitive. $K_I$s for palmitoyl-SCoA and decanoyl-SCoA were 593±150 μM and 76±6.0 μM. These $K_I$s agreed with expectations from Fig. 6.

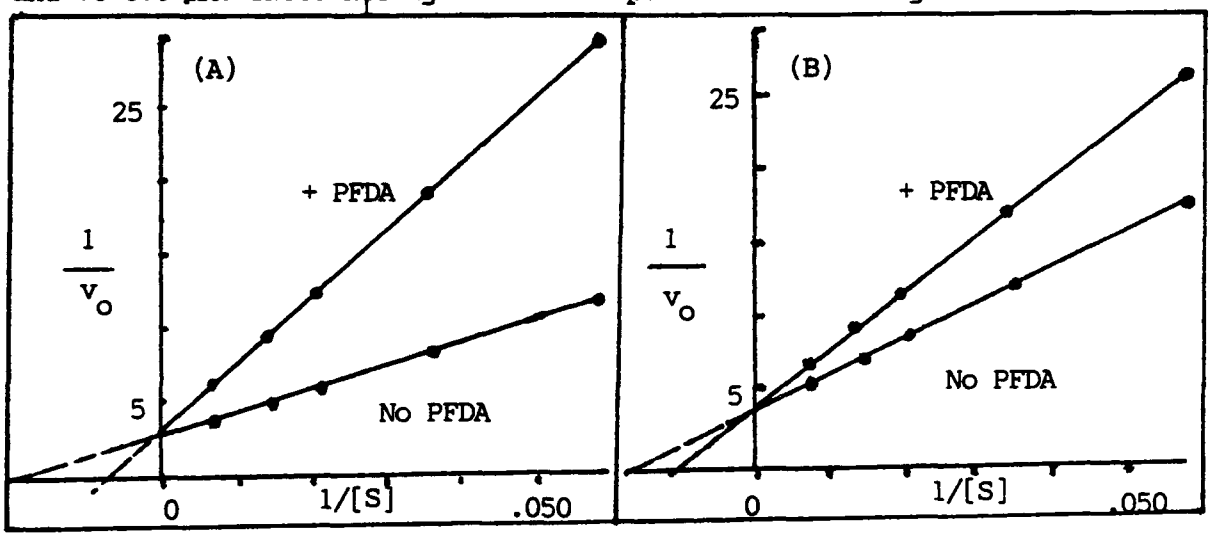



Fig. 7. Double Reciprocal Plots of Inhibition of Acyl-SCoA Oxidase- , Catalyzed Oxidation of Decanoyl-SCoA(A) and Palmitoyl-SCoA(B) by PFDA.

## V. STUDY OF CARNITINE ACETYLTRANSFERASE FROM PIGEON LIVER

### A. MATERIALS AND METHODS

Enzyme and Supplies(all from Sigma) The enzyme transports acyl-SCoAs of 2-10 C across mitochondrial membranes. Its study will model whether PFDA affects this transport in ways that explain observed changes of fatty acid metabolism and energy production after PFDA. Supplies are Carnitine Acetyltransferase(C-4899) 5,5'-Dithiobis-2-Nitrobenzoic Acid,DTNB(D-8130), L-Carnitine(C-0283), Acetyl-SCoA (A-2897), Butyryl-SCoA(B-1508),Hexanoyl-SCoA(H-2012), Tetrasodium EDTA(ED-455). Assay uses reaction of HSCoA(freed by transfer of acyl groups to carnitine)with 5,5'-dithiobis-2-nitrobenzoic acid to yield a yellow product,followed spectrophotometrically at 412 nm.

$$\text{Acyl-SCoA + Carnitine} \xrightarrow{\text{Acetyltransferase}} \text{HS CoA + Acyl-Carnitine}$$

$$\text{HS-CoA + NBA-SS-NBA} \xrightarrow{\text{Nonenzymatic}} \text{NBA-SS-NBA + NBA-SH(yellow)}$$

The Enzyme Assay Usually, each 1.5-mL reaction mixture contains 269 µmol Tris, 264 µmol DTNB, 1.5 µmol EDTA, 5.46 µmol carnitine, 300 nmol acetyl-SCoA* 0.9 µg enzyme, 0.005% Triton X-100, 3.9% propylene glycol, 5.0% glycerol. Procedure variations in kinetic and other studies can be found in USAF R & D Notebook 62640(***Note** that the enzyme is not saturated with acetyl-SCoA. Saturation requires 4 times more acetyl-SCoA. This was beyond our budget). **Enzyme activity in units per ml enzyme solution can be obtained from:**

$$U/mL = (\text{ Delta } A_{412} \text{ x vol rxn mix})/(13.6 \text{ x vol enzyme x min})$$

### B. RESULTS

1. Examination of the Enzyme Assay The method used elements of the procedure of Chase(**14**) and of that provided by Sigma. The routine assay we ultimately developed required much more carnitine and acetyl-SCoA than indicated by either source. In our reaction mixtures the use of 3.4 mM carnitine saturated the enzyme. We also included 200 µM acetyl-SCoA in reaction mixtures, although the enzyme was not saturated with this

amount of the acyl-SCoA(Saturation requires ~.80 mM acetyl-SCoA and this was beyond our budget).However, we obtained reproducible data, quite adequate for the inhibition studies planned. If absolute quantitation of tissue levels of carnitine acetyltransferase is required, we recommend use of 1.00 mM acetyl-SCoA. The observed enzyme activity increased in a fashion that allowed quantitation with up to 1 µg enzyme in reaction mixtures. Initial studies were limited to use of acetyl-SCoA because acyl-SCoAs possessing more than two carbons were reported**(14)** to be poor substrates.

2. PFDA Inhibition of Acetyl Transfer from Acetyl-SCoA to Carnitine. This was first examined with 100 µM acetyl-SCoA and 800 µM PFDA. Three experiments showed that inhibition was almost complete here($96.2 \pm 1.0$ %). Use of lower [PFDA] indicated that with 40 µM inhibitor([PFDA]/[acetyl-SCoA] = 0.2), acetyl transfer was diminished by $26.2 \pm 6.8$%.Carnitine acetyltransferase appeared to be more sensitive to PFDA than acyl-SCoA synthetase or acyl-SCoA oxidase. Study (4 exper) of the nature of the PFDA inhibition indicated that it was competitive with acetyl-SCoA(e.g., Fig. 8A), that the uninhibited $K_M$ for acetyl-SCoA was $339 \pm 50$ µM and that $K_I$ was $111 \pm 15$ µM. As might be expected, the PFDA inhibition was noncompetitive with respect to carnitine(e.g., Fig. 8B). Four experiments showed that the $K_M$ for carnitine was $483 \pm 47$ µM and $K_I$ was $140 \pm 6.5$ µM.

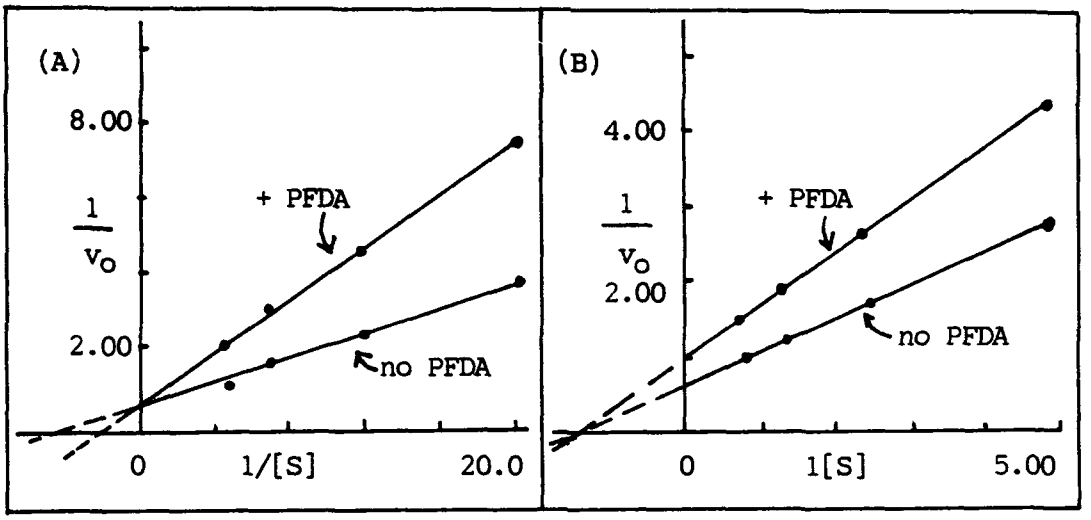



Fig. 8. Double Reciprocal Plots Showing the Effects of 60 µM PFDA on Acetyl Transfer from Acetyl-SCoA to Carnitine,with (A)Acetyl-SCoA and (B) Carnitine as Varied Substrate.

3. Comparison of the Effect of 80 μM PFDA on Acyl Transfer from 200 μM Acetyl-SCoA or Hexanoyl-SCoA to Carnitine. Here, we wished to ascertain the PFDA effect with a larger acyl-SCoA. We tested hexanoyl-SCoA first because it was reportedly(**14**)the best, larger acyl-SCoA substrate(13% of activity with acetyl- SCoA), C-10 and longer acyl-SCoAs being ineffective (**14**). We found(3 exper) that 80 μM PFDA([PFDA]/[acyl-SCoA] =.40)inhibited acetyl and hexanoyl transfer similarly, 43.4±.70% and 49.2±5.5%.

4. Comparison of Acetyl, Butyryl, Hexanoyl and Octanoyl Transfer.Here, (3 exper)transfer of C-2, C-4, C-6, C-8 acyl groups was 100%, 35.3±1.7, 18.9±1.8%, 9.03±1.7%. Inhibitory effects of 80 μM PFDA with the acyl-SCoAs ([PFDA]/[acyl-SCoA]=.4) were similar. We next examined the nature of octanoyl -transfer inhibition. To do this, we had to use octancyl-SCoA levels of 100 to 60C μM. Even so, the enzyme activity was quite low. Surprisingly, inhibition(e.g., Fig. 9)was noncompetitive. The $K_M$ for octanoyl-SCoA was 376±62 μM(similar to that for acetyl-SCoA). The $K_I$ was 76.0±28 μM. The last study suggests the need for further investigation of the enzyme.

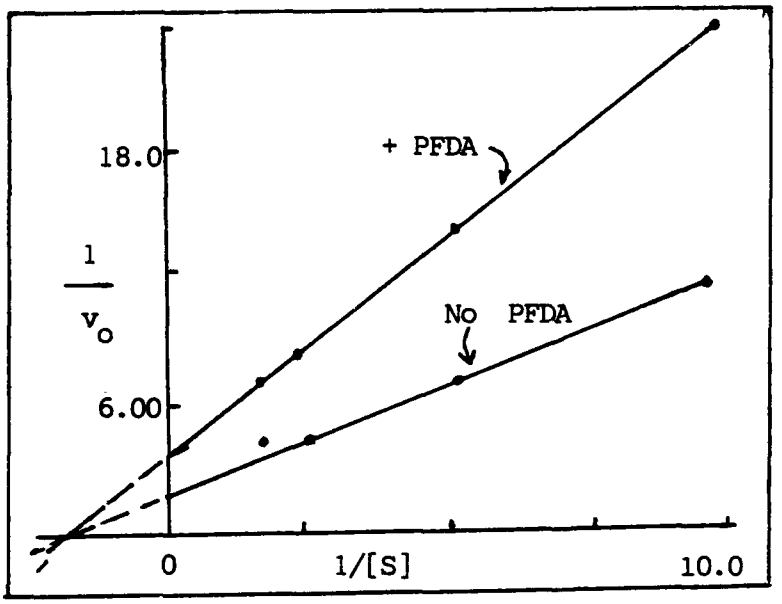



Fig. 9. Double-Reciprocal Plot of the Effect of 80 μM PFDA on Octanoyl Transfer to Carnitine from Octanoyl-SCoA.

**VI. EXAMINATION OF RAT LIVER ENZYMES:** The most desireable of these would be the enzymes isofunctional to those examined in **Sections III-V**, and rat liver palmitoyl-SCoA hydrolase. We had expected to begin purification and study of rat liver carnitine palmitoyltransferase near the end of the SFRP. However, we could not do this, as a WPAFB-wide spending freeze stopped purchase of the necessary supplies. The effort will be tabled for now.

## VII. DISCUSSION

In rats given 50 mg/kg PFDA, hepatic levels of the toxicant are quickly maximized at ~500 μM. Hepatic fatty acid levels(particularly, oleic and palmitic acids)are also elevated quickly. In time, cellular membranes become more fluid, less permeable and less fragile. These occurrences are deemed important to the toxicity of PFDA and related chemicals. It appeared that changes of the enzymology of liver lipids could be important components of the toxic response mechanism. Consequently, our primary aim here has been to examine PFDA interactions with appropriate enzymes of lipid metabolism and search for effects of interest, those that could explain the in vivo alterations.

We began our efforts by studying three central, fatty acid-metabolizing enzymes available in purified form, acyl-SCoA synthetase(Pseudomonas), acyl-SCoA oxidase(Candida) and carnitine acetyltransferse(pigeon muscle). If effects of interest were obtained, our next aim --planned to begin near the end of the SFRP and to continue on a more long-term basis-- was to purify and examine apropriate rat liver enzymes.

*Initial questions* that would be asked were:(1) Is PFDA converted well to PFDA-SCoA by the synthetase? (2) What are the effects of PFDA and PFDA-SCoA on activation of long-chain fatty acids(oleate and palmitate)and shorter-chain fatty acids(laurate and decanoate)? (3) How do PFDA and PFDA-SCoA affect the action on palmitoyl-, lauroyl- and decanoyl-SCoA of acyl-SCoA oxidase? This enzyme could be used to model both peroxisomal acyl-SCoA oxidase and isofunctional mitochondrial acyl-SCoSA dehydrogenase. (4) What would be the results of PFDA and PFDA-SCoA interaction with pigeon muscle carnitine acetyltransferase, model for carnitine acyltransferases of rat liver? It was to be remembered that observed effects of interest would mostly provide inferences that would make it potentially worthwhile to expend the time and expense needed to purify the rat enzymes in house for study of their interactions with PFDA. Our studies thus far have lead to the following observations of interest.

First, as to acyl-SCoA synthetase, we found(Fig. 1) that with 73 and 730 μM PFDA the enzyme made PFDA-SCoA very slowly compared to production of palmitoyl-SCoA . This suggested that PFDA-SCoA might be made only slowly by the comparable enzymes of rat liver and that it might be involved mostly

in "late"**(9)** effects of PFDA administration. By comparison(Fig.3), decanoate was almost as good a substrate as equimolar palmitate. PFDA inhibited palmitoyl-SCoA, oleoyl-SCoA, lauroyl-SCoA and decanoyl-SCoA formation in vitro. Palmitoyl-SCoA and oleoyl-SCoA formation were inhibited more than that of the shorter-chain fatty acids(Figs.2 & 3). These data, extrapolated to rat liver, have potential importance, in that extensive inhibition of palmitoyl-SCoA and oleoyl-SCoA formation in that organ could lead to elevation of free palmitate and oleate levels, as well as to less long-chain acyl-SCoa available for energy formation and/or membrane lipid synthesis.

It must be noted that PFDA is probably a competitive inhibitor of acyl-SCoA synthesis(Fig. 4). As such, it is [PFDA]/[fatty acid] that is important (e,g., Fig. 4 shows that with [PFDA]/[palmitate]= 3-4, formation of palmitoyl-SCoA was inhibited up to 30%). With 500 μM PFDA in the liver cell, significant inhibition could be expected with up to 300 μM palmitate. The total of the 6 major fatty acid levels in normal liver averages about 25 mM **(9)**. Because [PFDA]/[Total Major Fatty Acids] is therefore .02, it would seem that PFDA inhibition of the synthetase is probably potential rather than realized, if the enzyme approxinates the rat liver enzyme. However, the Pseudomonas enzyme is not kinetically the same as the rat enzyme. Perhaps the rat enzyme should be examined briefly for this reason. More importantly, examination of the rat enzyme should be undertaken to clarify issues relating to PFD-SCoA. That is: Is this compound, unavailable during my SFRP period, the inhibitor of real interest? Is it made by the rat enzyme? At what rate is it made ?

As to acyl-SCoA oxidase, PFDA-SCoA was not available for testing, but PFDA inhibited enzyme-catalyzed oxidation of palmitoyl-SCoA, lauroyl-SCoA and decanoyl-SCoA. Fig. 5 shows PFDA inhibition with 146 μM palmitoyl-SCoA was much weaker than with equimolar lauroyl-SCoA or decanoyl-SCoA. Kinetic examination of the PFDA effect with palmitoyl-SCoA and decanoyl-SCoA(repreentative long- and shorter-chain acyl-SCoAs)showed(Fig. 7) that the inhibitions(e.g., Fig. 7)were competitive and that the respective $K_I$s were $593 \pm 150$ and $76 \pm 6.0$ μM. Thus, once again, it is the [PFDA]/[substrate] that determines the extent of inhibition. Consequently, to acertain the relevance of the PFDA effect in vivo, it is necessary to know what the acyl-SCoA levels in rat liver are.

Recently, it was reported**(15)** that the hepatic long chain acyl-SCoA is 125 μM in rats. As the maximum early PFDA concentration in liver averages

about 500 μM, the [PFDA]/[Total Long Chain Acyl-SCoA] is near 4. This (see Fig. 6)would be expected to give about 40% inhibition of palmitoyl-SCoA oxidation and almost complete inhibition of oxidation of the shorter acyl-SCoAs. If the rat enzyme is similar, oxidation of palmitoyl-SCoA(and perhaps other long chain acyl-SCoAs)could be less extensive than usual and might diminish energy production from fatty acid oxidation. In addition, oxidation of long chain fatty acids would almost cease once C-10 or C-12 acyl-SCoAs were produced. Not only would this diminish energy production, but shorter acyl-SCoAs could accumulate and be incorporated into complex lipids more extensively. In turn, such incorporation could contribute to observed changes of properties of membranes and other complex lipids.

Finally, as concerns <u>carnitine acetyltransferase</u>, the transfer of acetyl butyryl, hexanoyl and octanoyl groups from acyl-SCoAs to carnitine was inhibited by PFDA. A [PFDA]/[acyl-SCoA] of .40, diminished hexanoyl and acetyl transfer by 49.2$\pm$5.5% and 43.4$\pm$.70%. The inhibition of acetyl transfer to carnitine was competitive with respect to acetyl-SCoA(Fig. 8), with a $K_I$ of 111$\pm$15 μM. Thus, the basis for acetyl-transfer inhibition by PFDA here is the [PFDA]/[substrate]. If the carnitine acetyltransferase models long-chain carnitine acyltransferases appropriately, then 500 μM PFDA in livers of treated rats would cause significant acyltransferase inhibition even if 2 mM acyl-SCoA or acetyl-SCoA were present. Thus, it seems possible that <u>in vivo</u>, PFDA inhibition of carnitine acyltransferases in rat liver might act to minimize acetyl and other acyl transfer across the mitochondrial membrane. This could diminish energy production and other important aspects of fatty acid metabolism/utilization necessary for normality. Even, if the puzzling, noncompetitive inhibition of octanoyl-ransfer we observed(Fig. 9) is the rule for longer acyl-SCoAs, its 76.0$\pm$28 μM $K_I$ would indicate that PFDA was a potent inhibitor of utilization of acyl-SCoA utilization and have similar results on fatty acid metabolism. Again it is unfortunate that PFDA-SCoA was not available for testing.

**VIII. RECOMMENDATIONS FOR ADDITIONAL STUDY** -This assumes funding of a RIP Grant.

1. Briefly examine the effect of PFDA-SCoA as an inhibitor of the 3 enzymes already tested. This will determine whether PFDA-SCoA is more

effective than PFDA.

2. Purify rat liver palmitoyl-SCoA synthetase, palmitoyl-SCoA oxidase palmitoyl-SCoA dehydrogenase and carnitine acyltransferases by methods reported by other labs.

3. Test these purified enzymes to identify the inhibitory effects of PFDA and PFDA-SCoA.

4. Examine the ability of rat liver palmitoyl-SCoA synthetase to make PFDA-SCoA.

5. Examine, or plan to examine, other crucial enzymes of fatty acid metabolism in rat liver(e.g., palmitoyl-SCoA hydrolase, acyl desaturase) and how to use the group of 7 or more enzymes assess the toxicity of potentially hazardous chemicals(e.g., CTFE) minimizing in vivo studies.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

GRADUATE STUDENT RESEARCH PROGRAM

Sponsored by the

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Conducted by the

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FINAL REPORT

# Perfluorodecanoic Acid Efflux from Cultured Primary Rat Hepatocytes


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| Date: | 29 July 88 |
| Contract No: | F49620-87-R-0004 |

# Perfluorodecanoic Acid Efflux from Cultured Primary Rat Hepatocytes

by

Andrew P. Whipple

## ABSTRACT

The interaction of perfluorodecanoic acid (PFDA) with cultured rat hepatocytes is very dynamic, with both uptake and efflux of PFDA occurring very rapidly. Just as earlier studies demonstrated nearly maximal uptake levels within one hour, and no significant increase in cell-associated PFDA over 24 hours, so is the rate of efflux of PFDA from rat hepatocytes cultured on collagen-coated dishes rapid. Regardless of whether the cells were loaded with $^{14}C$-labeled PFDA for 4 hours or 24 hours, when the cultures were switched to media without labeled PFDA a dramatic drop in cell-associated $^{14}C$ occurred. The loss of this PFDA from the cells was the same regardless of whether or not unlabeled PFDA was present in the medium. About half was lost in the first five minutes and by 40 minutes less than 5% of the $^{14}C$-PFDA was still cell-associated. By 4 hours less than 2% reamined and by 24 hours only about 1%, indistinguishable from background levels.

## Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this program, and Universal Energy Systems for their administrative leadership of this program.

I am grateful to Dr. Melvin Andersen for inviting me back to partake in in vitro toxicological research at the Toxic Hazards Division. Many thank you's to Miss Marilyn George who kept encouraging me as things fell apart time and again, and for explaining the PFDA story. Thanks to Nick Del Raso and Steve Godin for patient teaching, insights, discusion, and a most entertaining summer

## I. INTRODUCTION:

Perfluorodecanoic acid (PFDA) is a ten-carbon straight-chain perfluorinated fatty acid initially thought to exhibit little toxicity. The use of perfluorinated organics in ways leading to the exposure of personnel, such as in aqueous film-forming foams as fire extinguishants or possibly as vascular fluid replacement, demands an assessment of the toxicity of substances such as PFDA. PFDA may also serve as a model for the toxicity of other chemicals such as chlorotrifluoroethylene.

The Biochemistry Branch of the Toxic Hazards Division of the Air Force Aerospace Medical Research Laboratory at Wright-Patterson Air Force Base is actively involved in such an evaluation of the toxicological properties of PFDA and in building toxicokinetic models for this and other compounds. Whole animal studies have been conducted on rats, mice, guinea pigs, and hamsters in which the pathological effects on diverse body tissues, differential species morbidity and mortality, and alterations in lipid metabolism have been determined (1). The results of these studies suggest acentral role for the liver in the toxicity of PFDA and in the animals' response to this chemical insult via cellular and metabolic alterations (2).

In vitro toxicological studies have several advantages over in vivo (whole animal) studies. In vitro systems are less complex and more amenable to experimental manipulation than are intact animals. The amount of tissue is smaller and hence the amounts of intoxicants and of other materials such as radiotracers that are required are smaller and more easily contained than in whole animal systems. Disadvantages of in vitro work include the necessarily artificial setting in which isolated organs and tissues are maintained apart from the normal homeostatic mechanisms of the body, and hence the need for specialized equipment and for personnel with specific training and experience in the art of cell and tissue culture.

My research interests and experience are in the area of cell biology and especially in utilizing cell culture methodology to study cellular and metabolic events in response to alterations in the external environment. This matches well with the Toxic Hazards Division's recent move into utilizing in vitro techniques in addition to whole animal studies in its assessment of the toxicology of PFDA and other chemicals. My experience in manipulating cells and biochemicals and in studying their interactions allowed me to readily and fairly independently study the interaction of PFDA with two different cell types last year, and my experience working one summer with the Toxic Hazards Division has prepared me well for this summer's research activities.

II. OBJECTIVES OF THE RESEARCH EFFORT:

The first objective was to become proficient in the isolation of hepatocytes from perfused rat livers and in the initiation of cultures of these hepatocytes. The second objective was to obtain kinetic data on the rate of target cell uptake and efflux of radiolabeled PFDA and is an extension of last year's work. The third objective was to determine the intracellular distribution of radiolabeled PFDA in the target cells, primary rat hepatocytes, over time under uptake and efflux conditions in order to understand how hepatocytes handle the PFDA internally. Additional ancillary technical objectives were also a part of this summer's research activities and will be discussed.

III. a. Developing a proficiency in the techniques of rat liver perfusion and hepatocyte isolation and culture is simply a matter of doing same with the instruction and patient assistance of those already adept. Since I already am well trained in cell culture techniques and did culture hepatocytes last year the emphasis was on hepatocyte isolation and rat liver perfusion.

b. During the course of this summer I took part in about ten rat liver perfusions and performed every role several times, from injecting the anesthesia to teasing the hepatocytes free of the digested liver.

I also set up and took down the equipment and feel confident that I can set up a similar operation at my home institution. The isolation of the hepatocytes is a less complex procedure involving several centrifugations including a density-dependent centrifugation through Percoll, and I have performed all of these steps as well. I do think I am ready to start with a rat and successfully end up with primary hepatocyte cultures.

IV. a. Kinetic data on uptake and efflux were obtained in preliminary experiments designed to debug the system (not shown) and then experiments on PFDA efflux were performed as described in the legend to figure 1.

b. Hepatocytes were loaded with $^{14}$C-PFDA for 4 hours or 24 hours, and efflux was performed in a vast excess of $^{14}$C-PFDA free medium with or without 10 ug/ml cold PFDA (figure 1). Under all these conditions identical efflux curves were obtained (each data point is the mean $\pm$ 1 SD of 3 values). Efflux is quite rapid with about half the PFDA gone at 5 minutes and less than 5% remaining at 1 hour. By 24 hours only about 1% of the PFDA is still associated with the cells. A small amount of PFDA is present in the wells with no cells, constituting a small background relative to the cell-associated PFDA at early time points. By 4 and 24 hours of efflux the difference between cell-associated and background PFDA is very slight indicating that most of the cell-associated PFDA has exited the cells at these times. These results in combination with those for uptake obtained last year (5) demonstrate that the flow of PFDA in and out of cultured hepatocytes is very dynamic. Taken alone these efflux data suggest that only a very small amount of PFDA becomes stably associated with hepatocytes and that therefore only very small amounts are actually involved in PFDA toxicity. Hepatocytes do preferentially retain PFDA compared to other cells as determined both in vivo and in vitro even though the actual amounts retained may be quite small.

V. a. The determination of the intracellular distribution of PFDA was not attempted due to lack of time.

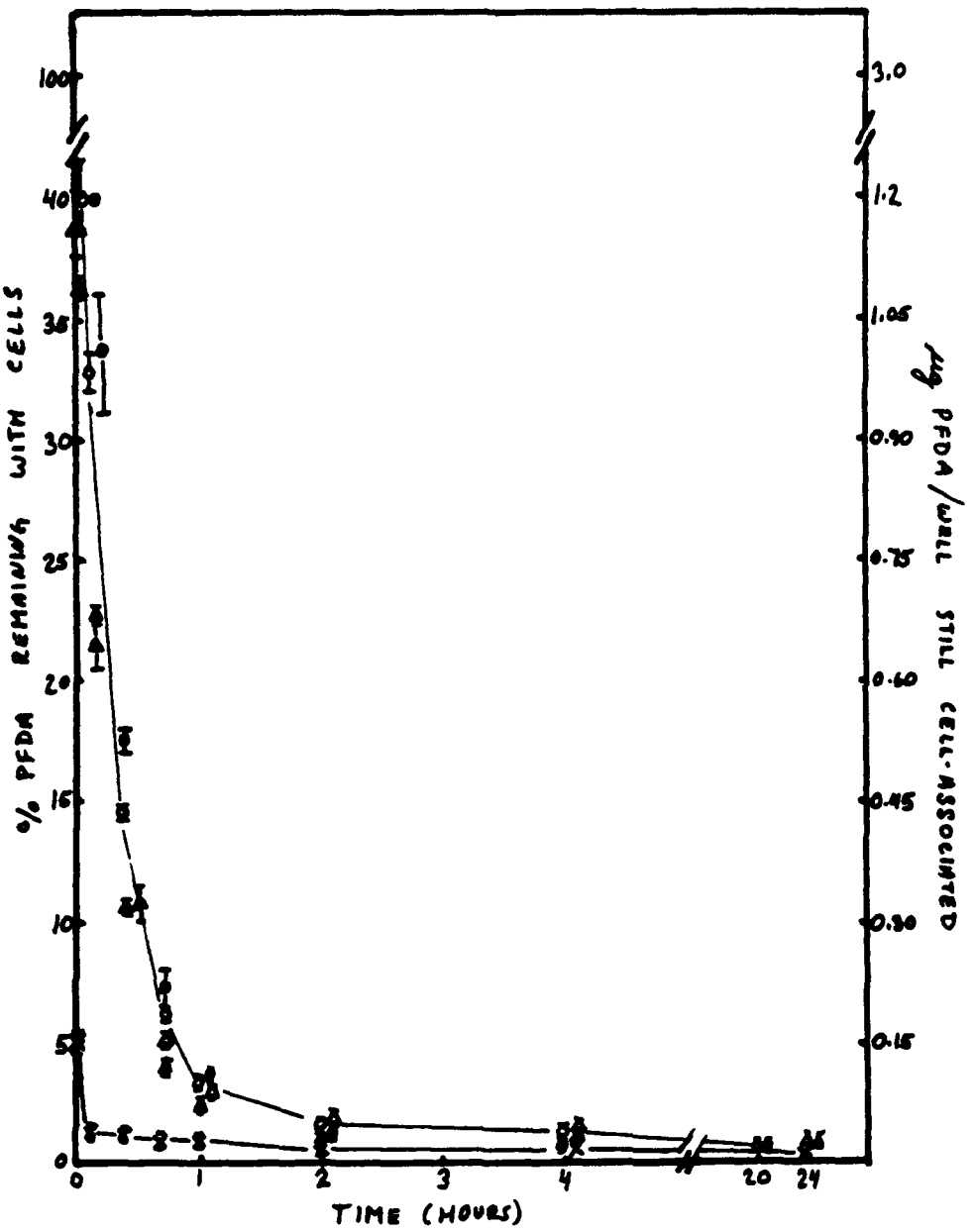



Figure 1. Efflux of $^{14}$C-PFDA from hepatocytes attached to collagen-coated multiwell plates. 0.3 ml of 10 ug/ml PFDA was added to each well to load the cells with PFDA for 4 hours (○,●) or 24 hours (△,▲) replaced with 2.0 ml/well of medium with 10 ug/ml unlabeled PFDA (○,△) or with 2.0 ml/well of PFDA-free medium (●,▲), and then the 2.0 ml/well removed at the times indicated and the attached cells harvested for counting. One set of wells without cells (x) was loaded for 18 hours and then processed as the others to determine non-specific PFDA association with the wells.

VI. a. The substantial effort in isolating viable hepatocytes is so successful, and the number of hepatocytes utilized in in vitro studies is so small, that many more cells are available from one liver perfusion than can be immediately used. Since cellular viability drops significantly within hours when the cells are kept in suspension, and since viability and metabolic integrity decreases significantly in a few days when the cells are cultured in collagen-coated dishes, there is a need to preserve the viability and integrity of the cells once isolated so all the isolated cells may be utilized. One approach is to alter culture conditions so that functional hepatocytes continue in culture for weeks rather than days, and another approach is to freeze the freshly isolated cells and then thaw them and initiate cultures at convenient times later on. In this study the latter was attempted.

b. Freshly isolated hepatocytes were resuspended at $10^6$ cells/ml in medium L15B supplemented with 10% fetal bovine serum and 10% dimethylsulfoxide and one ml aliquots placed into freezing vials. In lieu of a computer-controlled cooler used successfully for freezing hepatocytes ( 3) the vials were placed in the refrigerator for one hour, then the -20°C freezer for one hour, then the -40°C freezer for one hour, and then immersed in liquid nitrogen, a process which can slowly cool cells with retention of viability. After storage in liquid nitrogen for 24 hours vials were quickly thawed in a 37°C water bath and placed in 3 ml of medium L15B in collagen-coated 60mm dishes. The medium was supplemented with dexamethasone to enhance attachment. After 4 hours very few if any cells had attached or appeared viable by trypan blue exclusion. Since this was attempted with cells from PFDA-treated rats which were probably already weakened, greater success might be obtained using cells from untreated rats.

VII. a. Given that PFDA exerts toxic effects on the liver, and on hepatocytes, it becomes of interest not only to assess the nature of the association of PFDA with the liver and with isolated hepatocytes, but also to determine the kinetics of and nature of the PFDA association with different subcellular fractions of hepatocytes. This requires a

means of homogenizing cells rapidly and without damage to the nuclei followed by fractionation of the resulting homogenate. Since the association of PFDA with hepatocytes is so dynamic, with uptake and efflux occurring quite rapidly, it is particularly desireable that a means be found to rapidly yet gently disrupt the cells for centrifugal fractionation. Three methods were planned: sonication, Dounce homogenization, and a motor-driven Teflon pestle in a glass mortar (Potter-Elvehjem type homogenizer). In all cases isolated hepatocytes were suspended in 0.25M sucrose in pH 8.1 tris-acetate buffer at $10^6$ cells/ml for homogenization, and the homogenizations were performed on ice.

b. Sonication gave results best characterized as all-or-none. Varying the time from 5 to 10 seconds and the percent of full power from the lowest (20%) to 100% either gave suspensions without visible signs of disruption as viewed with phase contrast or Nomarski differential interference optics, or homogenates with few if any intact nuclei. Dounce homogenization of 10 ml of cell suspension was too gentle. The loose fitting pestle was without effect, and the tight fitting pestle, even when used in a very vigorous fashion for up to 50 strokes only broke open 50 - 80% of the cells and then only with more than 20 strokes. Nuclei did appear to be intact after Dounce homogenization. Attempts to weaken cells by first sonicating without disruption, followed by Dounce homogenization, did not improve the yield beyond that obtained through using the Dounce homogenizer alone. Due to a lack of adequate hepatocytes on two occaisions and at last to a lack of time, isolated hepatocytes were not subjected to homogenization in the motor-driven Teflon pestle / glass mortar homogenizer. It is puzzling and unfortunate that the Dounce homogenization did not work since this method was devised in order to maintain nuclear integrity.

VIII. a. Cell harvesting for counting cell-associated radioactive PFDA must be complete in order to obtain relaible results. Scraoing with a rubber policeman might work well for dishes but is inapprpriate for the much smaller weels of a 24 well multi-well plate. In current protocols the cells to be harvested remain attached to collagen-coated wells

without medium for up to 24 hours. This dessication renders the cells less easily removed by collagenase, as found in last year's study. Therefore another enzymatic preparation, a neutral protease mixture (Dispase) was tried as well as extended collagenase digestion.

b. Enough Dispase or collagensae (0.25 - 0.30 ml) to cover the bottom of the well was aliquoted per well and then the plates were incubated at 37°C. There seemed to be no difference in the efficacy of the two enzymes in releasing the cells from the collagen-coated plates. In cultures which were covered with medium up until the protease application, cells floated free within 30 minutes. In cultures which were dry for 24 hours no cells were released after 2 hours of protease treatment, although the monolayers could be removed after 30 - 60 minutes by scraping and pipetting with the plastic tip of the pipettor. Since collagenase at 600 mg/ml is cheaper, clearer, and contains less dissolved material than the Dispase solution, collagenase is preferable for this harvesting procedure where cell viability is not important. An incubation of 2 hours length followed by scraping and pipetting to release the hepatocytes has been incorporated into the standard protocol.

IX. a. In order to measure the radioactivity in cell suspensions harvested by protease treatment (see previous section), the suspensions were placed in combusto-cones (Packard), dried, and processed through the Packard tissue oxidizer. Although this device is designed to burn pieces of wet tissue repeatedly without becoming fouled and inoperational, the seemingly small amounts of cells/collagen/collagenase regularly befouled the oxidizer with soot and led to spec-check readings of 50% and lower. The following are empirically derived suggestions to lessen such problems.

b. First, use combustaid at 0.1 ml/cone. This cured the problem almost entirely, at least for several hours continuous operation while burning more than 100 samples. As per operating instructions the combustaid was added immediately prior to burning and as a result little soot appeared in the plastic lines of the oxidizer or in the wash/waste water. Second, carefully watch each burn itself, the bubbling of the waste container, and the accumulation of soot in the lines and in the waste

water. There should be little soot and lots of bubbles. If the bubbling seems weak, flush the long line on the right with water and check the pressure of this line and of the waste line. They should both be steady and at about 19 PSI (actuallly about 13 PSI but our pressure gauage is inaccurate). Third, periodically clean the lines with a pipecleaner wherever soot has collected - do this every 12 samples or so). Also clean the metal tubes leading from the burning chamber and to the waste vial. Once a day flush the condenser with methanol until satisfied that it is clean.

X. Recommendations. The following section will start with a consideration of possible research directly tied to the work of this summer and then will broaden into activites involving both cultured hepatocytes and in vivo studies. Some possible studies mentioned are already planned or underway and are placed in this report not to establish their origin with this author but rather to provide a context in which other related ideas may be developed. View this section as a literature review and idea source.

1. What is the nature of the interaction of PFDA with isolated rat hepatocytes? This is simply a recommmendation to continue studying the uptake, efflux, and subcellular distribution of $^{14}C$-labeled PFDA by cultured hepatocytes. Since transport mechanisms in isolated hepatocytes, the perfused liver, and the liver in vivo are experimentally equivalent ( ), this is a valid approach to understanding some aspects of the PFDA-liver interaction.

   a. Several recommendations regarding uptake and efflux studies are in my final report from last year (5 ).
   b. Since other substances are known to affect the binding of free fatty acids (FFA) to albumen ( 6 ), and since PFDA avidly binds to albumen which then affects its interaction with cells, it would be instructive to determine what effects PFDA has on albumen-FFA binding, and vice-versa.

c. Hepatocytes exposed in vivo to PFDA could be isolated and their PFDA handling characteristics compared to those of cells from unexposed animals. Does in vivo exposure to PFDA alter uptake/efflux charcteristics of PFDA measured in vitro?

d. The subcellular distribution of PFDA and its metabolites is as yet unknown. Studies on the subcellular distribution and metabolism of IgA ( 7), PCBs (8 ). astemizole (9 ), and plutonium (10) suggest such studies are feasible given successful cell fractionation (11).

2. How is PFDA metabolized by hepatocytes? An understanding of PFDA PFDA metabolism by hepatocytes would include identifying and quantifying metabolites, determining what enzymes of lipid metabolism are affected by PFDA and its metabolites, and assessing whether the synthesis of any of these enzymes is inhibited or stimulated by PFDA treatment. Since these studies have been planned by others or are already underway, there are only a few additional recommendations or suggestions.

a. PFDA might serve as essentially non-metabolized FFA analog for studies of FFA uptake and intracellular transport as well as other aspects of lipid metabolism.

b. Due to its relative metabolic inertness and its long-term retention in the body PFDA might provide a very stable, chronic modifier of lipid metabolism potentially useful in both research and clinical settings.

3. Is there a role for fatty acid binding proteins (FABP) in handling PFDA? Just as albumen and other serum proteins are involved in the transport and sequestration of FFA in the blood, several cellular proteins which preferentially bind FFA might be involved in the uptake (12), intracellular transport (13), and metabolism (14) of PFDA. Although the cytosolic FABP (15) and membrane FABP (12) of rat hepatocytes preferentially binds long-chain fatty acids, the observation that PFDA affects FFA metabolism by the liver (16) indicates that this could be a site of PFDA action.

a. Rat hepatocyte cytosolic FABP is increased by the following: starvation (17), clofibrate (17), and chemical carinogenesis by 2-acetylaminofluorine (18). Does PFDA cause increases in FABP?

b. Upon starvation the 14K FABP is converted to a 400K form (19). Does this occur with PFDA?

c. An assay for protein-bound versus free fatty acids using Lipidex 1000 (20) might be useful in these studies.

d. Aside from liver the other main target of PFDA is thought to be the kidney. There is a kidney FABP (21) so these studies could be extended to renal cells.

4. How is PFDA toxicity associated with peroxisome proliferation? PFDA induces peroxisome proliferation in rat liver in vivo (22,23) and in vitro (24). Other treatments that induce peroxisome proliferation include starvation, hypolipidemic agents, and high dietary fat (25). The common ground to all of these appears to be elevated cellular lipid levels which then in some manner stimulate peroxisome proliferation.

a. Specfic peroxisomal enzymes are increased as peroxisome proliferation is stimulated ( 26 ). Are these or other peroxisomal enzymes induced by PFDA?

b. Peroxisome proliferators increase the amount of FABP (27) and of FABP mRNA (28). Does PFDA cause such increases?

c. Inhibition of carnitine acetyl transferase blocks the benzafibrate induced peroxisome proliferation response (29). Will such inhibition block PFDA-induced peroxisome proliferation?

5. Are any aspects of PFDA toxicity, especially anorexia, weight loss, and loss of body fat and protein, mediated by the cytokine cachectin? Cachectin is known to mediate cachexia in certain parasitic and malignant diseases (30) and many aspects of this state are are similar to the condition in animals dosed with PFDA. It is possible that specific hepatotoxic effects of PFDA require only very low levels of hepatic PFDA and that many aspects pf PFDA toxicity result from a generalized systemic toxicity as excess PFDA spills over from the liver to other organs. PFDA-induced cachexia could be achieved by low levels of PFDA specifically altering liver lipid metabolism in such a way as to feedback and block further input of dietary lipid (that is, the continued presence of PFDA

sends the metabolic message that excess/saturating levels of FFAs are present). Alternatively PFDA cachexia might originate in several different organs but only in the presence of higher PFDA concentrations.

a. Dose animals with lower PFDA amounts to attempt a distinction between specific (low PFDA levels) and generalized (high PFDA levels spilling over into other organs) PFDA toxicity. This is in the context of cachexia and cachectin but could be used with other toxicologic parameters of interest.
b. Lipoprotein lipase is suppressed by cachectin in adipocytes (31). Does PFDA have this effect?
c. Cachectin alters gene expression by decreasing the expression of lipogenic genes in adipocytes (32). Does PFDA have this effect?
d. Hepatic acute phase gene expression is regulated by cachectin (33). Does PFDA have this effect?

6. How can hepatocyte cultures be maintained with greater hepatocyte integrity for greater lengths of time? This need was discussed earlier in this report; following are suggestions to achieve better hepatocyte culture.

a. Several suggestions including altering media composition, culture substrate and gasoeous environment were presented in last year's final report ( 5).
b. Sustitute ornithine for arginine in the medium. This prevents the overgrowth of hepatic parenchymal cells by other cell types and could be useful if not essential for long-term cultures(34).
c. Decrease or delete cystine in the medium in order to maintain P-450 levels (35). Other media formulations have also been reported to enhance P-450 maintenance (36).
d. Autophagocytic protein degradation is a significant problem in hepatocyte culture (37). Media designed to overcome this focus on amino acid supplementation (38).

7. Additional comments.

   a. Many of these suggestions require the use of $^{14}$C-PFDA which is in short supply. Careful consideration of its use will dictate what might be done.

   b. Several of the suggestions deal with the possible induction or repression of specific protein synthesis. In order to avoid an unfocused effort at establishing a role for alteration of gene expression as a component of PFDA toxicity the following are offered:

      1. Known proteins with established molecular weights (and possibly known isoelectric points) which might well be affected include a) peroxisomal enzymes, b) fatty acid binding proteins (both cytosolic and membrane), c)acute phase response proteins, d) enzymes of lipid metabolism. These are referred to elsewhere in this report.

      2. Utilization of antibodies to immunoprecipitate from a cell homogenate proteins of interest. This, combined with a radiolabel for de novo protein synthesis, could very nicely demonstrate effects of PFDA on protein synthesis.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/
GRADUATE STUDENT SUMMER SUPPORT PROGRAM

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FINAL REPORT

# DETERMINATION OF PERFLUORO-N-DECANOIC ACID TOXICITY IN VITRO AND IN VIVO VIA TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS


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| Date: | 29 July 88 |
| Contract No.: | F49620-87-R-0004 |

# DETERMINATION OF PERFLUORO-N-DECANOIC ACID TOXICITY IN VITRO AND IN VIVO VIA TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS

by

Frank A. Witzmann, Ph.D.

## ABSTRACT

Preliminary studies were conducted to assess the effect of perfluoro-n-decanoic acid (PFDA) on the two-dimensional protein pattern of cultured human peripheral lymphocytes and their spent media as well as rat liver cell fractions (cytosol, microsomes, and mitochondria) exposed to PFDA in vivo. Previous investigations of PFDA toxicity, which closely resembles that of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin), have been directed primarily at the liver though numerous other organs are PFDA targets. Among these is the thymus where PFDA-induced atrophy suggests immunodepressant activity. Despite such studies, specific PFDA effects on lymphocytes have not been well characterized. In the present studies, sample proteins from in vitro and in vivo experiments were separated by 2D-PAGE and visualized by polychromatic silver and Coomassie blue staining procedures. In the in vitro studies, various cellular proteins were altered as a result of PFDA exposure such that expression of specific proteins was either enhanced, diminished, or abolished entirely. Proteins of presumable cellular origin appeared in the spent culture medium as a result of PFDA exposure despite little or no in vitro cell lysis. Similarly, proteins from liver cells exposed to PFDA in vivo demonstrated several significant alterations. Foremost among the three fractions studied were protein additions and deletions in the microsomal fraction. While identification of the effected proteins awaits further study, these results lend support to the notion that in vitro toxicity testing methods may serve as viable adjuncts to traditional systems with the inclusion of two-dimensional polyacrylamide gel electrophoresis.

## ACKNOWLEDGEMENTS

I am most grateful to Dr. Sheldon A. London for his cooperation and collegial spirit during the tenure of this fellowship. His participation was both instructive and pleasureable. I am also indebted to the assistance of Dr. Shankar Bale who, in large measure, prepared the lymphocyte samples for culture and and whose wit, humor, and culinary expertise made this a gratifying experience.

I also gratefully acknowledge the contributions of Ms. Marilyn George, Mr. Nick DelRaso, and Dr. Steve Godin who prepared the liver cell fractions and isolated hepatocytes for the 2D studies.

Finally,I acknowledge the sponsorship of the United States Air Force Systems Command, the Air Force Office of Scientific Research, and the Toxic Hazards Division, Biochemistry Branch of the Air Force Aerospace Medical Research Laboratory.

## I. INTRODUCTION

The investigation of fluorocarbon toxicity has been of principal interest to the Aerospace Medical Research Laboratory's Toxic Hazards Division for nearly a decade. Previously, the toxicity of fluorocarbons had been thought to be negligible. The finding that the medium chain perfluorinated fatty acid, perfluoro-n-decanoic acid (PFDA), was not only toxic to rodents but closely resembled dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in its effects stimulated greater interest in this specific compound.

Since this original observation (1), numerous studies have characterized the systemic effects and in some cases the cellular effects of PFDA exposure in rodents. It is clear that PFDA exposure near the LD50 of 41 mg/kg in rats results in acute anorexia and weight loss, thymic and testicular atrophy, bone marrow depression, extreme hepatomegaly, loss of normal hepatic architecture (1,15), and peroxisome proliferation (12,13). Furthermore, a significant alteration in hepatic lipid metabolism has been indicated by qualitative and quantitative alterations in hepatic fatty acid composition and cholesterol levels (10,15).

In an effort to develop a new approach toward better understanding of PFDA toxicity, a year ago this author investigated the influence of PFDA on L5178Y mouse lymphoma cells in vitro (18). This study not only confirmed previous observations (14) but demonstrated that PFDA cytotoxicity was associated with altered expression of both intra- and extracellular protein species. While these results are enlightening, their extrapolation to human cytoxicity is of marginal value.

The present SFRP Project was thus designed to expose human peripheral lymphocytes to PFDA in vitro to determine not only the nature of its toxicity in human cells but also to verify its

potential immunosuppressant capability. In light of the previous work done on the rat liver, it was also the intention of this investigator to determine the extent of protein pattern alteration in liver cells exposed to PFDA in vivo. As in last year's study (18), qualitative and quantitative alterations in cellular protein pattern were designated as the primary indicators of toxic effect. As will be described, protein patterns were generated by two-dimensional polyacrylamide gel electrophoresis, a novel procedure which allows the separation of complex mixtures of proteins with high resolution and reproducibility. The method is characterized by the electrophoretic separation of proteins first on the basis of their individual charge characteristics (isoelectric focusing) in one dimension and then on the basis of their molecular weight (sodium dodecyl sulfate electrophoresis) in the second dimension. The implementation of this procedure results in distinctive patterns of protein spots which are visualized by sensitive staining procedures and compared within a large number of experimental conditions. Above all, this methodology is particularly suitable for toxicological studies in which one can expect alterations in regulatory cellular metabolic activities and/or architectural features, both of which are governed by the cell's protein complement.

## II. OBJECTIVES

### A. Preliminary Objectives.

1. One phase of the summer project related to the preliminary generation of protein patterns from rat hepatocytes and cell fractions from liver homogenates and the determination of in vivo PFDA exposure on those patterns. It was anticipated that 2D gels would be obtained from groups representing a variety of experimental and procedural conditions. These efforts were

intended to provide intial baseline observations regarding PFDA's in vivo effects on the rat liver.

2. A second phase was to involve the investigation of the influence of PFDA on human peripheral lymphocytes in vitro. As a result of the experimentation, 2D-PAGE electrophoretograms were to be generated, containing protein mixtures from either cell lysates or their corresponding spent culture medium. It was anticipated that alterations in the protein patterns so generated would significantly add to our current understanding of PFDA's toxic mechanism.

## III. APPROACH

A. Liver Sample Preparation. Rats used in this portion of the study were injected ip with either 20 or 50 mg PFDA/kg body weight. Twenty-four hours later, pair-fed control rats were injected with the 50% propylene glycol vehicle. Eight days after the PFDA or vehicle injections the rats were killed and the livers removed. Each liver was weighed and homogenized in four volumes of 0.25 M sucrose containing 1 mM EDTA and 0.1% ethanol using a Polytron homogenizer. The homogenates were then fractionated by differential centrifugation (11) and those fractions containing either cytosol, mitochondria, or microsomes collected. These fractions were solubilized for 2D-PAGE by mixing an aliquot of each with an appropriate volume of lysis buffer (7) to give a final protein concentration of 2 mg/ml.

Hepatocytes from similarly treated rats were isolated by a liver perfusion procedure previously described (17) and solubilized to give a final protein concentration of 2-3 mg/ml. All protein concentration determinations were made prior to solubilization using a commercial Coomassie Blue G-250 protein assay (Pierce Chemical Co.).

B. Lymphocyte preparation. Blood samples were obtained by standard venopuncture procedures from donors who signed appropriate informed consent forms. Equal quantities of blood were mixed with Sepracell-MC separation medium and centrifuged for 20 minutes at 1500 X g (3000 rpm). The band of monocytic cells were removed from each tube and resuspended twice in 3-4 volumes phosphate buffered saline (PBS) containing 0.1% albumin Following these washes, the cells were counted via Cou counter and adjusted to the required concentration with RPMI-16 medium plus pyruvate and Nutridoma HU. After mixing, 2 X $10^5$ cells were added to each of 24 wells on a multi-well plate along with either 0,2,6, or 8 ug/mL PFDA. Quadruplicate cultures were then incubated for 24 hours at 37° C under 5% $CO_2$ atmosphere.

Incubated cells were pelleted by centrifugation and washed with 1 mL PBS prior to solubilization. As with the liver samples, lysis buffer was added to the cell pellets such that the final concentration was approximately 2.5 X $10^7$ cells/mL (2 mg protein/mL).

C. Spent medium sample preparation. After the initial postincubation centrifugation step by which cells were collected, the supernate was collected and frozen at -70° C. To concentrate the extremely small quantities of protein in the spent culture medium, the frozen medium was thawed and to it an aliquot of a 10% diatomaceous earth suspension was added to make a final diatomaceous earth concentration of 2%. Proteins were adsorbed to the diatomaceous earth over a two hour period with gentle rocking, the mixture centrifuged at 13,000 X g for 10 minutes, and the proteins solubilized with the addition of lysis buffer (100 uL/per original 1.5 mL medium)(8).

D. Electrophoresis. Sample proteins were separated electrophoretically using a modification of the ISO-DALT technique (2,3) whose technical aspects are described in detail (7) and briefly described here. Polyacrylamide tube gels (ISO gels) were

cast simultaneously in each of 20 ISO tubes (1.5 mm dia.) by hydrostatic displacement. Gels were prefocused for 1 hour at 200V, samples added (20-30 uL), and focused for 14,000 V·hr (700V for 20 hours). After extruding the ISO gels from their tubes they were equilibrated for 15 minutes in an SDS and bromophenol blue containing buffer, frozen at -70° C, and stored for later separation in the second dimension. DALT slab gels were prepared pouring a 12.5% acrylamide mixture into a multigel plate casting chamber (BIO-RAD) and polymerizing for one hour. Subsequently, each DALT slab gel was loaded with an ISO gel and placed in a BIO-RAD multi-cell slab gel apparatus. Six slab gels were then run at 150V at 4° C until the bromophenol blue tracking dye ran off the gel (approx. 18 hours).

E. Protein visualization. 2D gels containing liver cell fractions were stained by either Coomassie blue (7) or polychromatic silver stains (16) and in some cases duplicate gels were stained with each procedure. Gels containing lymphocyte and spent medium proteins were stained exclusively by silver stain. Once protein spots were obtained, comparisons of gels representing different experimental conditions were made by visual inspection. Permament records of stained gels were made by phtographing the gels on a fluoresecent light box with Panatomic-X black and white film and with Ektachrome color slide film. Black and white prints were made by conventional processing techniques while color prints on Cibachrome paper were made from the slides by a color contact printing procedure (9).

## IV. RESULTS AND DISCUSSION

A. Liver studies. 2D gels from fractionated whole liver homogenates that were stained with the GELCODE system (16) displayed hundreds of protein spots throughout the MW and pH ranges incorporated on the gels. The cytosol fraction was relatively free of background staining and smearing and demonstrated only a few significant pattern alterations with PFDA exposure. Crowding of protein spots representing those with MW of 25 kD and above in the upper 67% of the slab gel suggests the need for exponential gradient gels in future experiments.

Mitochondrial fractions exhibited significant background staining and smearing, a problem likely due to the presence of nucleic acids (7). This troublesome contamination, peculiar to silver staining, can easily be remedied by improved differential centrifugation methods and including a minimum 100,000 X g spin after protein solubilization with lysis buffer. Despite the shortcomings of these particular gels, PFDA exposure did alter the protein pattern, qualitatively, in the region where $b_5$ cytochrome is typically located.

The most numerous alterations in protein pattern with PFDA exposure were observed in the microsomal fraction where at least 20 single or multiple spots were clearly different from control patterns. Again, severe background staining and smearing was seen in this fraction, presumably due to the large concentration of mRNA associated with the endoplasmic reticulum. One protein believed to be induced by PFDA exposure in the microsomes was seen only in Coomassie blue stained gels of this fraction. We have tentatively identified it as an 80 kD peroxisome-associated enzyme, enoyl-CoA hydratase whose induction by PFDA was previously documented (13). Since the induction of other detoxifying enzyme systems is based in the endoplasmic reticulum of the hepatocyte, the microsomal fraction seems a likely candidate for the observed protein pattern alterations and certainly warrants further investigation.

As expected, isolated hepatocyte protein patterns demonstrated an extremely complicated pattern of protein spots which included all of the previously mentioned fractional observations. Previous 2D electrophoretic studies of hepatotoxicity using whole homogenates or hepatocytes employed Coomassie blue staining procedures (4,5,6). This is necessarily associated with a high degree of loss in sensitivity yet a clearer picture of the effects on the most prevalent protein species is accomplished. In future PFDA investigations, it would be desirable to stain one set of such 2D gels with the more sensitive silver stain and another more heavily loaded set with Coomassie blue.

B. Lymphocyte studies. Under the experimental conditions described, PFDA exposure of nonproliferating human peripheral lymphocytes in vitro demonstrated a lesser degree of alteration than proliferating mouse lymphoma cells studied previously (18). That is not to say that protein pattern alterations were absent from these gels. Both cell lysate gels and spent medium gels exhibited a broad range of quantitative changes in a number of proteins. Since it was beyond the scope of this study to identify specific protein spots, no such attempt will be made here. However, we are encouraged by these findings in such short-duration incubations which suggest immunosuppressant potential and plan to extend and amplify these results in nonproliferating lymphocytes and proliferating cells (those exposed to mitogens) as well.

## V. RECOMMENDATIONS

A. Liver studies. With respect to the procedural difficulties which arose during the project, future liver 2D-PAGE studies must include the following technical improvements:

1. Liver subcellular fractions and isolated hepatocytes (fractionated or not) must be centrifuged at at least 100,000 X g

after solubilization to remove nucleic acid especially, and other insoluble materials which render the polychromatically silver stained gels with high background and smearing. This will significantly enhance both visualization and interpretation of the protein patterns.

2. All liver samples must be prepared in two distinctive concentrations, one for silver staining and the other (100X) for Coomassie blue stain.

3. Liver homogenates must be fractionated by a technique which clearly separates microsomes from mitochondrial contamination and vice versa. Furthermore, isolation of a relatively pure peroxisomal fraction would be very desirable.

4. The capability to pour DALT slab gels with an exponential polyacrylamide concnetration gradients must be obtained to improve resolution of heavier MW protein species. This would also greatly enhance pattern interpretation and set the stage for image ananlysis of such highly resolved patterns.

5. Finally, it would be of significant interest and relevance to obtain liver cell fractions from dioxin exposed animals for comparison.

B. Lymphocyte studies. Future investigations of PFDA toxicity on human peripheral lymphocytes must include the following:

1. Due to the time constraints associated with a short-term summer project it was not possible to evaluate significant variations in the culture medium. In the one set of incubations that were carried out, PFDA had little effect on cell number after twenty-four hours, leading us to conclude that little or no cell lysis occurred. Furthermore, as 2D gels indicate, alterations in protein pattern were similarly modest. Whether this implies a lack of PFDA toxicity is not known. More studies must be carried out in a variety of media with a variety of additives.

2. Future lymphocyte studies must evaluate PFDA's influence on proliferating cells. To this end it is necessary to evaluate the suitability of various mitogens alone and in combination with PFDA exposure.

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FINAL REPORT


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| Contract No: | F49620-87-R-0004 |

# Hypertext and Intelligent Interfaces for Text Retrieval

by

Patricia Ann Carlson

## ABSTRACT

The Integrated Maintenance Information System (IMIS) concept is to provide the technician with all logistical, operational, technical, training, and diagnostic information for aircraft repair. Because of the sheer amount of information being integrated, user overload is a significant concern. The traditional solution to this problem of presenting complex information in a timely fashion is to design a consistent display format and to employ standard commands. At a deeper level, however, questions of information integration become issues of information engineering and the nature of knowledge structures. At this level, the definition of user interface takes on a more sophisticated meaning.

The hypertext concept considers a body of knowledge as a database -- potentially a highly organized, compressed structure of richly interconnected "chunks" -- and allows for flexible indexing and retrieval by implementing a "smart" interface (a programmable "idea processing" mechanism). Hypertext, as the backbone for development philosophy, permits advanced design features -- such as enhanced functionality, customized views, and improved knowledge synthesis and representation -- which, in turn, increase the user's ability to interact productively with information.

Any successful electronic publishing system must provide access to text and graphics in a timely and meaningful fashion. This study identifies three categories of retrieval facilities and considers their efficacy when combined with hypertext. Because of its structure and connectivity, a hypergraph (hypertext web) incorporates intelligence, perhaps more than is possible with most current, static database storage representations. The purpose of this study is to suggest retrieval facilities that will squeeze as much functionality out of the hypergraph as possible. The end results of the investigation also include four "toy-world" interfaces built to demonstrate the characteristics of information retrieval using a hypertext database.

Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Also, I wish to thank the Human Resources Laboratory personnel at Wright-Patterson AFB (under the command of Colonel Donald C. Tetmeyer) for a rewarding and professionally enriching summer. In particular, I wish to acknowledge Mr. Bertram Cream (Technical Director of the Logistics and Human Factors Division) for serving as my Effort Focal Point.

Many people helped to make my experience both pleasant and challenging. David Gunning suggested the topic of "hypertext" and supplied the initial reading and bibliography. Robert Johnson (LRC Branch Chief) provided ideas for focusing the topic. Donald Thomas (Senior Scientist) provided help with the specifics of the project as well as guidance for the overall approach. To all these people, I express my gratitude for encouragement and support. Additionally, I express my appreciation to all personnel in Building 190 for their willingness to help in a variety of ways and for making my stay at Wright-Patterson both pleasant and productive.

## I. INTRODUCTION:

The Air Force Human Resources Laboratory (AFHRL), Logistics and Human Factors Division is working on a prototype information management system for aircraft maintenance. Proof of concept, development, and implementation involve parallel research on several software and hardware projects. A partially ruggedized, portable computer/display unit (PCMAS), an authoring system for creating online technical orders (APS), and an automated fault isolation system for troubleshooting (MDAS) are examples of issues in hardware design, software tools, and AI applications currently being examined.

The functionality envisioned for the full IMIS implementation is summarized in the following excerpt:

> The system will display graphic technical instructions, provide intelligent diagnostic advice, provide aircraft battle damage assessment aids, analyze in-flight performance and failure data, analyze aircraft historical data, and access and interrogate on-board built-in-test capabilities. It will also provide the technician with easy, efficient methods to receive work orders, report maintenance actions, order parts from supply, and complete computer-aided training lessons and simulations. The portable computer will make it possible to present quality information by taking advantage of the computer's ability to interact with, and tailor information to, technicians with varying levels of expertise.
>
> "IMIS: Integrated Maintenance Information System -- Maintenance Information Delivery Concept"

For the past few years, I have focused my research on methods to improve technical documentation. I have been particularly interested in document design -- a discipline concerned with the substance, structure, syntax, and style of text written for a specific function.

In the past, I have done research projects in document design under the NASA/ASEE (American Association for Engineering Education) Summer Faculty Research Program. Much of my work has been in the area of system design for online technical and instructional data. Representative areas of my research include:

- Screen format for online text -- including issues of menu design, error messages, and data entry.
- User support requirements in large, complex computing facilities.
- Rhetoric and software problems in the design of a "dynamic" book.

- o Modeling user interaction with text under a variety of conditions and constraints.

I was a participant in the 1987 Air Force Summer Faculty Research Program, assigned to the Human Resource Laboratory at Wright-Patterson AFB. My project was to examine and report on the benefits of using hypertext (non-linear text storage and retrieval) as a design philosophy for IMIS.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

As they currently exist, TOs (Technical Orders) represent a paper textbase (usually multi-volumed, complex, and highly interconnected). Typically, a technician accesses the material in search of instructions to perform a specific maintenance or repair task. His/her success in finding the information in a timely fashion depends, in part, upon an understanding of the organization of the document(s) and on the amount of experience he/she has had with the system. In short, users of traditional TOs develop an expertise for finding information in the paper system. Encapsulating user expertise and providing ease-of-access to electronic information are priority items for IMIS development.

Traditionally, information retrieval systems have consisted of relatively structured databases (such as library card catalogs, legal citations, scientific or medical journal abstracts) which are searched by using keywords, inverted indexing, cross-referencing, string searches, and pattern matching. These methods can be refined through Boolean logic connections (AND, OR, NOT), by designating a proximity (a numerical boundary within which the combination of query terms must appear), and by indicating scope (the set of entries over which the search will take place). Clearly, for large, full-text databases blind search is not feasible. Currently, meaningfully narrowing the search may require the services of a skilled researcher, who -- in consultation with the end user -- formulates a search strategy based on preplanning, use of thesauri, and successive iterations.

Low-cost, high-capacity storage devices (particularly CD ROM) have increased interest in information retrieval (IR) and documents as databases or, more specifically, textbases. Vannevar Bush's 1945 call for automated, global libraries of text and graphics was refined by Alan Kay (1975) into the "dynabook." Today, various disciplines of information sciences have pushed these concepts into the realm of reality. Examples of large textbases now or soon-to-be available on CD ROM include the Oxford English Dictionary, Bowker's Books in Print, and Grolier's Encyclopedia. However, any electronic information delivery system

must be able to -- at the very minimum -- duplicate the capabilities provided by the combination of an experienced reader and a well-designed paper text. Anything less degrades the system: leaving at best, an electronic page-turner; at worse, even less than the paper version. Furthermore, in order to justify abandoning the conventional method and medium, an electronic system should offer improvements, such as increased flexibility, reduction in storage, and convenient document development/maintenance.

This study assumes that paper TOs will be automated for IMIS using the concept of hypertext. Documents stored in hypertext have active cross-references. Each entry in the textbase is called a node; the combination of the nodes and their connecting links form a hypertext network (hypergraph). Both nodes and links can be typed. Given these features of hypertext, online information can be characterized by structure and by connectivity. The focus of this study is to determine how the innate features of a hypertext can augment traditional search techniques (such as those used in Database Management Systems), as well as facilitate the integration of more advanced search procedures now being developed in Artificial Intelligence (AI) research. Figure 1 delineates the specific area of my interest -- the intersection of three research domains:

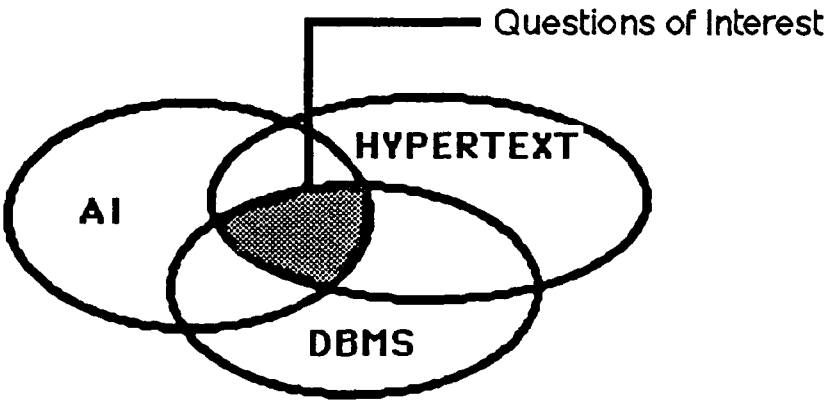



FIGURE 1: Study Domain

During the ten-week fellowship, I examined some of the more common search techniques available on an electronically supported textbase and wrote "toy world" demonstrations for a representative sample of "smart interfaces" to hypergraphs. Specifically, I used HyperCard (Apple Computer, Inc.) and KnowledgePro (Knowledge Garden, Inc.) software to develop four demonstrations. One of the demonstrations was implemented using the "Illustrated Parts Breakdown" segment of a TO ("Fire Protection System," USAF/EPAF Series F-16A and

F16-B). Others were based on the repair guide for a GM automobile. This switch in source documentation was made to accommodate the study's limited, ten-week timeframe. Most Air Force TOs run into the hundreds of pages, containing separate sections on checkout and analysis, theory, troubleshooting, schematics, and procedures. The automobile guide, on the other hand, is much shorter and is broken down into small, logical units. Therefore, its knowledge structure was more easily assessed; the segment selected (clutch repair) is self-contained; and finding experts and engineering a knowledge base for a car clutch was easier than finding experts for weapon systems.

## III. THE HYPERTEXT CONCEPT: FOUR CENTRAL ISSUES:

My project for the 1987 SFRP was to explore the benefits of using hypertext (non-sequential information storage and retrieval) as a design philosophy for IMIS. Since last summer, a significant hypertext community has emerged. The number of conferences and application products announced over the last few months reflects the intensity of industry and academic interest. However, the field is still new and lacks a history of experience and a systematic assessment. In fact, detractors point out that much of the interest in hypertext is on a theoretical level. They charge that few rigorously specified systems exist, and many of the commercial products which pose as hypertext are not (Raskin, p. 330). Since hypertext systems have a brief history of application, we have sparse evidence for their effectiveness, let alone proven principles to guide design.

Four major issues being debated in the literature are central to the implementation of a hypertext document database.

**Authoring versus Browsing:** A major debate in the literature concerns whether or not -- in order to be genuine -- a hypertext systems must support user-initiated links. Purists contend that unless the user can make bi-directional links -- or chart new paths through the information space -- the system is not truly hypertext. This camp sees hypertext not as a tool for information management, but as an environment for knowledge development and open-ended problem exploration. In their view, constructing the web -- or graph of links -- which conjoins the fragmented chunks represents learning, creativity, and/or collaboration. Viewing hypertext as a convenience for storing and accessing huge bodies of text, they charge, reduces the concept to database management system (DBMS) technology.

Both approaches share common goals of improved search and navigation; however, one is intended for accessing existing documents while the other is better suited for authoring new information structures. Clearly many applications of threaded text

fall somewhere between absolute freedom and rigorous structure, and thus could benefit from an interface which facilitates information retrieval. Useful (but relatively simple) automated features to help users traverse a body of information include string and keyword search capability, cross-referencing, and indexing. More powerful navigational devices -- whether we call them embedded expert systems, smart filters, or interactive interfaces -- can guide readers through a textbase and increase the user's ability to interact productively with information.

**Mental Model and Metaphor:** These two interrelated concepts are of great significance to system design. The goal of each is to accommodate the user's preconceptions and to increase the ease-of-use. Users normally approach a task with some concept of how to proceed. This model, or image, comes either from knowledge or experience with the task domain. When the domain is unknown to the user, the user may develop a mental model through an analogy with a familiar domain.

Proponents of hypertext contend that non-linear information processing mirrors two natural patterns of human information processing -- association networks and hierarchies. Furthermore, hypertext systems are more malleable and can mold themselves to user expectations more easily than paper or early electronic text storage formats. However, it is important to note that hypertext is not a form of artificial intelligence. AI tries to encapsulate human knowledge in a form which can then be used by a machine. Hypertext, on the other hand, is intended to augment human thinking by providing a dynamic platform for presenting data.

**Search and Navigation:** Just as any database, a hypertext information retrieval system has two central components:

- o a document database: which can be -- (1) a large, loosely structured library of documents which have been placed online directly from hardcopy, (2) a highly organized, compressed, structure of richly interconnected "chunks," which have meaningful boundaries between sets and subsets and logical relationships among elements, (3) a variation of organization somewhere between these two extremes.

- o an assistance processor: a retrieval mechanism (or a collection of retrieval mechanisms) for effective access to and management of the database.

As a rule-of-thumb, one can say that there is a reciprocity between the amount of design put into each element. Relatively unformatted libraries require more complicated software in the retrieval mechanisms or the assistance of a knowledgeable research specialists, if the user requires fine-grained applications. Text which has been specifically preprocessed for the system service requires less complicated software. A hypertext-flavored IMIS should be a symbiotic relationship between the intelligence built into the web and the capabilities of a smart interface.

**Data Preparation -- Translating Text in Hypertext:** The written word -- as it exists today -- has been molded to suit a paper delivery system. While in some applications, it may be feasible to construct text especially for a hypertext representation, most implementations will only be cost-effective if documents

authored for paper can be modified for a hypertext environment (Raymond and Tampa, p. 143). This translation process raises questions of rhetoric and knowledge structure.

These are not trivial problems. Extracting a workable hypertext representation from an existing document can be difficult. In most cases, the author(s) are not available for consultation, so answers to questions of meaning and form may have to be inferred from the paper representation. In many cases, form does not reflect logic because the requisites of paper and pen, or the press, exert powerful influences on text formatting. [For example, the original paper Oxford English Dictionary was restricted to a fixed number of pages by the publishing contract. Therefore, every effort was made to conserve space -- hence the dense typesetting, extensive use of abbreviations and symbols, and limited use of meaningful white space (Raymon and Tompa, 146 )].

Additionally, not all texts are suitable for hypertext representation. Hypertext makes the implicit knowledge structure of a text explicit. Although there is no rigorous model for hypertext implementation, experience suggests that if the document is closely interwoven through rhetorical devices, then decomposition into chunks and links will be difficult, with lose of information and confusion of meaning a result. For some documents, this conversion is either impossible or not desirable because is destroys the subtle interconnections of theme, argument, metaphor, and word choice.

## IV. HEURISTIC SEARCH TECHNIQUES:

Heuristic search methods -- long the purview of specialists in online services -- need to be automated and presented as a collection of information extraction tools. One impressive example of an intelligent interface to TO information is the MDAS (Maintenance Diagnostic Aiding System) project. A fully automated diagnostic system guides the technician to the most likely fault and then, on request, displays the appropriate rectification procedure (TO text). In a sense, the automated troubleshooting tree becomes a filter for the textbase.

However, this intelligent interface would be inadequate and perhaps even an impediment to accessing information if the user were motivated by a different information need -- say, for example, the need to use the TO for reference purposes only or to browse through the textbase while following a hunch based on past experience. Since there are different scenarios for information use patterns (see "Integrated Maintenance Information System: Training Technology Scenarios," AFHRL - TP- 87 - 18), different intelligent retrieval mechanisms should be developed to facilitate each situation.

Because brute force or blind searches through all nodes in a database produce a combinatorial explosion, almost all research into automated information extraction focuses on ways to more fruitfully direct the search algorithm. Attempts to produce a "superbook" have

merged the forces of IR, DBMS, and AI to focus on the single crucial issue of implementation: how to provide search procedures which are accurate, complete, and comfortable for the end user.

The remainder of this report suggests a series of "smart" interfaces for a hypertext-based IMIS. These examples are divided into three categories, based on relative degree of sophistication and the extent to which they approximate an expert system. Examples in the first category have their roots in traditional DBMS techniques. Examples in the second category draw heavily upon recent research in cognitive science. Examples from the third category model intelligence using AI practices.

All hypertext systems are characterized as a collection of nodes (modules) and links (webs), allowing for three-dimensional navigation through a body of data.

- Modules: pools of information collected in one anthology, labeled or typed, and electronically stored as nodes in a database.
- Webs: the pattern of links among the nodes. The links can be predefined by the hypertext system designer or the user(s) can establish the links as part of walking through the information space.

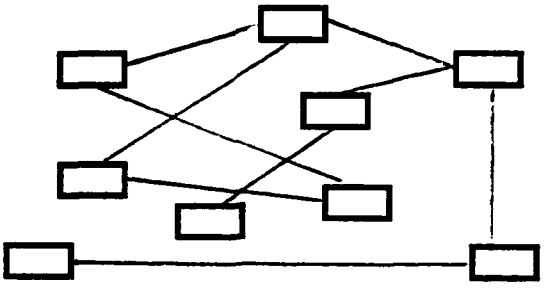


FIGURE 2: 3-D Information Processing

Since the intent of my research is to demonstrate how hypertext enhances retrieval mechanisms, each of the following three categories is illustrated by a variation of Figure 2, showing how the interface is grafted onto the concept of a hyper-web.

CATEGORY I: Until recently, large textual databases involved mainframes, magnetic storage, and complex user interfaces operated by trained researchers and skilled librarians. Because of the expense, such systems were found only in large institutions or time could be purchased through several online services. Typical retrieval devices in such systems are

based on keyword search techniques. Speed is enhanced through inverted indexing and query language which supports Boolean operations. Unfortunately, these methods do not produce ideal rates for completeness or accuracy. A typical query hits only about 50 percent of the relevant texts and finds about the same percentage of irrelevant texts (Colvin, p. 131).

Using an inquiry-based interface has been the dominant concept in IR since the 1950s. However, improvements to the query form have been made over time. Methods to improve completeness and accuracy include enhancements to the basic search algorithms of the system. Areas of intensive research today include natural language query, vector and probabilistic models, weighted keyword searches, and layered information structures (as represented by embedded menus). The latter two are of interest to this project.

> Weighted Keyword Search: Keyword search is known to be flawed. Imprecision and incompleteness can be attributed to choice of search terms by users, lack of semantic analysis of texts, and the fact that relevance is difficult to model as a yes/no choice. Weighted keyword search offers improvements. Coupling weighted keyword search with hypertext links can improve performance in two ways. First, if links joining related concepts are part of the system, finding only a subset of nodes through algorithmic search would suffice since the user could then follow links to other semantically related nodes. Second, because the links indicate other related nodes which were not part of the "hit" list, the user can overcome the false impression of completeness which is a danger of full-text search (Oren, pp. 297-298).
>
> Embedded Menus: The advantages of menu systems over command driven systems have been adequately documented. In general, menus reduce cognitive overhead by limiting the need to memorize commands, by reducing training time, and by providing a structure for decision making in a computer session.
>
> An alternative to explicit menus is to embed the menu choices within the information being displayed. With this approach, the user is less likely to be confused by menu labels because contextuality is not lost. The user is able to make an informed choice about whether s/he wishes to pursue a particular path. Embedded menus naturally lead to a kind of layered approach, where detail is hidden unless specifically requested. For a textbase, however, the danger is that the user is not given specific guidance as to which selections to pursue. This could be partially overcome by careful layering of the text, or by having only subsets of keywords highlighted during a given session. But these concepts require considerable time and skill in designing the knowledge structure of the textbase, and would prove inefficient in all but the smallest and perhaps simplest domains.

Figure 3 shows the paradigm for the search techniques of Category I. In essence, the user is guided toward a node or subset of nodes which, in turn, provide an entree for traversing the web in a meaningful fashion. Though slightly different in final implementation, each facilitates access to the database, thereby positioning the user to make use of the knowledge structure built into the hypergraph.

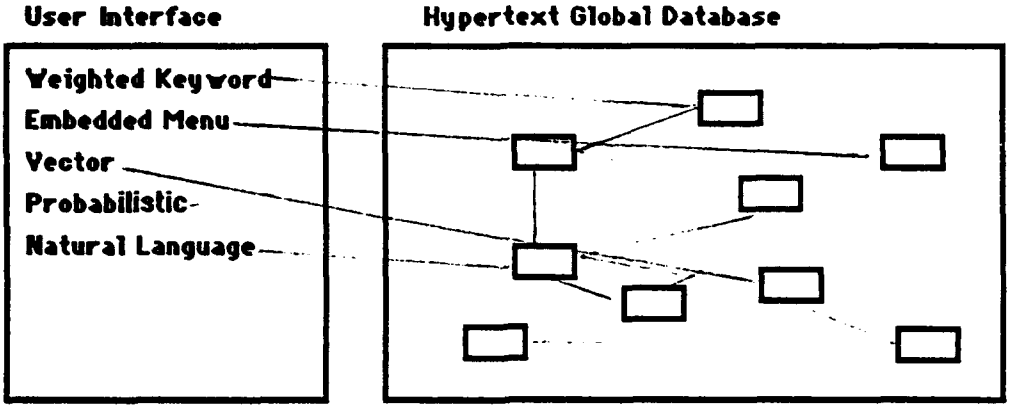



FIGURE 3: Category I Search Techniques

CATEGORY II: Category I search techniques help the user to enter the hypergraph at an appropriate point; after that, navigation is a function of the hyper-web rather than the interface. As such, they are analogous to indexing and cross referencing in conventional paper texts. Category II search techniques help the user to visualize the knowledge structure of the textbase; as such, they are analogous to various location signals in paper text (running headers and footers, chapter titles, section headings, type fonts, indentation, proximity, and physical features such as number of pages and binding).

Location is one of the most difficult problems in hypertext. Electronic presentation of text removes most of the location cues available in paper. Adding to the confusion, hypertext encourages the proliferation of small nodes. The result can create chaos for the end user. Adopting some form of visualization vastly improves system usability. Three candidate methods include (1) an iconic interface, (2) a filtered view based on one or more specific criteria, such as proximity, (3) a contiguity or logic map.

Spatial Data Management Systems (SDMS): User overload is a major human factors concern in any information integration system. Both common sense and scientific evidence supports the contention that visuo-spatial (e.g. use of icons) representation and retrieval are easier than symbolic (words and numbers), and the success of workplace metaphors in commercial systems warrants considering spatial management devices in the IMIS interface. Huge portions of our brains are dedicated to processing visual-spatial data. Additionally, people retain a cognitive image of information contained in a fixed area, for example in an office or in a book.

One "toy world" demonstration designed as part of this project is an automated Illustrated Parts Breakdown (IPB). In its paper form, the IPB consists of hierarchical graphics and alphanumeric tables which are keyed to the graphics by callouts. The tables contain the weapon system's model number, a vendor-supplied part number, the federal supply category number, a brief description, the number of parts per assembly, and the applicable tail numbers.

In the hypertext version, the user moves from a global view of the weapon system down levels of specificity by clicking on segments of the visual representation. An information entry screen asks the user to select (from visual representations) the part category, the weapon systems number, the tail number, and a more specific indication of location. From this information, the software generates a list of "hits." By clicking on an item in the list, the user goes to a close up graphic of the specific part, along with all stock information, and instructions for installation.

Advantages of such direct manipulation interfaces have been outline by Ben Shneiderman (1984). Succinctly, spatial management cuts down on cognitive overload because the user approaches the system with a familiar, high-level problem domain view. Computer dictated concepts (e.g. command language syntax) are minimized. Actions create immediate and comprehensible reactions. Stress is reduced, especially for individuals whose preferred method of working is visual/concrete rather than language/abstract.

Fisheye or Distorted View Filters: This approach -- as its name indicates -- is based on a metaphor. Researchers have noticed that humans represent information/knowledge using a prioritizing scheme. If this perceived relevance were represented visually, the picture would resemble the view from a wide angle lens. In other words, things of greater importance would be at the center, while items of lesser attention would be on the periphery -- and the image would become increasingly blurred as one moved away from the focal point.

George W. Furnas of Bell Communications Research has suggested an algorithm to simulate this purposeful distortion (Furnas, p. 17). The program generates a "neighborhood" image by computing a relationship between a priori importance of an element to a knowledge structure and the distance between that element and the current position in the body of information. Empirical evidence suggests that such filters are valuable information-handling aids. For example, holophrastic viewing techniques (or hiding designated levels) have been used to help debug long and complex segments of code (Koved and Shneiderman, p. 315).

Graphical Browsers: While direct manipulation tries to use a real world, physical model, graphic browsers may use a more synoptic approach. The idea of overview displays for bodies of information also appears in traditional text design, where the table of contents serves as a map for the organizational structure of a document. Graphic browsers are also visual methods of information representation and compression, and may resemble such devices as charts, diagrams, graphs, decision tables, and taxonomies.

Content-oriented search mechanisms receive much attention. Hypertext systems, because of the web-like structure of connectivity, lends itself to a less well-known search mechanism -- that of structure search. If both nodes and links are typed, user could ask for a diagram of all subnetworks that match a given pattern.

The three representatives illustrated in Figure 4 demonstrate a central feature of Category II interfaces -- they are holistic or synoptic in nature. They provide a higher-level view of the information structure and, thus, should improve ease-of-use. They conform to natural models and metaphors, making information access and manipulation easier. They minimize the impediments an electronic delivery system may have, and they allow the user to maximize whatever expertise he or she may have. For example, this type of interface fosters intuition and discovery. Additionally, users may follow up on hunches and educated guesses more readily.

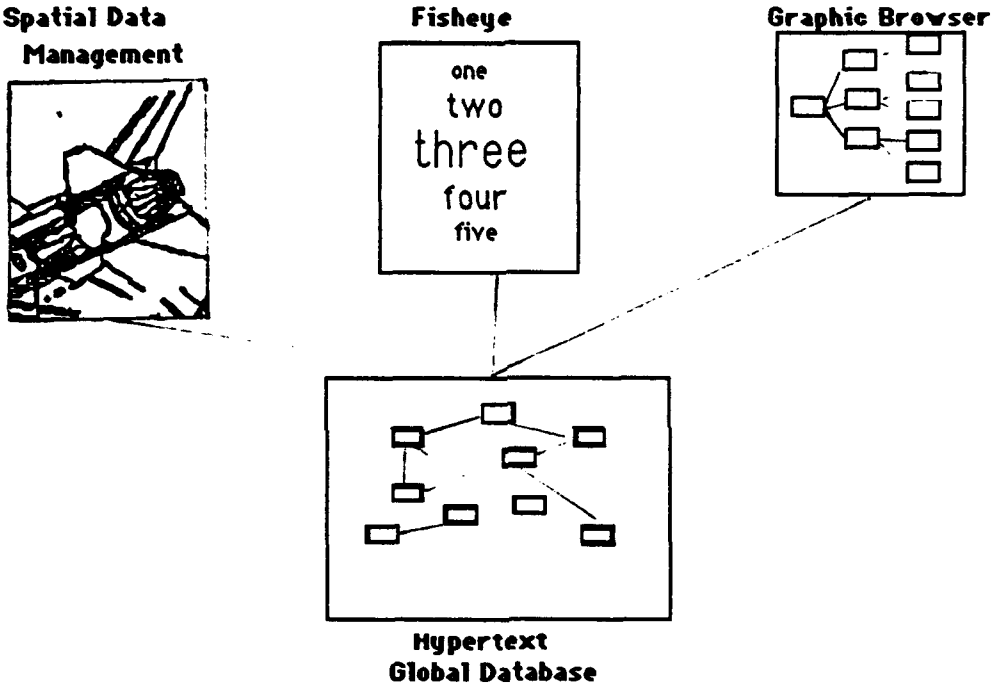



FIGURE 4: Category II Search Techniques

CATEGORY III: Conjoining certain AI models with information retrieval research has produced intriguing hybrid systems. Of particular interest are expert systems which constitute intelligent interfaces to a document database. Several efforts attempt to capture the expertise of search intermediaries in online systems. More ambitious efforts integrate browsing and automated retrieval, even to the point that the system has rudimentary models of categories of users and can select search strategies based upon a specific situation (Fox, p. 163).

In many cases, the end user may not be able to tell if an application is an expert system, a

decision support system, or a database management program. The latter two use conventional algorithmic approaches to problem-solving, while the former uses heuristic symbolic processing. The former have two major components, the database and the retrieval mechanism. Adding the intelligence of an expert system requires the use of additional components -- a knowledge base and an inference engine. The expert system embodies the facts, information, knowledge, and rules of thumb and other elements of heuristic expertise. The user may tap this expertise as an expert advisor or a consultant to solve problems or make decisions.

At least three approaches to conjoining expert systems and hypertext are possible.

Separate Knowledge Base and Hypergraph: This configuration uses the knowledge base an an interactive interface to filter the information-rich chunks of information in the hypergraph. In this case, the expert system and the hypertext are separate components, as represented in Figure 5. After a consultation, the system automatically calls up the appropriate segment of the TO. This selection can be as generic as an entire subnet (or stack) or as fine-grained as a single chunk (card).

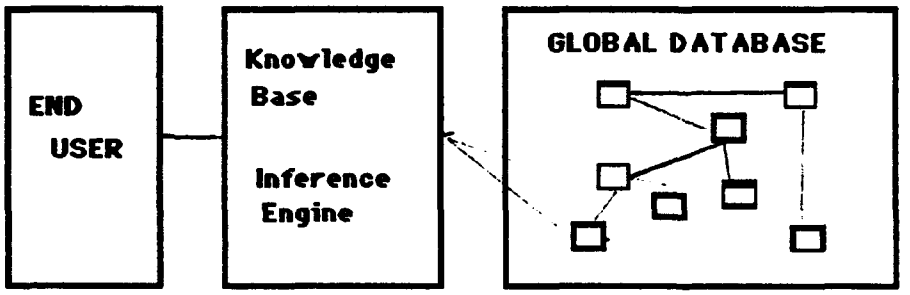



FIGURE 5: Separate Expert System and Hypergraph

MDAS (Maintenance Diagnostic Aiding System), developed by the Air Force Human Resources Laboratory, is a good example of an expert system which sits outside the hypergraph and acts as an intelligent interface to point the user to relevant documentation. MDAS replaces the troubleshooting section of the TO with a sophisticated software model which advises a technician on fault isolation by taking into account availability of parts and test equipment, mission criticality of components, time to perform tasks, and component failure rates.

Merge Knowledge Base with the Hypergraph: Critics of expert systems charge that most systems are too brittle; that the user is led, lock-step, through a series of information-extracting questions without adequate opportunity to interject intuition or to adapt the system to the particular situation. Eventually, the user begins to feel like a slave. In fact, the educational value of an expert system is limited, if the user cannot understand the rationale behind the various steps in the consultation session.

One approach to overcome some of these limitations is to design the knowledge base as a hypertext. At any point in the consultation, the user may ask for additional information on domain concepts, on particulars of the problem, or on the operation of the expert system.

KnowledgePro (from Knowledge Garden, Inc.) is a software platform for building expert systems, although it is not, strictly speaking, an expert shell. The software combines an expert system, a programming language, and a hypertext product into one seamless and highly modular environment (Shafer, p. 31). The expert builds text blocks in a manner similar to how she would sit down and tell someone what she knows. The expert also links these chunks of knowledge to others. Eventually, the expert adds rules and an inference engine to boost the intelligence of the system. Figure 6 represents the basic structure of this potentially important new trend in expert development tools.

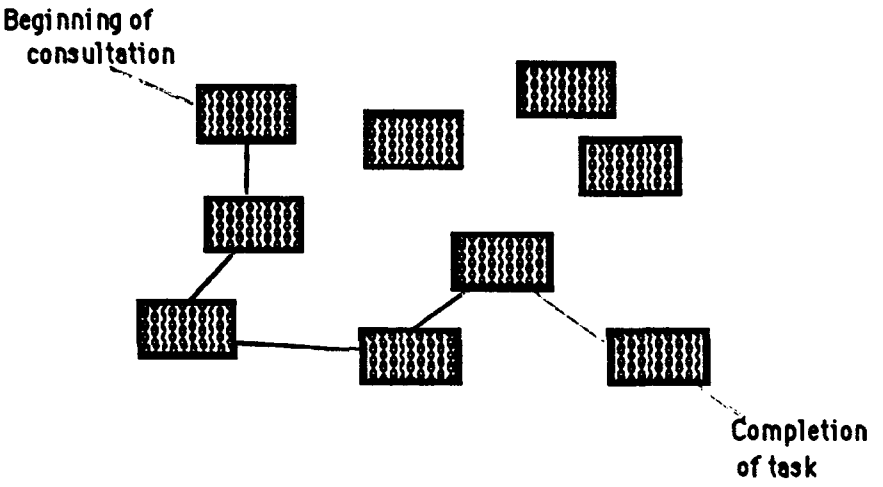



FIGURE 6: Merged Expert System and Hypergraph

Embed or Distribute Expert Systems within the Hypergraph: This approach is something of a combination of the previous two. The hypergraph retains its integrity, but the user is able to call up search aids at given choice points in the textbase or the search aids may acts as demons which awaken given a particular set of circumstances.

This configuration has many intriguing possibilities. Since nodes in a hypergraph can be anything from text to procedures, small knowledge bases can be stored and threaded like any other modules in the web. (In other words, expert systems can call other expert systems.) Additionally, this conjunction of hypergraphs and smart interfaces may be appealing to users if the intelligent filters can be viewed as a collection of selectable information extraction tools.

Figure 7 represents a hypothetical web with embedded expert systems. Advocates of a "new-generation retrieval system" describe a multi-faceted system which accommodates

individual users and specific situations. Edward A. Fox delineates the system of the future as "having multiple experts, each with private rule bases, as well as blackboard-based strategists for both analysis and access of documents, that can run as separate processes on multiple machines" (p. 164). While embedded expert systems may not deliver all these capabilities, they do provide more flexibility and functionality than traditional search techniques.

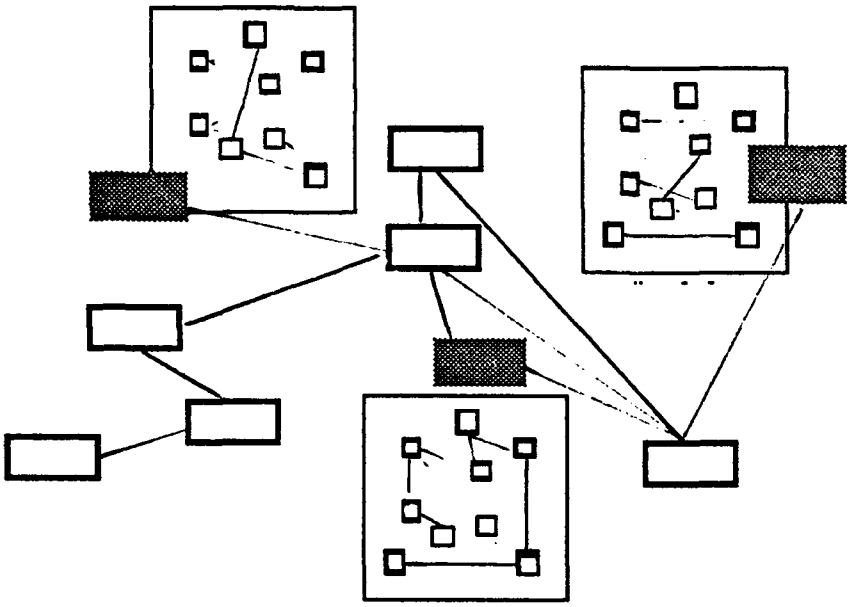


FIGURE 7: Embedded or Distributed Expert Systems

## V. RECOMMENDATIONS:

The future for document databases looks bright. The availability of CD ROM will bring down the cost of storage. An added push in the information sciences will increase research into such design issues as data models, user interface, and authoring. Based on the reading and design work I did during the past ten weeks, I recommend continued IMIS research in four areas:

- **Usage Model for Paper Documentation:** Thomas Duffy, in "Preparing Technical Manuals: Specifications and Guidelines," describes a session in which a maintenance technician consults several volumes and many pages of text as part of a particular repair task. (Duffy's purpose was to demonstrate that the

organizational schemes of paper manuals do not match the task requisites imposed by the real situation.) Duffy's scenario, while brief, is informative.

Full IMIS integrates a number of functions (job aiding, training, troubleshooting, collecting logistical data, displaying personnel management information, etc.). Deciding how these functions might be bundled in the user interface is crucial to the total IMIS knowledge synthesis. More specific scenarios, based on observation and giving details of how technicians integrate maintenance tasks and information-seeking tasks, will uncover both the strategies and the tactics for using TOs which technicians learn through experience. At some point, these scenarios might even lead to a more formal taxonomy of usage categories and/or a model of information-seeking patterns.

- **Translating Text to Hypertext:** Several historians of ideas have written eloquently on the growth of knowledge in human culture. For example, James Burke, in The Day the Universe Changed, points out that the invention of cross-referencing accelerated the accumulation of knowledge because old concepts could now be connected in new ways. Similarly, studies of the technology of text point out that changes in medium precipitate different habits of mind, to accommodate the remodeled modes of communication. Hypertext may be the beginnings of a paradigm shift in information processing.

  Be that as it may, the majority of human knowledge is now archived in books, journals, monographs, collections of papers, various microforms, films, videos, and audio disks and tapes. If this vast body of text is to be placed online, will significant retrofitting be required? Or can search software be designed to overcome some of the drawbacks of a knowledge structure which might be rendered incomprehensible by the transition. More to the point, what is the balance between search software and remodeled text for a TO?

- **Continued Research Into Applied Hypertext:** Hypertext is still more of a notion than a developed idea. Although the concept receives much attention in the literature, few articles report systematic efforts to answer some of the fundamental questions of application. If possible, HRL/LRC should sponsor an experimental platform, involving both internal and external personnel.

  For a start, the Laboratory could develop a relatively large document database, drawn from existing TOs. This collection of rhetorically neutral, reusable, information-rich chunks could then be threaded into various webs. Tests of various combinations of search mechanisms and interfaces could be run. Each combination must be rigorously assessed using human factors criteria. Such empirical studies are invaluable for testing the claims made about hypertext, for guiding the direction of future development, and for becoming smart shoppers for future Air Force system purchases.

- **Design for a Range of Interactivity:** Clearly, full IMIS requires support for a number of user classes and information processing schemes. Specifically, the system (interfaces and databases) must be versatile enough to accommodate the user actively searching for information as well as the learner acquiring concepts and experience. Although the system will be built in pieces and the development will be incremental, periodic modeling of how it will all play together should benefit both near- and far-term design.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

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FINAL REPORT


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| Date: | 2, Sept 88 |
| Contract No: | F49620-87-R-0004 |

# Linking Training Evaluation to Training Needs Assessment:

## Development of a Conceptual Model

by

J. Kevin Ford, PhD.

## ABSTRACT

A critical linkage in training systems is the translation of training evaluation information for reassessing training needs and for making training program changes. This report presents the development of a conceptual framework for examining the job relevancy and efficiency of training and the linkage of this evaluative information to training needs reassessment. How to integrate job performance information into the existing training evaluation system for identifying over- and under-trained tasks is also described. Recommendations for future research to identify the content domain of an Airman Basic in Residence (ABR) Training program and to integrate performance information into the evaluation system is presented.

## Acknowledgements

I am grateful to the combined efforts of Universal Energy Systems and the Air Force Office of Scientific Research for providing the opportunity to spend a ten-week period concentrating on a single research project. Various members of the Human Resources Laboratory helped make this research project an enjoyable and productive experience. Lt. Colonel Ovalle, Mark Teachout and Winston Benett provided help and guidance. Captain Marty Pellum arranged various meetings and was a valuable source of information about the Air Force training system. Thanks also to the members of the Occupational Measurement Center at Randolph AFB (Col. Baker, Mr. Tartell, Captain Agee, Lt. Col. Houtman, Dr. Aslet) and personnel at Chanute AFB (Col. Zeitler, Col. Robbins, Msgt. Watt, Mr. Predmore, Mr. Gissing, Mr. Willis, Captain Reed, and Mr. Greer) who gave up their valuable time to provide information regarding the Air Force training system. Finally, special thanks to Dr. Hendrick Ruck for his insights into the research issues relevant to training.

I. INTRODUCTION:

The quality of training programs is critical in the Air Force to maintain personnel readiness. To insure readiness, training must be viewed as a continuously evolving process which uses evaluative information to adapt and to improve its quality. Therefore, a critical linkage in training systems is the translation of training evaluation information for reassessing training needs and for making training program changes.

The Air Force Human Resources Laboratory (AFHRL) at Brooks AFB has recently developed a job performance measurement system (JPMS) to validate selection standards. AFHRL personnel contend that JPMS data can also be used as evaluative information for improving the quality of Airman Basic-in -Residence (ABR) technical training. AFHRL is particularly interested in developing a methodology for using JPMS data to identify tasks which are over- or undertrained in ABR training programs.

My research interests include organizational training and criterion measurement. In training, I have been involved in evaluating the job relevancy of training programs and in the development of a methodology for identifying knowledges and skills that might be over- or undertrained. My work on linking training evaluation to training needs reassessment contributed to my assignment to the ID Training Systems Division of AFHRL.

II. OBJECTIVES:

Currently, there is no systematic procedure for identifying over- or undertrained tasks in the ABR courses. There is also a lack of high quality job performance information available in the training evaluation system to allow for informed decisions regarding course content changes.

My assignment as a participant in the 1988 Summer Faculty Research Program (SFRP) was to develop a conceptual framework for understanding the linkage of training evaluation information to training needs reassessment. The specific focus of the paper is to develop a framework for addressing the issues of the job relevancy of training and the over- and undertraining of tasks. The conceptual framework includes a focus on the integration of job performance-

based information into the existing training evaluation system for identifying over- and undertrained tasks.

## III. A TYPOLOGY OF TRAINING EVALUATION PURPOSES

A comprehensive analysis of training needs is critical to training program development. Similarly, an initial assessment of training needs should also provide information for the development of a training evaluation system. The key question asked during this evaluation development phase is "what do we want to know about the training program that provides information about the quality of the program?" Decision makers may be interested in answering one or more evaluation questions.

Depending on the purposes of the evaluation, different kinds of information need to be collected to answer the evaluation question being addressed. Thus, if the purpose(s) behind the development of an evaluation system for a particular training program is not clearly specified during the needs assessment phase, it is unlikely that the appropriate information will be collected to link the evaluation data with training needs reassessment for program redesign. Without a clear sense of purpose, modifications to enhance the quality of the training program must rely heavily on the intuition of the people who develop the training program (Montague and Wulfeck, 1986).

The purposes for conducting training evaluation is conceptualized as consisting of five components. Table 1 presents the five purposes, the types of questions asked for each purpose and the information needed to answer the questions posed by the evaluation purpose.

One purpose for conducting training evaluation is to determine the content validity of the training program (Goldstein, 1986). Content validity asks the question "is the training content job relevant?". To answer that question, information about the tasks being performed on the job must be matched with the tasks being trained in the training program. Then a content validity ratio can be calculated to determine the extent to which the content of the program is job relevant (Ford & Wroten, 1984).

A second purpose is to examine training efficiency. Training efficiency asks the question "is the training program over- or undertraining certain tasks (or Knowledges, Skills and Abilities

(KSAs))?". To answer this question, information regarding the importance of tasks in the job domain and the emphasis of those tasks in the training program must be acquired. A matching technique can then be applied to examine areas of over- or undertraining (Ford & Wroten, 1984).

A third purpose for conducting training evaluation is to determine training validity. Goldstein (1986) states that this purpose asks the question "did the trainees learn the material that was being trained?". To answer this evaluation question, one must collect information about the performance of the trainees during and/or at the end of the training program. The analysis determines the extent to which learning has taken place in comparison to a specified standard or criterion of success.

A fourth purpose of evaluation is to determine transfer validity (Wexley, 1984). Transfer validity asks the question "are people performing well on the job after training?". To answer this question, information regarding performance on the job must be collected. Job Performance must then be compared to some criterion of success to determine the extent of successful transfer. Experimental designs can be used to determine if the transfer of knowledges and skills have actually occurred and whether the change is attributable to the training program.

A fifth purpose of training evalution is to determine the predictive validity of a training program. At times, training is used as a device to select or place individuals into a particular position or job. Therefore, the question that must be answered with this evaluation purpose is "does training performance predict job performance so that selection/placement decisions can be made?". The information that must be collected to conduct this analysis includes training and job performance information.

## IV. A CONCEPTUAL MODEL OF EVALUATIVE INFORMATION

An examination of Table 1 indicates that the information available for answering the questions posited for each evaluation purpose can be conceptualized as consisting of two major dimensions. One dimension is the source of the information that is gathered. The two sources of information available are the job domain and the

training domain. A second dimension is the type of information gathered. The type of information is conceptualized as being task based or performance based. Task information focuses on what an individual "does do" while performance information examines "how well an individual does the specified tasks".

Figure 1 presents the four domains that result from combining the two types of sources with the two types of information available. The four domains are the: (1) job content domain; (2) training content domain; (3) job performance domain; and the (4) training performance domain.

The job and training content domains are conceptualized as consisting of a task and an emphasis component. The "task" component of the job content domain involves the identification of what tasks (or KSAs) are performed on the job. The "task" component of the training content domain includes information regarding what tasks (or KSAs) are taught during training.

The "emphasis" component of the job content domain involves an analysis of how "important" the various tasks are for job performance. Importance can be operationalized in a number of ways including "importance to job performance" and "task difficulty". The "emphasis" component of the training content domain concerns the amount of effort devoted to training the various tasks included in the training program. Emphasis can be operationalized as a subjective assessment of effort devoted in training to each task or as the actual amount of time spent training each task.

The job and training performance domains are conceptualized as consisting of a knowledge and a performance component. The "knowledge" component of the job performance domain focuses on how well the individual knows the appropriate ways to perform job tasks. Job knowledge tests are often used to address this issue. The "performance" component specifies how well the individual actually performs the job tasks. Information from job performance ratings, work samples and job indices such as quantity or quality of work can be gathered to determine the level of job performance.

For the training performance domain, the "knowledge" component examines how well the trainees have learned the material that has been

taught. Pencil and paper tests on the material covered in the course are typically used to examine the knowledge component. The "performance" component focuses on how well the trainees can perform the tasks that have been trained. Performance ratings, work samples and other objective indices can be used to determine performance level in training.

In summary, the model in Figure 1 provides a conceptual framework for describing the information available to answer training evaluation questions. The specific information gathered is dependent upon the purpose(s) for conducting training evaluation. An examination of Figure 1 also indicates that a critical boundary condition surrounding these four domains is information quality. This means that the usefulness of any information collected for answering the evaluation question posed is heavily dependent upon the quality of that information.

## V. APPLICATION OF THE CONCEPTUAL MODEL TO TRAINING EVALUATION

Discussions with researchers of the Training Systems Division (ID) indicated that one key issue is the need to incorporate job performance information into the design of training programs. Such performance information is useful to determine the relevancy of training content as well to identify training inefficiencies (i.e., over- or undertraining of knowledges and skills). Prior to using job performance information, a better understanding of the conceptual issues involved is needed.

### Content Validity

One purpose for conducting training evaluation is to assess content validity ("is the training content job relevant?"). The information needed to address this question includes an analysis of the "task" components of the job and training content domains. Thus, the question of content validity must be answered through a direct comparison of the tasks being performed on the job with the tasks being taught in the training program.

There are three possible outcomes of this comparison process. The first outcome is the area in which there is an overlap between the job and training content. The percentage of overlap is directly related to the content validity of the program. When the overlap is

high, tasks performed on the job are, for the most part, taught in the training program. A small degree of overlap indicates a low degree of content validity.

The second outcome of this comparison process is the area in which tasks are performed on the job but are not taught in the training program. These "training omissions" should be examined for possible inclusion into the training program. The third outcome is the area in which tasks are taught in the training program that are not performed on the job. These "training excesses" should be examined for elimination from the program.

The three outcomes of the comparison of the job and the training content provide useful information to training researchers. It is important to note, though, that the comparison of the job content and the training content can only be as good as the quality of the data collected. The type of information (tasks, KSAs) collected on the job and training content domains must also be the same. The tasks or KSAs must also be measured at the same level of specificity to make this direct comparison. If the job domain is defined in terms of tasks, the training domain must also be defined in terms of tasks at the same level of specificity.

Training Efficiency

A content valid training program indicates that the tasks being performed on the job are the same tasks being trained. Once content validity is established, a second issue is training efficiency. Training efficiency asks the question "is the training program over- or undertraining certain tasks or KSAs?". Thus, a training program may have a high degree of job relevancy (it is training the appropriate tasks) but may or may not be placing an appropriate amount of emphasis on the various tasks during training to match their "need" for training.

Training and Job Content Domain Information. The information needed to address the training efficiency question includes an analysis of the "emphasis" components of the job and the training content domains. The question of efficiency can be addressed through a direct comparison or matching of the emphasis placed on the tasks during training with training "needs" i.e., how important the task is

in the job domain.

Ford and Wroten (1984) developed a methodology called the Matching Technique to link the "emphasis" components of the job and training content domains to determine training efficiency. The Matching Technique is conceptualized in Figure 2 as a matrix in which training emphasis is directly compared to training "needs" (i.e., how important the task is for job performance).

The comparison of emphasis with needs identifies training "hits" and "misses". Training hits refer to those tasks where the emphasis received in training appropriately reflects training needs. Training misses can involve areas of deficiences (undertraining) and excesses (overtraining). Training deficiencies are content areas whose high training needs are not matched by a high degree of emphasis in the training program. Training excesses are tasks that are receiving an excessive amount of emphasis relative to their need to be training. The greater the number of misses (both difficiences and excesses) uncovered, the less efficient the training program is and the greater need for reassessment and redesign of the program.

Any measure of training "needs" or training "emphasis" can be used to apply the Matching Technique as long as the measures reliably quantify the extent to which areas need to be trained or emphasized in the training program. Nevertheless, as noted when discussing content validity, the comparison of training "need" (i.e., job emphasis or importance) and training emphasis can only be as good as the quality of the data collected. Also the type of information (tasks, KSAs) collected on the job and training content domains must be operationalized at the same level of specificity to make this direct comparison possible.

Integrating Efficiency and Performance Information. The Matching Technique was developed to compare information from the job content and training content domains. Information from the job and the training performance domains were not integrated with the information from the Matching Technique. Nevertheless, information from the job performance domain may be quite useful in providing data for identifying over- and undertrained tasks. Figure 3 presents a 2 X 2 matrix which combines information on training efficiency with

information on job performance. An analysis of training efficiency can identify tasks that are excesses and deficiences. Performance level is conceptualized at the group level of analysis i.e., how well the group of individuals trained are performing various tasks on the job. For purposes of this example, performance is dichotomized into the categories of "not performing well" and "performing well". The four components of the matrix in Figure 3 derived by combining training efficiency and performance level information provide data relevant to identifying over- or undertrained tasks. As an example, let us assume that a number of tasks were identified through the Matching Technique as being deficient. The next step is to examine how well the individuals are actually performing those tasks on the job. If the tasks are not being performed well, then the need for more training emphasis during the training program is indicated. If the tasks are being performed well, this indicates that training deficiency is not an important issue to consider. Such a result may suggest that formal training of the tasks is not necessary.

The Matching Technique may also identify some tasks as training excesses. An examination of job performance may indicate that some tasks that are training excesses are performed well and others are not be performed well (see Figure 3). If a task is not performed well on the job, this indicates that there is a serious problem that is best addressed through other means such as on-the-job training. If a task is performed well, this suggests that consideration be given to examining whether training emphasis placed on the task could be reduced without detrimental effects on job performance.

## VI. ANALYSIS OF THE AIR FORCE ABR TRAINING EVALUATION SYSTEM

The conceptual models developed in this paper highlight the important role evaluation systems have for enhancing the quality of training. The issues of content validity and training efficiency were used to illustrate the concept of linking training evaluation purpose with the kinds of information that need to be collected. Based on this conceptual development, this section provides a brief critique of the existing evaluation system for examining training efficiency in the Air Force ABR courses. A more detailed analysis in relation to a particular ABR course (Aerospace Ground Equipment) is presented in a

companion paper (Sego, 1988). The conceptual models indicate that there are three key requirements that need to be met to address the issue of training efficiency. First, the job and training domains must be defined using the same type of information e.g., either task statements or KSA statements. Second, the job and training domains must be defined at the same level of specificity. Third, the quality of the information must be high i.e., we must have confidence that the information collected is an accurate reflection of the job and training domains. When these three requirements are met, the Matching Technique can be used to link the emphasis placed on tasks in the job content domain with the emphasis placed on tasks in the training content domain. Performance information can then be integrated into the results of the Matching Technique to identify over- and undertrained tasks.

The "emphasis" component of the job content domain for Air Force Specialties is defined at the task level through rating responses. Information from the Occupational Survey Report (OSR) includes "training emphasis" (from no training is needed to training need is extremely high) and "task difficulty" (from extremely low to extremely high) for each task. The emphasis component of the training content domain is not systematically collected for analysis. Information on the training content domain comes from the Specialty Training Standards (STS) which provides information for each task on issues such as "task knowledge" (nomenclature, procedure, operating principles and advanced theory) base, "proficiency levels" (limited, partially proficient, competent, highly proficient) to be obtained through training, and "subject knowledge proficiency" (facts, principles, analysis, evaluation). These indices are useful when developing a training plan. Nevertheless, this does not provide the information needed regarding how much emphasis is placed on each task once the POI has been developed.

The system for evaluating training efficiency can be evaluated according to the three requirements discussed above. Both the job and training content domains are defined in terms of tasks. Unfortunately, the information from the OSR on training "needs" for use in a Matching Technique is not matched by useful information from

the training content domain. One problem is that the STS tasks are not at the same level of specifity as the OSR. The second problem is that the emphasis given to the tasks in the training domain is not being systematically gathered.

Consequently, this situation does not allow for a direct comparison of training "need" (the information from the OSR) and training emphasis through the use of a Matching Technique. A direct comparison is only possible if data from the training domain on the amount of emphasis placed on each task during training is collected. This information may be in the form of the amount of training time allocated to each task or through emphasis ratings by instructors and trainees.

VII. RECOMMENDATIONS:

Based on the conceptual models developed and the analysis of the Air Force ABR Training system, two research needs are identified: (1) to systematically identify the training content domain in terms of tasks and emphasis placed on those tasks; and (2) to incorporate job performance information into the training system to address the issue of training efficiency.

Identification of the Training Content Domain

The job content domain in terms of the tasks performed on the job and the emphasis that should be placed on training those tasks during the ABR course are identified through the OSR process. The OSR process represents a large commitment of resources to develop systematic data on the job domain which can be used for aiding training personnel of the ABR courses in updating training to maintain a high quality program.

A major problem identified in this review (also see Sego, 1988) is that similar efforts to systematically identify the tasks taught and the emphasis placed on those tasks in the training content domain are lacking. Information about the ABR course is available through the POIs and lesson guides but is not at the level of specificity as the OSR data. In addition, no data is gathered on how much emphasis is actually placed on each task that is part of the training content domain. Without such information, decision makers can not link the job and training content domains to answer the question of whether

tasks are being over- or undertrained.

Consequently, the first effort should be devoted to developing a methodology for identifying the training content domain in terms of tasks and emphasis. It is suggested that Training Branch personnel, instructors, students and recent graduates are all potential sources of information regarding the training content domain. Multiple small group sessions can be conducted to identify the OSR tasks which are actually taught in the training program. Surveys can then be conducted to determine whether there is substantial agreeement among the various sources that the tasks identified are actually taught in the training program. In addition, the tasks can be rated in terms of the emphasis placed on each task in the training program. Stability of judgements over time and interrater agreement across sources (staff members, current students and recent graduates) can be examined to determine the quality of the information gathered.

The Matching Technique (Ford & Wroten, 1984) can then be used to link the data from the OSR report on the emphasis (or task difficulty) that should be placed on a task during training with the ratings of the actual emphasis placed on each task during the training program (small group sessions could also be conducted to determine the actual amount of time devoted to each task during the training program). Such a matching of training "need" with training emphasis provides the necessary information to identify possible areas of over- and under-training.

Integration of Job Performance Information

Figure 3 presented a conceptual model which integrates job performance information with the information from the Matching Technique. Currently, job performance information is not used to make decisions regarding training efficiency. The JPMS developed by AFHRL provides the type and quality of job performance data needed to identify over- and undertrained tasks.

The JPMS contains three types of information. First, participants complete "a hands on" portion of the Walk-Through Performance Testing procedure in which participants actually perform certain tasks and are scored by trained observers on whether the tasks are performed correctly. A second portion of the Walk-Through

Performance Test is an interview portion which asks participants to explain how they would go about completing certain tasks rather than actually performing those tasks. These responses are scored by trained interviewers. Both the hands on testing and the interview method are scored at the Technical Order (subtasks are performed correctly or not) which are then summed for each task to provide a score at the OSR (task) level. The third type of information is a Specialty Job Knowledge Test which covers a number of tasks in the career field. Participants must respond to a number of multiple choice questions derived from the task content domain at the task level of specificity.

Given the development of the conceptual model, the next step is the development of the methodology to integrate JPM data into the training evaluation system to address issues of over- or under-training. If the training content domain is specified at the task level of specificity, then the integration of performance data must be at the task level also. Nevetheless, for tasks that are identified as over- or under-trained, job performance at the subtask (Technical Order) level can then be examined to determine which subtasks are most problematic. Research could examine the impact of feeding back job performance information to training personnel on ABR course changes.

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Table 1. The Purposes for Conducting Training Evaluation

| Purpose of Evaluation | Type of Question Asked | Information Required |
|---|---|---|
| 1. Content Validity | Is Training Job Relevant? | Job Content<br>Training Content |
| 2. Traning Efficiency | Are We Over- or Under Training Certain Tasks? | Job Importance<br>Training Emphasis |
| 3. Training Validity | Did the Trainees Learn the material Trained? | Training Performance<br>Criterion of Success |
| 4. Transfer Validity | Are People Performing well on the Job? | Job Performance<br>Criterion of Success |
| 5. Predictive Validity | Does Training Performance Predict Job Performance? | Training Performance<br>Job Performance |

## SOURCES OF INFORMATION

### Job Content Domain

Task: What are the tasks performed on the job?
Emphasis: How important are the tasks to job performance?

### Training Content Domain

Task: What are the tasks taught in the training program?
Emphasis: What is the emphasis placed on the tasks included in the training program?

### Job Performance Domain

Knowledge: How do individuals know how to do the job?
Performance: How well do individuals actually perform job tasks?

### Training Performance Domain

Knowledge: How well have trainees learned the trained material?
Performance: How well can the trainees perform the trained tasks?

Figure 1. The sources of information and type of information available to conduct training evaluation.

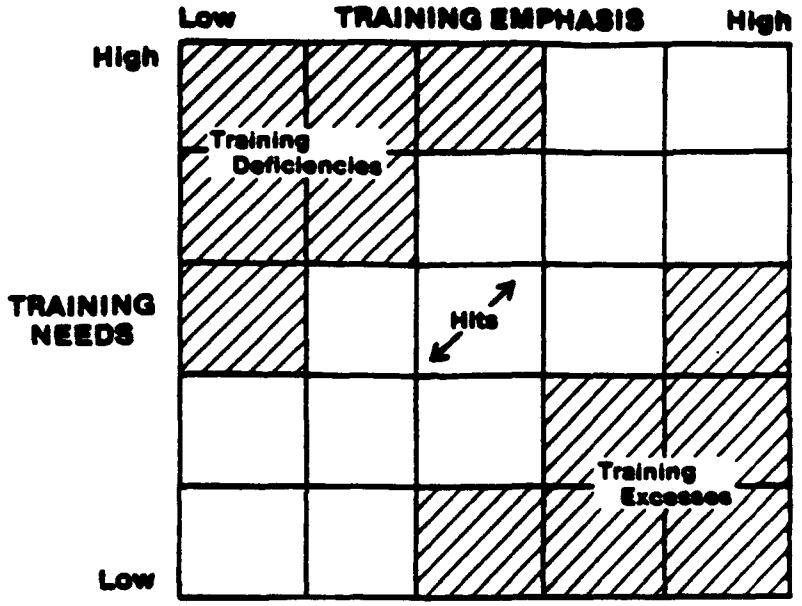



*Figure 2.* Model of the matching technique.

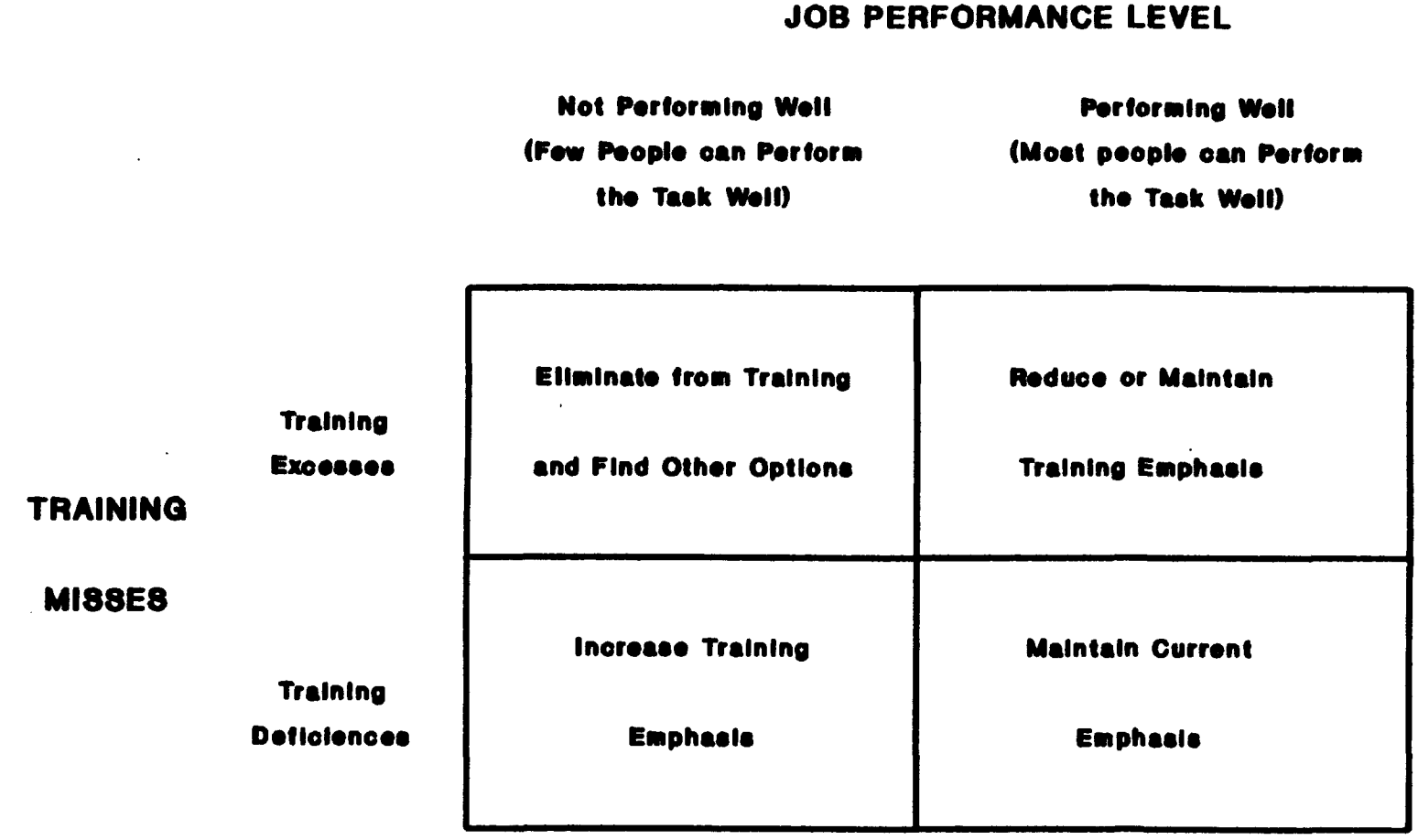



**Figure 3. An Integrative Model of Training Efficiency Information and Job Performance Information.**

1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

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FINAL REPORT

# A CONCEPT FOR AN INTELLIGENT TOOL TO FACILITATE THE DEVELOPMENT OF QUALITATIVE PROCESS MODELS IN NOVICE PROGRAMMERS


| | |
|---|---|
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# A CONCEPT FOR AN INTELLIGENT TOOL TO FACILITATE THE DEVELOPMENT OF QUALITATIVE PROCESS MODELS IN NOVICE PROGRAMMERS

by

Hugh P. Garraway

## ABSTRACT

This document relates the learning environment for computer programming and the development of students' mental models for programming. Problems with this environment are discussed through a review of previous research and a design is proposed for a software tool, an intelligent debugging assistant incorporating artificial intelligence techniques, to advise and direct novice programmers through strategic debugging paths. This tool will allow a student to confer with an "expert" during the debugging of programs under development. This tool should help students to more quickly develop an accurate qualitative process model for programming and thus resolve a problem area associated with learning to program in the Air Force and academia. The resulting tool could become a component of an Intelligent Tutoring System.

Acknowledgements

I would like to thank the United States Air Force Systems Command, the Air Force Office of Scientific Research, the Training Systems Division of the Human Resources Laboratory, and especially the Intelligent Systems Branch. Universal Energy Systems was most helpful in providing administrative support for this program.

The creative and professional atmosphere within the Intelligent Systems Branch at AFHRL was most conducive to my research efforts and is greatly appreciated. The efforts of all of the members to include me as a team member, not just an observer, has been of great benefit to me and, I hope, to the Branch.

The research schedule offered by branch chief Lt.Col. Hugh Burns was very helpful in the organization of my work. The frequent interchange of ideas with all of the branch was most rewarding and I would like to personally thank Lt.Col. Burns, Major Jim Parlett, Dr. Wes Regian, Dr. Kurt Steuck, Capt. Kevin Kline, Capt. Jeff Noone, Lt. Chuck Capps, Ms. Melinda Lobley, Mr. Elliot Schindler, Mr. Randy Morlen, and Ms. Rose Reyes for providing such a hospitable and productive work environment. The encouragement offered by Col. Gene Berry, Lt. Col. Roger Ballentine and Dr. Henk Ruck is also deeply appreciated.

## I. INTRODUCTION:

The Intelligent Systems branch of the Training Systems Division of the Human Resources Laboratory at Brooks Air Force Base is interested in the research and application of artificial intelligence techniques to learning, specifically those related to Air Force training.

My research activities have dealt with computer-based learning and intelligent authoring systems. My teaching experience has been in the areas of instructional technology and computer science. It was the overlapping of these areas and my interest in artificial intelligence and its role in teaching/learning that precipitated my assignment to the Intelligent Systems Division.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The primary goal of this research was to identify an area of training which could benefit from artificial intelligence-based computer-assisted instruction and design a software tool which could attempt to enhance the teaching/learning environment for this area . It was decided that the instruction of programmers would be the target area as it is an area of interest to the Air Force and an area relative to my expertise. An exhaustive search of past research in this area was performed and the highlights are reported in this document. A design for an intelligent teaching/learning tool was then made. The

following sections are the result of this research and synthesis.

## III. INTELLIGENT TOOL FOR DEBUGGING ASSISTANCE:

This document presents a design for ITDA (Intelligent Tool for Debugging Assistance). ITDA will be an intelligent software tool integrated into a programming environment. Some of the expertise of a human expert relative to programming, novice programmer errors, misconceptions, and debugging skills will be 'captured' so that all students will have instant access to a consultant when a programming impasse is met. ITDA will incorporate artificial intelligence techniques and will increase its knowledge as it interacts with students and, when necessary, a human expert.

Specific goals of this research will be:

1) Address a stated basic research need in the development of intelligent tutoring systems: Implement a practical intelligent tool and thus gather knowledge on how the engineering of such a system will proceed.
2) Provide an application of research that will:
   a. Aid in reducing a teaching/learning bottleneck that often causes introductory computer programming courses to be less effective.
   b. Function as a job aid to instructors.
   c. Serve as an intelligent, embedded learning aid to practicing programmers.

A Practical Intelligent Tool : According to Anderson (1988), most of the work done with intelligent tutoring systems has had the status of basic research, more concerned with gathering basic knowledge than providing useful learning experiences. "The point has been reached," he states, "where a few applications are feasible and it might be worthwhile to pursue some of them both for the relatively immediate benefit and for some sense of how the engineering of these projects will progress." ITDA will address this issue in that one goal of its design will be to create a practical intelligent tool to fill an existing need.

Teaching/Learning Bottleneck: The bottleneck occurs when a student, who has reached an impasse with a programming problem, must wait until an appointment with the instructor or teaching assistant can be arranged to obtain expert assistance. Even when expert assistance or perceived expert assistance (often in the form of the upperclassman) is available, another problem may arise, the 'quick fix'. The quick fix may occur when the expert simply corrects the problem for the novice without imparting the knowledge of how the problem was corrected or possibly of more importance, how the problem was diagnosed. The quick fix can also introduce confusion and frustration when the expert uses a programming construct not yet intended by the instructor to be a part of the student's knowledge. In an optimal learning environment for programming, quality expert assistance should be available on request to guide the student to the solution of a problem.

Instructor's Aid: ITDA should reduce the number of student/instructor consultations caused by problems within ITDA's knowledge base. ITDA will also point the instructor in the direction of a bug that presently eludes a correct diagnosis.

Embedded Learning Aid to Programmers: ITDA's scanning tools will be available to programmers. For example, a programmer may directly invoke ITDA's uninitialized variable detector to quickly search for a variable that is assigned an initial value based on an expression containing a reference to itself.

IV. Rationale:

Although out-of-lab instruction attempts to provide students with the declarative and procedural knowledge necessary for creating correct computer programs, the experiential learning that takes place in the lab plays an important role in the development of a student's qualitative process model. Conceptual and mechanical errors often present major problems for novice programmers (students) who are trying to complete programming assignments. Novice programming students may not have yet developed the debugging skills which evolve as a programmer's qualitative process model for programming grows.

There seems to be an ironic recursive relationship between a student's qualitative process model of programming and his

programming/debugging skills. A definition of this relationship might be:

> In order to successfully write programs, i.e. gain experience, a student must have a valid qualitative process model for programming.
>
> In order to have an appropriate qualitative process model, i.e., know how to program, a student must successfully write and maintain programs.

Thus a student's qualitative process model for programming evolves through the experience of successfully writing and maintaining programs which requires a pre-existing qualitative process model. This relationship may be the root of the learning problems that so often bring even the brightest students to their knees in the early stages of learning to program. When this problem arises, motivated students will seek a consultation with an expert, in many cases the instructor.

During a consultation, the student's responses to the expert's questions may identify a misconception held by the student or suggest the cause of an identifiable mechanical, typographic, or syntax error. Through dialog with the student, the instructor is often able to coach the student to discover the source of the bug as well as the logical path its location. If a misconception is determined to be at fault, then a direct tutoring or clarification activity can be initiated by the instructor. The student, through

this guided diagnosis and resolution of the problem begins to build a valid causal relationship between the symptoms of a particular bug and possible sources of the problem thus adding to the student's qualitative process model of programming. In a sense, the expert has given a portion of his qualitative process model to the student.

A problem exists in this system of learning in that genuine expert advice is not always available to students as they begin to develop their own expertise. In fact, some 'perceived expertise' can prolong a student's frustration and confusion. If it were possible for the instructor to be available to all students at all times in all labs and at all personal workstations, then the level of students' experiential learning might be expected to increase. It is impossible for a human expert instructor to assume this omnipresent role, and there lies the major implications for this research and development proposal.

Previous efforts at using a software tool to help debug programs has taken the approach of looking at a student's buggy program to identify problem areas for tutoring. Although ITDA will have tools to look at a student's program, its first course of action will be to interact with the student in an effort to identify bug or misconception types. A metaphor for ITDA's interaction with a student might be the dialog that takes place between a physician or physician's assistant and a patient previous to a physical examination.

Before describing ITDA in any greater detail, it is appropriate to briefly describe some of the related research and comment on its relevance to the ITDA concept.

V. Literature Review:

Several researchers have studied problems encountered by novice programmers and others have developed intelligent tutoring systems (ITS) or intelligent tools for programming and other areas relative to the concept for ITDA. Naturally, the results of this research will influence the development of ITDA.

Brown, Burton, and De Kleer's (1982) work with tutors (SOPHIE I, II & III)) for electronic troubleshooting provides a foundation of research and application in tutoring diagnostic skills for systems that can be represented within a tutor. Burton (1988) states:

> "It is important that these new systems be built on effective environments, that is, ones that present relevant problems and provide pedagogically appropriate tools. ... The environment in many ways defines the way the student looks at the problem. ... Empowering environments that make explicit the process the student has to do should be developed and their use explored."

MENO II (Soloway,1982) and PROUST (Johnson and Soloway, 1985) are bug-finding programs developed with support from the Office of Naval Research. Both MENO II and PROUST

compare a student's program with a library of bugs and misconceptions associated with a programming assignment dealing with rainfall. An inference is then made by MENO II or PROUST regarding the suspected underlying misconception and a report in the form of comments is generated for the student. In MENO II An attempt was made to use context-independent bug templates, but according to the developers the effort was less than successful. PROUST has been given the ability to access knowledge bases for several programming assignments and thus may be used on more than one assignment although its application is limited to the programs it "knows."

GUIDON (Clancey, 1983) was designed to teach diagnostic problem-solving to medical students. The subject material for GUIDON is the rule base for MYCIN (Shortliffe, 1976), a medical consultation system for diagnosing infectious diseases. Although diagnostic rules could be produced for particular cases, they were not presented in the hierarchical, top-down order as would generally be followed by an expert. This was a function of the unstructured set of production rules within MYCIN.

Anderson's (1985) LISP tutor is claimed to approach the effectiveness of a human tutor for teaching the LISP language. It provides a structure editor which produces construct templates that are completed by the student. The tutor provides assistance when a student has problems in correctly completing functions. Immediate feedback is given to the student as each element of a program is entered so

that only a correct program can be constructed. As a program is being developed, the tutor can provide examples of correct code for the student.

Perkins (1986) classifies programming students into groups of stoppers, movers, and extreme movers. The stoppers simply give up and disengage from the problem when a programming impasse is met. The movers think and try new ideas and sometimes break the impasse and carry on to completion. The extreme movers seem to experiment without thinking. Sometimes the extreme mover will find a correct solution but often a logical path to the solution will be overlooked or possibly be prematurely abandoned. Perkins observed that some students who initially tended to disengage from a problem proved capable of solving the problem when encouraged by a researcher.

Pea (1986) has classified language-independent-conceptual bugs based on his observation of novice programmers. He has labeled three major categories of conceptual bugs as Parallelism Bugs, Intentionality Bugs, and Egocentrism Bugs.

The success of Brown, Burton, and De Kleer's work in providing reactive environments for experiential learning suggests further development of tutors or intelligent tools to provide similar environments for other types of labs where it may not be desirable or possible to model a static system such as an electronic device. This could be the case with a tutor or tool, such as ITDA, to assist in the development of debugging skills.

Soloway, in his concluding remarks about MENO II, briefly discusses the role of the human program consultant (the expert instructor) and the possibility of incorporating the expertise and dialog management of the expert into future intelligent tutoring systems.

Clancey's research with GUIDON points out the necessity of providing a data structure for rule representation that contains not only the discreet rules but the expert diagnostic paths through the rules.

The reported success of Anderson's LISP tutor supports further research and practical application of artificial intelligence to the area of learning to program.

ITDA's presence should serve as encouragement to Perkins' potential stoppers and keep them moving. Extreme movers might benefit from the direction offered by ITDA since it will keep them "on track" in a diagnostic path.

In a system such as ITDA, identifying a misconception as a member of a group, as defined by Pea, might serve as the top level starting point for a dialog with the student in which subsequent bug subset group types could be identified.

VI Programming Environment:

Programming environments have in the past required significant cognitive overhead for students who have had to

wrestle with complex system specific commands and directives to enter, compile, link, and run programs (duBoulay, 1986). To many students, learning the system "incantations" caused at least as much stress as learning to program. Today, products such as Borland's Turbo compilers have reduced this overhead as integrated program development environments combining editors, compilers, linkers, and pop-up help screens are commonly used in teaching programming. These environments provide novice and experienced programmers with easy to learn and use, efficient tools for entering, testing, and experimenting with programs.

Assuming that students have been challenged with a problem to solve, the addition of an immediately accessible expert (ITDA) to such an environment should bring the lab programming environment closer to Burton's vision. By integrating ITDA into an existing successful programming environment, its effect as a component of an instructional system can be observed and fine-tuned. This will allow ITDA to not only fulfill a practical role, but also provide more basic research for future inclusion of such components in intelligent tutoring systems.

## VII. Design of ITDA:

ITDA will be an intelligent tool to facilitate the development of qualitative process models in novice student programmers. Specifically, ITDA is designed to enhance the learning environment of student programmers by providing instant access to the knowledge and collected diagnostic

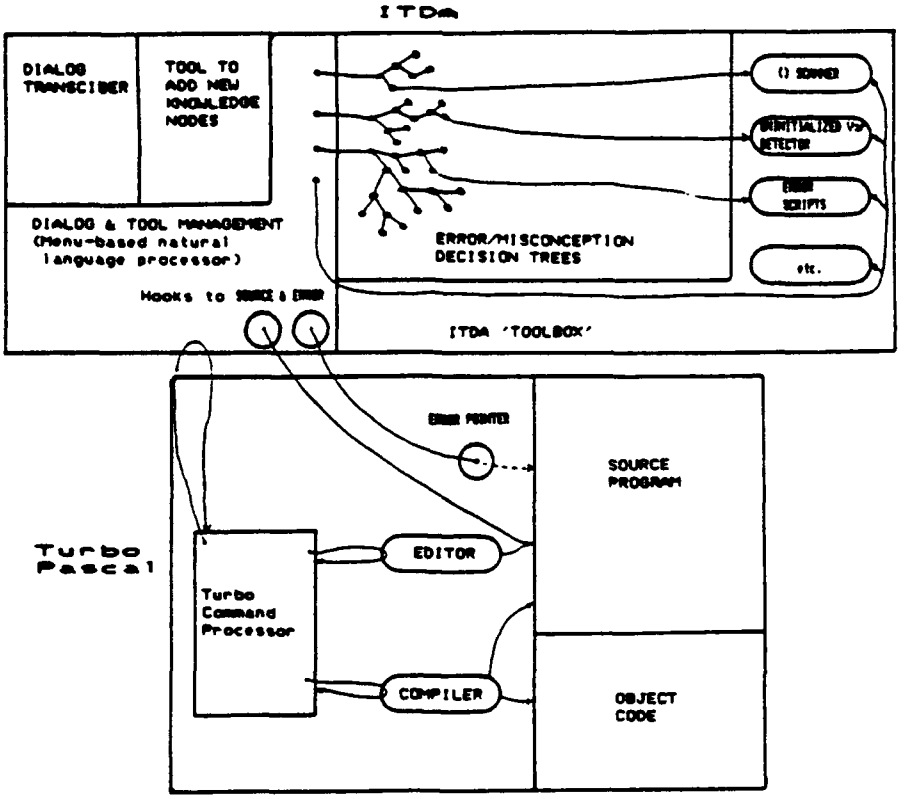
experiences of an expert programmer/debugger. This expert knowledge will be made available to students through a program using artificial intelligence techniques to manage the application of several diagnostic tools to students and their buggy programs. Students will 'consult' with ITDA in a manner similar to a consultation with a live expert.

ITDA's pedagogical strategy will be to engage the student in a menu-based natural language dialog to determine the overall class of advice needed. The student's responses during the dialog will help ITDA select the proper decision tree to traverse in an effort to lead the student as close to the problem as possible. Each decision tree consists of nodes in which the student is guided by ITDA to make specific observations in the program and respond accordingly. If deemed appropriate, ITDA can execute one of its scanning tools to help identify a problem.

When an interaction with ITDA fails to bring the student to the successful resolution of a problem, the student will be directed to consult with the instructor. When a student is interacting with ITDA, a transcript of the dialog will be made and stored on the student's disk. This transcript will be available to the instructor when a student has encountered a problem not currently covered by ITDA's expertise. The transcript will serve two purposes. First, it will help the instructor quickly identify and eliminate some solution paths. Second, it will indicate the need and mark the logical position for a new knowledge node in ITDA's expertise. After the student's problem is resolved, the

instructor may execute a command to add a knowledge node to ITDA so that future problems of this nature can be diagnosed under ITDA's direction. ITDA therefore dynamically, and in a heuristic manner, increases its expertise as its experience grows. This will constitute a modification of what Feigenbaum (in Shea, 1983) calls a "graceful failure mode", an instance in which the system reaches the end of a solution path and still fails to successfully diagnose a problem.

ITDA will consist of diagnostic, explanatory, example, and tool modules tied together by a management module. ITDA will have access to the student's source program and Turbo Pascal's error pointer. ITDA's internal structure will be such that additional tools may be easily added and managed. The following diagram illustrates the components of ITDA.



The beginning knowledge base for ITDA will be created by identifying the major classes of possible bugs, i.e. runtime, syntax, I/O, inconsistent results etc. and incorporating them into a menu-based natural language command module. This will define a number of empty decision trees. An expert (instructor) will then assume the role of a novice programmer and begin an interaction with ITDA. During this interaction, the expert will simulate the most common problems experienced by students. When ITDA reaches "graceful failure mode" in a decision path, the human expert will provide the appropriate diagnostic script and expected response for a knowledge node to identify this class or instance of a bug. Implementation in a course should produce additional problems and solutions which will be incorporated into ITDA's knowledge base.

## VIII. Concomitant benefits:

Although much of the research in artificial intelligence has been done using the LISP language, successful practical applications development for microcomputer-based systems has been hindered by the time and space requirements of LISP. The resources of microcomputer systems are growing and several versions of LISP have been developed specifically for microcomputers but some professionals in the AI industry feel that a better solution to the LISP problem is to implement AI techniques in less resource-demanding languages. (Kozlov, 1988) ITDA will be implemented in Pascal and possibly use parsing routines in Prolog in some of its scanning tools. The procedures developed for ITDA

will provide a library of AI functions that may be used in the development of other projects.

ITDA will also function as an intelligent, embedded job aid and possibly be a source for limited on-the-job training for programmers who have been placed in a production role while still developing an operational qualitative process model. In addition to its role as an advisor, ITDA will respond to commands from experienced programmers who wish to use selected tools from the toolbox module.

## IX. Recommendations

The recommendation of this research is simply that ITDA be built. A more detailed description of ITDA is contained in a proposal which is being submitted for a mini-grant .

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FINAL REPORT


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# A Tool for Studying the Effect of Range Restriction on Correlation Coefficient Estimation

by

Douglas E. Jackson

## ABSTRACT

It frequently happens that one must try to estimate the correlation coefficient between two random variables, X and Y, in some population P using data taken from a population Q, where Q is a proper subset of P. For example X and Y might be performance scores, P the set of individuals trying to gain acceptance into the armed services, and Q the subset of P consisting of those accepted. If X or Y or both are not part of the screening tests used as the basis for selection, then for at least one of these scores we have no data outside Q. We can administer tests to the members of Q and hence obtain data which may be used to estimate $\rho_{X^*,Y^*}$ [$X^*$ and $Y^*$ are X and Y restricted to Q]. Now suppose that X is a criterion variable and we wish to measure the value of Y as a means of selecting individuals who will have high X scores. Obviously we want to know $\rho_{X,Y}$ and not $\rho_{X^*,Y^*}$. This paper has two purposes. The first is to present the equations involved in such a way that the problem becomes more intuitively understandable. The second is to describe a monte-carlo program written to simulate repeated sampling from Q. This program displays the sampling distribution of the traditional estimator for $\rho_{X^*,Y^*}$ and of a proposed statistic for estimating $\rho_{X,Y}$. This proposed statistic is sometimes called the Pearson correction formula for range restriction. Presently the program assumes that the joint distribution of all variables is multinormal.

## Acknowledgments

I wish to thank the MOA staff for many stimulating discussions on the range restriction problem. They also provided me with valuable references describing previous work on the problem.

I would also like to thank the Air Force Office of Scientific Research for sponsoring this work and UES for administrative support.

I. Introduction

Let X and Y be two random variables defined on a population P. Let Q be a subpopulation of P and suppose that we have a random sample selected from Q. $X_1, \ldots, X_n$ and $Y_1, \ldots, Y_n$ will denote the X and Y data collected from this sample. The traditional statistic for estimating the correlation between $X^*$ and $Y^*$ [ $\rho_{X^*, Y^*}$ ] is ...

$$r = \frac{\sum_i (X_i - \bar{X})(Y_i - \bar{Y})}{n - 1}$$

However, this may not be a very good estimate of $\rho_{X,Y}$ . The need to know $\rho_{X,Y}$ when you only have a random sample from Q is a problem that occurs quite naturally and it has been investigated for some time. The most widely used method to deal with it has been to use a correction formula first developed by K. Pearson[1], and then extended by Lawley[2]. This formula applies when certain assumptions are satisfied. These assumptions are basically those associated with the classical linear regression model, and will be described in detail later. The formula only applies when $\rho_{X^*, Y^*}$ is known exactly. It is not uncommon to take a formula that holds for population parameters and apply it instead to statistics used to estimate those population parameters. Unfortunately, this approach comes with no guarantees. It is not assured to provide an unbiased or even a very good estimation. Finding a mathematical description for the sampling distribution of the Pearson statistic appears to be very difficult. At least it has defied solution so far. Rather than seeking a mathematical solution we decided instead to take a computational approach and write a monte-carlo simulation program. The purpose of this program is to evaluate, under

varying conditions, the accuracy of the traditional r statistic and of the Pearson statistic in estimating $\rho_{X,Y}$ . It will also be useful for testing statistics that use correlation coefficients as inputs.

## II. Notation and Objectives.

We will use the notation of Lord and Novick[3]. We assume that the members of an organization were admitted to the organization by virtue of having passed a battery of tests. These members are called the selected group or the restricted population and will be denoted by Q. These members plus those that were denied entry constitute the applicant group or the unrestricted population and will be denoted P. The tests that were used as a basis for selection are viewed as random variables on P and are called the explicit selection variables. Any other tests that are given to the members of the selected group Q are called incidental selection variables. We assume that all random variables are defined on P. If X is a random variable on p then the restriction of X to the selected group Q will be represented by the notation $X^*$.

Our objective is to study the sampling distribution of two statistics. The first is the standard sample correlation coefficient, r, which is calculated using a random sample from Q. The second is the Pearson correction formula for range restriction. It is calculated using the sample covariance matrix for the explicit selection variables based on data from the applicant group P, plus the sample covariance matrix for all variables based on the selected group Q.

In this study we will assume that the most general type of selection criteria is

$$L <= C_1X_1 + \ldots + C_{nve}\,X_{nve} <= H,$$

where H may be infinity, L may be negative infinity, and nve is the number of explicit selection variables.

III. Correction in the two variable case.

Although I have referred to the correction formula as the Pearson formula, the most general version is actually due to Lawley[2]. This is the version used in our simulation program. In order to understand the general theorem in its matrix form, we need to look at a couple of special cases in this and the next section. The proof of the general theorem is obtained by generating functions and is not a very instructive proof. The proof of the present special case however is very instructive and we will give an outline of it here.

Let X be the only explicit selection variable and let Z be the only incidental selection variable. Hence we have X and Z defined on P, $X^*$ and $Z^*$ defined on Q, and the members of Q are selected on the basis of their X score.

Assumption 1.(Linearity) The true regression function of Z on X is linear. In other words we have

$$Z = a + bX + E,$$

where a and b are constants, E is a random variable, and the expected value of E given x is zero for all x.

Note : It is not necessary to assume that X and E are independent. Linear regression is enough to imply that cov(X,E) = 0, which is needed for the proof of theorem 1. The proof that cov(X,E) = 0 follows directly from the definition of covariance and hence is omitted.

Assumption 2.(Homoscedasticity) The conditional variance of Z given x, does not depend on x. In other words, $\sigma_E$ does not depend on x.

Note : Assumption 2 still does not imply that X and E are independent.

Theorem 1 : Under assumptions 1 and 2

$$\rho^2_{X,Z} = \left[ 1 + \frac{\sigma^2_{X^*}}{\sigma^2_X} \left( \frac{1}{\rho^2_{X^*Z^*}} - 1 \right) \right]^{-1} \qquad (1)$$

Proof: Given that cov(X,E) = 0 it is a matter of simple algebraic manipulation and the relationship

$$cov\left(\sum_i a_i X_i, \sum_j b_j Y_j\right) = \sum_i \sum_j a_i b_j cov(X_i, Y_j)$$

to show that

$$b = \rho_{X,Z} \frac{\sigma_Z}{\sigma_X} \qquad (2)$$

and

$$\sigma^2_E = \sigma^2_Z (1 - \rho^2_{X,Z}). \qquad (3)$$

But now assumption 1 and equation 2 imply that

$$\rho_{XZ} \frac{\sigma_Z}{\sigma_X} = \rho_{X^*Z^*} \frac{\sigma_{Z^*}}{\sigma_{X^*}}, \qquad (4)$$

while assumption 2 and equation 3 imply that

$$\sigma_z^2\left(1 - \rho_{x,z}^2\right) = \sigma_{z^*}^2\left(1 - \rho_{x^* z^*}^2\right). \qquad (5)$$

These two equations are exactly equivalent to the conclusion of the theorem. That is to say, you get the conclusion by solving for $\sigma_{z^*}^2$ in equation 4 and putting that in equation 5 and solving for $\rho_{x,z}$.

It is important to understand that the conclusion depends exactly on linearity and homoscedasticity and the fact that Z is not explicitly restricted. We need no assumption of normality. We will, of course, not know any of the population parameters that appear on the right side of the formula and so the statistic based on this theorem becomes ...

$$\left[1 + \frac{S_{x^*}^2}{S_x^2}\left(\frac{1}{r_{x^*,z^*}^2} - 1\right)\right]^{-1},$$

which is the Pearson statistic for two variables. The sampling distribution of this statistic does depend on the joint distribution of X and Z. The simulation program described later assumes that this distribution is bivariate normal. From looking at a few examples using that program it appears that the corrected statistic is always a slight under estimate. In the cases we examined, this downward bias seems to be so small that it could easily be ignored.

Notice that if $\sigma_{x^*}^2 < \sigma_x^2$, as it will be for the type of restrictions we are considering, then $\rho_{x^*,z^*}^2 < \rho_{x,z}^2$. So if we use $r_{x^*,y^*}^2$ instead of the correction formula we will always be getting estimates for a parameter that is smaller than the one we want

to estimate. This last statement is not true when there are more than two variables. We will say more about this in the next section.

The effect of range restriction on population parameters in the two variable case is easily visualized. Think of a correlation coefficient as a measure of how well we can perform the following task. We may administer test X to two randomly chosen individuals and we wish to predict which of these individuals would score highest on test Z. There are three characteristics of the joint density function between X and Z that determine how well we can predict. The first is the slope of the regression line. This does not change with a restriction on X. The fact that it does not change is reflected by equation 4, which is part of the proof of theorem 1. It is obvious that a greater slope leads to greater chance of success in our task. The second is $\sigma_E$ . This does not change with a restriction in X. The fact that it does not change is reflected in equation 5, which is the other significant equation in the proof of theorem 1. It is clear that a smaller $\sigma_E$ leads to a greater chance of picking the correct individual. The way this is reflected in the equation

$$\rho_{X,Z} = b \frac{\sigma_X}{\sigma_Z} ,$$

is that if we make $\sigma_E$ smaller without changing $\sigma_X$ or b, then $\sigma_Z$ will become smaller. The third factor is the variance of X. It is clear that we have a better chance of choosing the correct individual if the X values of randomly chosen people are spread out rather than being packed together. This is the factor that is depressed as a result of selection. As already mentioned, for the type of selection in common use, the variance of $X^*$ is always smaller than the

variance of X. Hence, in the two variable case selection always causes under estimation of the correlation coefficient if the correction formula is not used.

One of the benefits of presenting a proof of theorem 1 and then discussing how the three factors affect correlation coefficients is that one can see how correlation estimation depends on linearity and homoscedasticity. If one or both of these conditions fails drastically then it will be very difficult to get a decent estimate. We will make a few comments about this problem at the end of this paper.

## IV. THREE VARIABLES WITH ONE EXPLICITLY RESTRICTED.

Let X be the explicit selection variable and let Y and Z be incidental selection variables.

**Assumption 1 :** The true regressions of Z on X, and Y on X, is linear.

**Assumption 2 :** The variance of Y given x, the variance of Z given x, and the covariance of Z and Y given x, do not depend on x.

**Theorem 2 :** Under assumptions 1 and 2

$$\rho_{Y,Z} = \frac{\rho_{Y^*Z^*} + \rho_{X^*,Y^*}\rho_{X^*,Z^*}\left(\frac{\sigma_X^2}{\sigma_{X^*}^2} - 1\right)}{\left[1 + \rho_{X^*,Y^*}^2\left(\frac{\sigma_X^2}{\sigma_{X^*}^2} - 1\right)\right]^{1/2}\left[1 + \rho_{X^*,Z^*}^2\left(\frac{\sigma_X^2}{\sigma_{X^*}^2} - 1\right)\right]^{1/2}}$$

This correction formula is slightly more complex and it allows us to construct examples where the correlation in the restricted population is larger than the correlation in the unrestricted population. Levin[4] refers to these cases as 'Pseudo-Paradoxical'. The terminology probably stems from the fact that it is widely assumed in

the literature that restriction always causes an underestimate. We would certainly predict this on the basis of our discussion in the two variable case. Notice that with the formula appearing in theorem 2 and a little algebra it is easy to characterize these 'Pseudo-Paradoxical' situations in the three variable case. In these cases the uncorrected estimation is an over estimation. Taking examples from Levin[4], we tried using the correction formula and the simulation showed each time that the corrected value was very good. It seemed that the corrected estimate was slightly low in each case but the estimate was so close that this low estimation might not be a real effect. In any case the bias appears to be so slight that it is not significant. The interesting fact is that the correction statistic based on theorem 2 works well in these cases, at least when the joint distribution of the three variables is multinormal.

## V. THE GENERAL CASE.

Let X be the p-element vector of explicit selection variables, and Y the n - p element vector of incidental selection variables on the applicant group. Then $X^*$ and $Y^*$ represent the explicit and incidental selection variables on the selected group. Let

$$V = \begin{bmatrix} V_{p,p} & V_{p,n-p} \\ V_{n-p,p} & V_{n-p,n-p} \end{bmatrix}$$

represent the variance-covariance matrix for $X^*$ , $Y^*$. The first p rows and columns refer to the components of $X^*$. So $V_{p,p}$ is the variance-covariance matrix of $X^*$, $V_{n-p,n-p}$ is the variance-covariance matrix for

$Y^*$, $V_{p,n-p}$ gives the covariances between $X^*$ and $Y^*$, and $V_{n-p,p}$ is the transpose of $V_{p,n-p}$. In this discussion V refers to selected data and W refers to applicant data. In our application V will be the estimates of the variance-covariance of all tests and it is based on selected data. The restricted population consist of those who were accepted into the organization so we have data on all tests for these people.

Let

$$W = \begin{bmatrix} W_{p,p} & W_{p,n-p} \\ W_{n-p,p} & W_{n-p,n-p} \end{bmatrix}$$

be the matrix of variance-covariances for the unselected data. We will estimate $W_{p,p}$ from the data since we have data for the explicit selection variables on all applicants. The $W_{p,n-p}$, $W_{n-p,p}$, and $W_{n-p,n-p}$ are the matrices that we wish to know and will be given to us by the theorem. $W_{n-p,p}$ is, of course, the transpose of $W_{p,n-p}$ so we will just give an expression for $W_{p,n-p}$ when we state the theorem. The following statement of the theorem is taken from [5].

Assumption 1 :(Linearity) For each j the true regression of $Y_j$ on X is linear.

Assumption 2 :(Homoscedasticity) The conditional variance-covariance matrix of Y given X does not depend on X.

Theorem 3 : Under assumptions 1 and 2

$$W_{p,n-p} = W_{p,p} V_{p,p}^{-1} V_{p,n-p} \quad \text{and}$$

$$W_{p,p} = W_{n-p,n-p} - V_{n-p,p}\left(V_{p,p}^{-1} - V_{p,p}^{-1} W_{p,p} V_{p,p}^{-1}\right) V_{p,n-p}$$

Lawley proved this theorem in 1943 using moment generating functions.

Both of the earlier theorems(1 and 2) are just special cases of theorem 3. With some algebraic manipulations the reader can verify this by writing out the entries of the matrix and comparing them with the formulas in the earlier theorems. Remember that the matrices of Lawley's theorem are variance-covariance matrices and so should be converted to correlation coefficients for the purposes of comparison.

Notice that the theorem says nothing about the types of restriction that are allowed. Restrictions of any type on the X variables will preserve the linearity and homoscedasticity. However a few runs of our simulation program seems to imply the following. If there are explicit restriction variables that are not known and hence not included in the equations of theorem 3, then the accuracy of the corrected statistic suffers. When you use population parameters of course this is no problem since we know that the formulas are exactly correct. However, we will always be using estimates of the population parameters as input to the equations and the empirical evidence indicates that missing explicit restriction variables cause problems. This conclusion certainly has intuitive appeal and it seems to agree with Mifflin and Verna[6].

## VI. GENERAL DESCRIPTION OF THE SIMULATION PROGRAM.

The program was written in PASCAL and is currently running on an IBM compatible micro computer. The joint distribution of all of the random variables is assumed to be multinormal in the unrestricted population. The inputs to the program are listed here for reference. They will be explained later as we discuss the program.

: the number of variables[nv] and their names[vname] :

: the mean and standard deviation of each variable in the unrestricted population[mu , sig] :

: the correlation coefficients of each pair of variables in the unrestricted population[rho] :

: the number of explicitly selected variables[nve] ; they are assumed to be the first nve variables that were entered :

: the number of restrictions[nr] :

: the coefficients of the explicitly selected variables and the cutoff value for each restriction[ncoeff , cutoff] :

: size of the unrestricted population[nwp] :

: number of people in the restricted population[nvp] :

: the number of times the experiment will be repeated[reps] :

: the positions of the two variables of interest in the list of variables[int1 , int2] :

Figure 1 on the next page is an example of a file describing the input to a run. The first line says that there are 3 variables in this case. The next three lines give the name, mean, and standard deviation of the three variables. In this case they each have mean 0.0 and standard deviation 1.0. The next three lines give the correlation matrix for the three variables. So the correlation coefficient for (x,y) is 0.86, for (x,z) it is 0.0, and for (y,z) it is 0.426. The next line gives the number of explicit selection variables. There is 1 in this case and so x is the only explicit selection variable. Next we see that there is only 1 restriction(selection) and then we see that the restriction is

$$x >= 0.0.$$

```
3
x       0.0   1.0
y       0.0   1.0
z       0.0   1.0
        1.0   0.86   0.0
        0.86  1.0    0.426
        0.0   0.426  1.0
1       # of explicitly restricted variables
1       number of restrictions
1.0     0.0
2  3    variables of interest
1  50   100
```

Figure 1.
AN INPUT FILE

So the selected group will consist of those persons getting a score of zero or greater on the X test. The second to last line says that the variables of interest are 2 and 3[Y and Z]. Data and a histogram of the distribution will be given for the uncorrected r between X and Z and the same information is given for the Pearson correction statistic. The program calculates the Pearson correction statistic using theorem 3 from the last section. The last line will be explained after the following discussion.

Creating a multinormal observation is equivalent to simulating one individual. In the above case this means getting three values, one for each of the three test scores X, Y, and Z. Each multinormal observation is part of the applicant group and is also a member of the selected group if the scores satisfy all of the restrictions. For the present case this means that the score on the X test must be at least zero.

One experiment is simulated by generating observations until two conditions are satisfied. There must be at least nwp observations in the applicant group and there must be at least nvp observations in the selected group. For most cases we set nwp = 1 and then the only restriction is that we have at least nvp observations in the selected group. One run of the program consists of simulating reps experiments. The last line of a file which describes a run gives nwp, nvp, and reps in that order. In figure 1 nwp = 1, nvp = 50, and reps = 100.

When program corr begins it will ask if the user wants to enter the data necessary to describe a run or to give the name of a file which contains the data in the expected format. The file in figure 1 is called test4 in my directory and so I can just give that name to corr and the run is specified by the input parameters in figure 1. The reason I know that test4 is in the expected format is because corr wrote the file in the first place. It was written when I ran corr and specified that I would enter the input data from the keyboard and that I wanted this data saved in a file named test4. Now if one is familiar with PASCAL read statements they could use a text editor to change some of the parameters and use test4 for another run. After corr executes, the data necessary to produce the histograms of the corrected and the uncorrected statistics are in two internal files and one must run program plot which will read these internal files and display this data on the printer.

For each experiment corr calculates each of the following quantities. Hence corr will generate reps copies of each of these parameters. In each case the two implied variables are int1 and int2, and the regression parameters are for int2 on int1.

: $b_0$ and $b_1$ = the estimates of the regression parameters :

: statu = the uncorrected estimate of correlation coefficient :

: statc = the corrected estimate of the correlation coefficient calculated with the equations of theorem 3 :

In the case of $b_0$ and $b_1$ the only values retained are the totals so that after the reps experiments have been generated the mean values of these parameters may be calculated. In the case of statu and statc each observed value is retained and written to the files pltu.dat and pltc.dat respectively. As mentioned earlier the user can run plot to have all these results displayed.

## VII. PROGRAM METHODOLOGY

One can see that the correction procedure, as specified in theorem 3, requires taking the inverse of a matrix. This is accomplished with the Gauss-Jordan matrix inversion algorithm in unit matops. This unit also contains algorithms to multiply and to subtract matrices.

Unit normgen includes all of the routines necessary to generate a multinormal observation with the correlations specified in the input file. Suppose that there are nv variables. The first step is to generate nv independent standard normal observations. This is accomplished by repeated calls to algorithm p in Knuth[7]. The desired multinomial transformation results from taking a linear transformation of these independent standard normal observations. This transformation is obtained by multiplying the independent observations and the matrix A which is defined to be that unique matrix which is upper triangular and satisfies $AA^T = C$. In this last equation $A^T$ refers to the transpose of A, and C is the variance-covariance matrix of all

variables in the unrestricted population. For a complete discussion of this procedure consult [8] or [9]. The matrix A is calculated by the recursive procedure solve called by transpar in unit normpar.

## VIII. Recommendations

Most of my time has been spent in writing the program. Hence most of my recommendations have to do with proposed applications of the tool. However, based on a limited amount of experimentation, a few observations seem appropriate.

The correction statistic seems to work well under the conditions of the theorem. It seems to have a downward bias but, for the cases we considered, it was always preferable over the uncorrected statistic. As can be seen from the proof of theorem 1, neither the corrected nor the uncorrected statistic will be accurate if the joint distribution of all variables fails to satisfy the linearity condition or the homoscedasticity condition. After fully understanding the theorem, and a little experimentation with the simulation program, it seems likely that the best strategy is to always use the Pearson statistic instead of the uncorrected statistic.

There are a number of studies that could be pursued with the use of the simulation program. I tried plotting the sampling distribution of the Fisher Z-transformation of the corrected statistic and it looked approximately normal as might be expected. We could find the mean and standard deviation of this normal distribution as a function of selection ratio, sample size, and other parameters that might be discovered to be significant. This work could form the basis for a procedure that could be used to construct confidence intervals for the true correlation coefficient based on the Pearson statistic. It might

be instructive to modify the program slightly so as to allow the joint distribution of all variables to be specified in the input. This would allow one to test the confidence intervals procedure using actual data instead of stochastically generated multinormal data.

It would be useful to know how much accuracy is lost in the corrected statistic when one or more explicit selection variables have been omitted from the model. With the multinormal distribution the hypothesis of theorem 3 is satisfied even if some explicit selection variables are omitted. However, based on a few experiments, the accuracy of the corrected statistic is diminished by the omission of explicit selection variables. It would be of value to know just how drastic this effect is. This is important since some people still use the two or three variable formulas even when there is more than one explicit selection variable. The simulation program is ideally suited to answer this question.

The program can be used to test the validity of the common F test. Using the uncorrected statistic to calculate an F value is probably a bad practice because of the inaccuracy of the uncorrected statistic. If the corrected statistic is used one would hope that the sampling distribution of F behaves as it should under the null hypothesis. This could be tested using the program with a slight modification.

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FINAL REPORT

# Evaluation of a Methodology for Estimating Cross-AFS Transferability of Skills


| | |
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| Date: | 19 August 1988 |
| Contract No: | F49620-87-R-0004 |

# Evaluation of a Methodology for Estimating Cross-AFS Transferability of Skills

by

Charles E. Lance

## ABSTRACT

A Skills and Knowledge Questionnaire (SKQ) was designed for the collection of experienced airmen's ratings of the job content of 47 selected Air Force Specialties (AFSs) for the purposes of: (a) evaluating the usefulness of an Occupational Measurement Center (OMC) skill/task taxonomy for assessing cross-AFS job content similarity, (b) examining the feasibility of measuring skill requirements using Subject Matter Expert (SME) judgments, and (c) identifying procedures for calculating cross-AFS relative ease-of-movement predictions. 'Part-of-Job,' 'Relative Time Spent,' and 'Months to Proficiency' ratings on 26 task categories were completed by 675 7-skill level respondents in 47 AFSs. Results indicated that (a) experienced airmen made reliable judgments about the task content of AFSs, (b) SKQ ratings effectively distinguished among AFSs on the basis of task content, and (c) one method for estimating cross-AFS relative ease-of-movement produced predictions which were consistent with AFS differences in Occupational Learning Difficulty and ASVAB aptitude area qualifying scores. Ease-of-movement predictions need to be validated against the actual ease with which airmen are able to attain proficiency in a new AFS.

## Acknowledgements

I thank the Air Force Systems Command, the Air Force Office of Scientific Research and AFHRL/MOD for sponsoring this research.

I also thank those that gave us the technical, informational, and moral support we needed to complete this research, including Mr. Bill Phalen, AlC Robert Zook, Ms. Connie Villareal, Mr. Bill Haynes, Mr. Cal Fresne, Ms. Doris Black, Ms. Janice Lazenby, Mr. Glen Ware, and Lt.Col. Rodger Ballentine.

Special thanks to SQNLDR Phil Davis for writing a BASIC program we needed to complete this research, for an introduction to Australia, and for social support this summer, to Dr. Jacobina Skinner for her assistance in finding, interpreting, and generating a copy of the Retraining Study data, to Lt.Col. Larry Short for supporting our summer work, Capt. Joe Filer for his ideas on estimating cross-AFS transferability of skills, and to Mr. Wayne Archer for his critical and insightful contributions at various stages of this research.

Finally, I am very grateful to Drs. Bruce Gould and Michael J. Kavanagh for the advanced planning of our summer work, for providing us with background literature prior to our summer work, for their work in designing and overseeing the administration of the SKQ, and for their support and critical contributions during the summer research period. Drs. Gould and Kavanagh helped make this summer work possible.

## I. INTRODUCTION

The question of the transferability of job skills has surfaced in the context of civilian occupational mobility (Byrne, 1975; Fine, 1957a, 1957b) transitions from military to civilian occupations (Mangum & Ball, 1987) design of formal educational curricula (Altman, 1976; Dillon & Horner, 1968; Pratzner, 1978) and skills obsolescence from changing technologies (Downs, 1985; Fossum, Arvey, Paradise, & Robbins, 1986; Rumberger, 1981).

U.S. Air Force (USAF) interest in estimating the transferability of job skills across Air Force Specialties (AFSs) stems from their potential use (a) as a component of the retraining Person Job Match system (PJM, Hendrix, Ward, Pina, & Haney, 1979) for optimizing reassignments, (b) to aid in AFS restructuring as in RIVET Workforce, (c) as input to long-range human resource planning (Dyer, 1982; Milkovich, Dyer, & Mahoney, 1983), for example, in the integration of Manpower, Personnel and Training (MPT) planning into the Weapon System Acquisition process (Askren & Eckstrand, 1980; Eckstrand, 1981), and (d) for consolidation of training courses for jobs that share common training requirements.

The present work was designed to extend research ongoing in the MPT Technology Branch of the Manpower and Personnel Division, Air Force Human Resources Laboratory (AFHRL/MOD) by (a) evaluating the appropriateness of a skill/task taxonomy developed by the USAF Occupational Measurement Center (OMC) (1984) for making cross-AFS comparisons of the similarity of job content, (b) examining the feasibility of measuring skill requirements using Subject Matter Expert (SME) judgments, and (c) identifying

procedures for estimating cross-AFS relative-ease-of-movement.

## II. OBJECTIVES OF THE RESEARCH EFFORT

The USAF Occupational Research job analysis data base is probably the largest of its kind in the world (Christal, 1974). However, its usefulness for assessing job content similarities across AFSs is extremely limited, since separate occupational surveys are developed for each USAF career ladder.

Earlier, Capt. Joe Filer and Dr. Michael J. Kavanagh (AFHRL/MOD) reviewed published and unpublished literature describing existing knowledge, skill, and ability taxonomies (e.g., Fleishman & Quaintance, 1984) which might facilitate cross-AFS job content comparisons. In a study conducted by USAFOMC (1984), a 26-category task taxonomy was developed that appeared particularly appropriate for this purpose (see Table 1). In earlier work, Capt. Filer demonstrated procedures by which the transferability of skills across task training modules, or AFSs, could be estimated as a function of (a) SME allocation of job tasks to the 26 OMC categories, (b) task learning difficulties, and (c) the amount of task content overlap and non-overlap.

Two questions addressed in the present research concerned (a) the usefulness of the OMC 26-category task taxonomy for estimating cross-AFS job content similarity, and (b) the feasibility of obtaining SMEs' direct judgments of the extent to which these categories were descriptive of the task content of incumbents' jobs. The third research objective was to suggest procedures by which SMEs' direct judgments could be used to predict relative transferability of skills across AFSs.

## III. PROCEDURE

During my pre-summer visit to AFHRL/MOD, I worked with Drs. Bruce Gould and Michael Kavanagh in designing a Skills and Knowledges Questionnaire (SKQ) which asked 7-skill level raters to complete a standard Background Information form, and make three judgments about tasks performed by fully qualified 5-skill level incumbents as they related to each of the 26 OMC task categories: (a) whether a typical 5-skill level incumbent performs tasks in the category (a binary Part-of-Job rating), (b) Relative Time Spent performing tasks in the category (0 = Not Performed, 1 = Very Small Amount, to 9 = Very Large Amount), and (c) typical Months to Proficiency on tasks within the category (1 = 0-1 months, 2 = 2 months, to 9 = 9 or more months).

Earlier, Dr. Kavanagh obtained data from the Military Personnel Center (AFMPC) summarizing frequencies of transfers (movements) across AFSs. In this study, 47 AFSs with the highest frequencies of movement (either 'out of' - to another AFS, or 'in to' - from another AFS) were targeted for data collection. Thirty 7-skill level incumbents within each of the 47 AFSs were randomly selected from airman personnel files as potential respondents. There were fewer than 30 incumbents in four AFSs targeted for data collection. In these cases, all available 7-skill level incumbents were selected to participate. A total of 1356 questionnaire packets containing a cover letter, an SKQ, rating instructions, definitions of the 26 task categories, and a return envelope, were mailed to selected participants the third week of June 1988. Participation was voluntary. A total of 675 questionnaires were returned by mail for a response rate of 50%.

## IV. ANALYSES AND RESULTS

Most respondents were pay grade E-6 (mean = 6.32, sd = .63), male (91%), High School graduates (mean education = 13.49 years, sd = 1.46) and supervised four others (mean = 4.09, sd = 7.69). Respondents' mean job tenure was 3.12 years (sd = 2.68), and mean Total Active Federal Military Service was 15.42 years (sd = 5.19).

Table 1 shows descriptive statistics for the Part-of-Job (POJ), Relative Time Spent (RTS), and Months to Proficiency (MTP) ratings. Here, means and standard deviations were calculated for RTS and MTP ratings only if the task category was indicated as being part of a typical 5-skill level job. Relatively few respondents endorsed POJ ratings for the Medical and Special Talents categories, whereas most respondents endorsed more general categories (e.g., Clerical, Computational, Physical Labor and Communication). Nearly all respondents reported that 5-skill level incumbents engage in some type of Training.

Respondents indicated that 5-skill level airmen spend relatively larger proportions of their time in Mechanical, Electrical/Electronic, Communication, problem solving, Supervising and Training activities. They also indicated that tasks in most of these areas required relatively longer training times to attain proficiency, while Physical Labor, and Clerical and Medical tasks were reportedly more quickly learned. These results are consistent with occupational learning difficulties established for AFSs comprised of tasks requiring longer learning times (Weeks, 1984), and thus provide general support for the validity of the ratings.

Table 1

Descriptive Statistics and Intraclass Correlations for SKQ Ratings

| Category | Part of Job | Relative Time Spent Mean* | Relative Time Spent S.D.* | Months to Proficiency Mean* | Months to Proficiency S.D.* | ICC (1,1) | ICC (1,k) |
|---|---|---|---|---|---|---|---|
| 1. Clerical | 82% | 4.66 | 2.32 | 3.14 | 2.22 | .294 | .853 |
| 2. Computational | 71% | 4.17 | 2.26 | 2.90 | 2.20 | .220 | .797 |
| 3. Office Equip Oper. | 71% | 4.19 | 2.31 | 2.46 | 1.99 | .285 | .847 |
| 4. Mechanical | 51% | 5.27 | 2.58 | 4.25 | 2.67 | .381 | .985 |
| 5. Simple Mech. | 55% | 4.92 | 2.31 | 3.31 | 2.32 | .292 | .851 |
| 6. Complex Mech. | 40% | 5.66 | 2.35 | 5.56 | 2.73 | .232 | .808 |
| 7. Mech-Electrical | 31% | 4.79 | 2.30 | 4.78 | 2.70 | .395 | .901 |
| 8. Mech-Electronic | 28% | 5.01 | 2.34 | 5.00 | 2.63 | .249 | .821 |
| 9. Electrical | 30% | 4.76 | 2.46 | 4.57 | 2.58 | .323 | .869 |
| 10. Electronic | 34% | 6.50 | 2.67 | 6.50 | 2.71 | .742 | .976 |
| 11. Electrical-Mech | 25% | 5.14 | 2.49 | 5.17 | 2.62 | .326 | .871 |
| 12. Elec'l-Elec'c | 26% | 5.38 | 2.67 | 5.38 | 2.70 | .452 | .920 |
| 13. Electronic-Mech | 30% | 5.20 | 2.52 | 5.12 | 2.85 | .493 | .931 |
| 14. Physical Labor | 71% | 4.40 | 2.31 | 1.62 | 1.47 | .272 | .838 |
| 15. Med-Patient Care | 5% | 3.92 | 3.42 | 3.11 | 2.76 | .461 | .972 |
| 16. Med-Equip Orient | 4% | 3.73 | 3.28 | 3.55 | 2.71 | .656 | .964 |
| 17. Med-Procedures | 6% | 3.75 | 2.69 | 2.63 | 2.56 | .461 | .922 |
| 18. Simp Nontech Procs | 68% | 4.78 | 2.18 | 2.66 | 2.04 | .045 | .395 |
| 19. Commun-Oral | 75% | 6.36 | 2.28 | 4.34 | 2.77 | .296 | .854 |
| 20. Comm-Written | 67% | 5.87 | 2.28 | 5.02 | 2.75 | .324 | .870 |
| 21. General Tasks | 63% | 5.67 | 2.14 | 4.12 | 2.45 | .070 | .510 |
| 22. Reasoning/Planning | 65% | 6.14 | 2.14 | 6.01 | 2.75 | .264 | .833 |
| 23. Science/Math | 35% | 5.03 | 2.36 | 4.97 | 2.70 | .309 | .862 |
| 24. Special Talents | 21% | 5.57 | 2.51 | 5.94 | 3.10 | .123 | .662 |
| 25. Supervisory | 67% | 5.27 | 1.89 | 6.51 | 2.66 | .085 | .564 |
| 26. Training | 90% | 6.06 | 1.95 | 6.42 | 2.56 | .098 | .602 |

*Note: Zeros were treated as 'missing data' in these columns.

Next, Intraclass Correlations (ICCs) were calculated for RTS ratings to assess (a) the extent to which AFSs can be differentiated on the basis of the ratings, (b) within-AFS interrater agreement on the time spent performing tasks in each category, and (c) potential rating unreliability due to ambiguity in the content or definition of rating categories. Responses that were 'missing' because a respondent indicated that the task category was not part of the job being rated, were coded '0' to indicate 'No time spent,' and treated as valid values. ICC(1,1) in Table

1 indexes the reliability of a single rater's rating, and ICC(1,$\underline{k}$) the reliability of the mean of $\underline{k}$ judges' ratings (Shrout & Fleiss, 1979). In the present case, there was an mean of 12.9 respondents from each AFS surveyed (i.e. $\underline{k}$ = 12.9).

ICCs were relatively low for two categories having high mean POJ ratings: Supervisory and Training. This could indicate that (a) nearly all of the AFSs surveyed have training and supervisory requirements, (b) actual requirements vary with specific job assignments within an AFS, or (c) agreement among respondents on these requirements was low. ICCs were also lower for the Simple Nontechnical Procedures, General Tasks, and Special Talents categories. This is likely due to ambiguities in these categories' task content and definitions in the rating instructions. ICCs for the remaining categories were high.

Zero-order (Pearson) correlations among RTS ratings were computed to assess potential redundancy among the SKQ rating categories. Note that high correlations among the categories could either indicate category redundancy, or a high degree of co-performance between tasks in different categories. On the other hand, low correlations could either reflect the independence of the categories, or restricted ranges of values on one or more of the categories. Correlations among the RTS ratings are shown in Table 2.

Correlations were relatively high (a) between Clerical and Office Equipment (1 and 3), (b) between Computational and Scientific Math Reasoning or Calculations (2 and 23), (c) between the two Communication categories (19 and 20), (d) between Supervision and Training (25 and 26), (e) among Medical

Table 2

Zero-Order Correlations Among SKQ Relative Time Spent Ratings

| Task Category | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. Clerical | 1.00 | | | | | | |
| 2. Computational | .31 | 1.00 | | | | | |
| 3. Office Equip Oper. | .53 | .30 | 1.00 | | | | |
| 4. Mechanical | -.24 | -.08 | -.21 | 1.00 | | | |
| 5. Simple Mech. | -.16 | -.04 | -.15 | .62 | 1.00 | | |
| 6. Complex Mech. | -.20 | .01 | -.22 | .53 | .51 | 1.00 | |
| 7. Mech-Electrical | -.24 | -.04 | -.21 | .62 | .55 | .60 | 1.00 |
| 8. Mech-Electronic | -.17 | .02 | -.16 | .50 | .41 | .55 | .72 |
| 9. Electrical | -.16 | .04 | -.15 | .46 | .37 | .44 | .64 |
| 10. Electronic | -.20 | -.01 | -.22 | .28 | .13 | .30 | .33 |
| 11. Electrical-Mech | -.17 | .00 | -.16 | .41 | .35 | .41 | .60 |
| 12. Elec'l-Elec'c | -.16 | .04 | -.17 | .30 | .24 | .32 | .46 |
| 13. Electronic-Mech | -.18 | .01 | -.21 | .35 | .24 | .35 | .47 |
| 14. Physical Labor | -.09 | -.01 | -.11 | .45 | .45 | .30 | .31 |
| 15. Med-Patient Care | .13 | .11 | .04 | -.04 | -.01 | -.04 | -.05 |
| 16. Med-Equip Orient | .08 | .10 | .00 | -.03 | .00 | -.04 | -.04 |
| 17. Med-Procedures | .05 | .06 | .02 | -.02 | -.00 | -.03 | -.01 |
| 18. Simp Nontech Procs | .04 | .05 | .05 | .14 | .18 | .04 | .14 |
| 19. Commun-Oral | .31 | .26 | .34 | -.11 | -.11 | -.10 | -.09 |
| 20. Comm-Written | .38 | .37 | .38 | -.13 | -.15 | -.13 | -.11 |
| 21. General Tasks | .12 | .14 | .16 | .02 | .10 | -.02 | .05 |
| 22. Reasoning/Planning | .26 | .32 | .29 | -.22 | -.14 | -.12 | -.14 |
| 23. Science/Math | .10 | .50 | .11 | .01 | -.02 | .10 | .04 |
| 24. Special Talents | .07 | .15 | .13 | -.01 | .02 | .08 | -.00 |
| 25. Supervisory | .10 | .03 | .12 | .12 | .10 | .13 | .11 |
| 26. Training | .01 | .02 | .02 | .22 | .13 | .14 | .13 |

Table 2 (continued)

| | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|
| 8. Mech-Electronic | 1.00 | | | | | | |
| 9. Electrical | .63 | 1.00 | | | | | |
| 10. Electronic | .50 | .59 | 1.00 | | | | |
| 11. Electrical-Mech | .61 | .80 | .59 | 1.00 | | | |
| 12. Elec'l-Elec'c | .56 | .70 | .70 | .76 | 1.00 | | |
| 13. Electronic-Mech | .60 | .64 | .75 | .73 | .77 | 1.00 | |
| 14. Physical Labor | .20 | .19 | .10 | .16 | .10 | .13 | 1.00 |
| 15. Med-Patient Care | -.05 | -.04 | -.07 | -.05 | -.07 | -.07 | .04 |
| 16. Med-Equip Orient | .00 | -.03 | -.06 | -.03 | -.04 | -.05 | .03 |
| 17. Med-Procedures | .02 | .01 | -.05 | .02 | -.04 | -.03 | -.01 |
| 18. Simp Nontech Procs | .10 | .09 | -.09 | .03 | .00 | -.03 | .29 |
| 19. Commun-Oral | -.06 | -.07 | -.18 | -.07 | -.10 | -.12 | -.04 |
| 20. Comm-Written | -.06 | -.07 | -.15 | -.07 | -.07 | -.13 | -.11 |
| 21. General Tasks | .01 | .00 | -.18 | -.02 | -.05 | -.09 | .10 |
| 22. Reasoning/Planning | -.06 | -.06 | -.14 | -.09 | -.06 | -.12 | -.17 |
| 23. Science/Math | .12 | .12 | .11 | .11 | .11 | .14 | -.08 |
| 24. Special Talents | .08 | .01 | -.02 | .01 | .01 | -.01 | -.05 |
| 25. Supervisory | .15 | .13 | .05 | .14 | .13 | .12 | .11 |
| 26. Training | .13 | .17 | .14 | .14 | .12 | .16 | .18 |

Table 2 (continued)

| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|
| 15. Med-Patient Care | 1.00 | | | | | | |
| 16. Med-Equip Orient | .72 | 1.00 | | | | | |
| 17. Med-Procedures | .59 | .70 | 1.00 | | | | |
| 18. Simp Nontech Procs | -.00 | .04 | .05 | 1.00 | | | |
| 19. Commun-Oral | -.00 | -.03 | -.01 | .16 | 1.00 | | |
| 20. Comm-Written | .06 | .01 | -.02 | .09 | .59 | 1.00 | |
| 21. General Tasks | .00 | -.02 | .03 | .30 | .30 | .29 | 1.00 |
| 22. Reasoning/Planning | .07 | .02 | .01 | .05 | .46 | .48 | .28 |
| 23. Science/Math | .08 | .11 | .08 | .00 | .18 | .26 | .10 |
| 24. Special Talents | .07 | .05 | -.01 | .07 | .25 | .24 | .23 |
| 25. Supervisory | .08 | .02 | .06 | .10 | .21 | .19 | .13 |
| 26. Training | .02 | .01 | .01 | .14 | .24 | .19 | .16 |

Table 2 (continued)

| | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|
| 22. Reasoning/Planning | 1.00 | | | | |
| 23. Science/Math | .31 | 1.00 | | | |
| 24. Special Talents | .28 | .23 | 1.00 | | |
| 25. Supervisory | .11 | -.01 | .16 | 1.00 | |
| 26. Training | .15 | .08 | .11 | .46 | 1.00 |

For $|r| \geq .08$, $p < .05$, for $|r| \geq .10$, $p < .01$, two-tailed.

categories (Categories 15-17), and (f) among Mechanical and Electrical/Electronic categories (4-13). That several of the SKQ category correlations were very high suggested that some of them could be collapsed with little loss in power to discriminate among AFSs or groups of similar AFSs.

To further assess commonalities among the SKQ categories, a Principal Components Analysis (PCA) of RTS ratings was conducted. An examination of a plot of the first 15 eigenvalues of the 26 x 26 correlation matrix suggested four to six components should be retained. The six component solution, with components rotated to the VARIMAX criterion, provided the most meaningful and interpretable solution. Table 3 shows significant loadings (approximately .400 or higher) from the six-component solution.

Table 3

Principal Components Analysis of Relative Time Spent Ratings

| Task Category | Principal Component I | II | III | IV | V | VI |
|---|---|---|---|---|---|---|
| 1. Clerical | | | | | .800 | |
| 2. Computational | | | | .523 | .475 | |
| 3. Office Equip Oper. | | | | | .763 | |
| 4. Mechanical | | .713 | | | | |
| 5. Simple Mech. | | .780 | | | | |
| 6. Complex Mech. | .393 | .571 | | | | |
| 7. Mech-Electrical | .544 | .626 | | | | |
| 8. Mech-Electronic | .688 | .411 | | | | |
| 9. Electrical | .807 | | | | | |
| 10. Electronic | .826 | | | | | |
| 11. Electrical-Mech | .850 | | | | | |
| 12. Elec'l-Elec'c | .880 | | | | | |
| 13. Electronic-Mech | .880 | | | | | |
| 14. Physical Labor | | .692 | | | | |
| 15. Med-Patient Care | | | .922 | | | |
| 16. Med-Equip Orient | | | .870 | | | |
| 17. Med-Procedures | | | .853 | | | |
| 18. Simp Nontech Procs | | .469 | | | | |
| 19. Commun-Oral | | | | .477 | .419 | .387 |
| 20. Comm-Written | | | | .525 | .499 | |
| 21. General Tasks | | | | .409 | | |
| 22. Reasoning/Planning | | | | .651 | | |
| 23. Science/Math | | | | .687 | | |
| 24. Special Talents | | | | .653 | | |
| 25. Supervisory | | | | | | .744 |
| 26. Training | | | | | | .741 |

The first two components were clearly interpretable I - Electrical/Electronic, and II - Mechanical/Maintenance, but there was some overlap in the content represented by the components (i.e., Items' 6-8 loadings). Component III represented Medical-related duties, Component IV - Technical activities, Component V - Clerical, and Component VI - Managing/Developing others. General Verbal and Quantitative activities (Categories 2, 19, and 20) overlapped more than one content area. These results corroborate conclusions suggested from examination of category intercorrelations: Electrical/Electronic (Categories 9-13),

Medical (Categories 15-17), Clerical and Office Equipment (1 and 3), and Managing/Developing items (25 and 26) appeared to relate to similar task content areas.

A reduced set of unit-weighted SKQ rating composites was defined from PCA results in Table 3. Electrical (Items 9-13), Mechanical (Items 4-6, 14, and 18), Medical (Items 15-17), Clerical (Items 1-3), Technical (Items 22-24), and Managing (Items 25 and 26) composites were formed on the basis of items' significant univocal component loadings in Table 3. Separate Mechanical/ Electrical (Items 7 and 8) and Communication (Items 19 and 20) composites were formed because these items appeared to overlap more than one task content area. AFSs were also grouped according to their ASVAB MAGE qualifying aptitude area: Mechanical (MECH), Administrative (ADMIN), General (GEN), or Electrical (ELEC), to determine the extent to which the SKQ composites differentiated among the MAGE areas. Mean RTS composite ratings and ICCs are shown in Table 4 [for ICC(1,k) in Table 4, k = 148.5].

RTS composite means tended to be highest in appropriate MAGE areas. However, Means for the Managing composite were fairly uniform across MAGE areas, indicating common supervisory requirements across most AFSs. For the most part, ICCs for the RTS composites were also high, indicating reliable cross-MAGE area differences in task content, and high within-area aggreement. Two exceptions were (a) the Medical composite, on which RTS ratings' ranges were restricted, and (b) the Managing composite, on which MAGE areas differed little.

Table 4

SKQ Relative Time Spent Composite Means and ICCs for MAGE Areas

| Rating Composite | SKQ Relative Time Spent | | | | Intraclass Correlations | |
|---|---|---|---|---|---|---|
| | MECH | ADMIN | GEN | ELEC | ICC(1,1) | ICC(1,k) |
| 1. Electrical | 5.15 | 1.43 | 2.75 | 23.18 | .590 | .995 |
| 2. Mechanical | 26.54 | 9.90 | 11.41 | 16.29 | .285 | .983 |
| 3. Mech/Elec | 6.24 | .62 | 1.50 | 5.19 | .266 | .982 |
| 4. Medical | .16 | .48 | 1.15 | .18 | .019 | .743 |
| 5. Clerical | 6.48 | 13.40 | 10.94 | 7.39 | .201 | .974 |
| 6. Communication | 5.80 | 9.57 | 10.84 | 6.32 | .144 | .962 |
| 7. Technical | 2.98 | 7.16 | 9.69 | 5.26 | .158 | .966 |
| 8. Managing | 9.13 | 8.31 | 9.11 | 9.75 | .009 | .587 |

A final goal of this study was to suggest procedures for predicting cross-AFS ease-of-movement (EOM). This work drew on Capt. Joe Filer's earlier work, and was aided by a BASIC program written by SQNLDR Phil Davis (AFHRL/MOD). Input data were SKQ category MTP ratings in which nonresponses (indicating that tasks in a category were not performed) were coded '0' to indicate 'Zero months to proficiency.' First, mean SKQ category MTP ratings were computed for each AFS in which there were at least five respondents. Next, AFS mean vectors (e.g., X1 and X2) were compared pairwise to calculate two sums: (a) differences between X1 and X2 values for which X1 values were larger (i.e. $\Sigma_i (X1_i - X2_i)$, only if $X1_i > X2_i$, and zero otherwise, indicating skills to be acquired in moving from AFS2 to AFS1, and (b) differences between X1 and X2 values for which X2 values were larger (i.e. $\Sigma_i (X2_i - X1_i)$, only if $X2_i > X1_i$, and zero otherwise, indicating

skills to be acquired in moving from AFS1 to AFS2). A total of 1806 EOM predictions were calculated for 43 AFSs.

Table 5

Relative Transferability Predictions Across Selected AFSs

| "From" AFSC | OLD | MAGE | "To" AFSC 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. 411x1c | 130 | M-51 | 0 | 12.0 | 22.7 | 18.3 | 32.5 | 6.3 | 23.2 | 25.1 |
| 2. 426x2 | 154 | M-44 | 6.9 | 0 | 21.0 | 17.7 | 32.1 | 7.0 | 26.0 | 26.4 |
| 3. 661x0 | 125 | A-61 | 30.6 | 34.0 | 0 | 9.2 | 24.6 | 3.8 | 39.0 | 29.7 |
| 4. 732x0 | 80 | A-45 | 25.8 | 30.3 | 8.8 | 0 | 24.0 | 1.7 | 34.5 | 26.5 |
| 5. 272x0 | 98 | G-43 | 19.8 | 24.4 | 3.9 | 3.8 | 0 | 0.6 | 27.4 | 16.9 |
| 6. 811x0 | 92 | G-35 | 28.8 | 34.7 | 18.4 | 17.7 | 35.8 | 0 | 40.1 | 35.7 |
| 7. 305x4 | 120 | E-67 | 10.9 | 18.8 | 18.8 | 14.7 | 27.9 | 5.4 | 0 | 11.8 |
| 8. 493x0 | 126 | E-67 | 14.3 | 20.6 | 10.9 | 8.2 | 18.9 | 2.3 | 13.2 | 0 |

For illustrative purposes, Table 5 shows relative transferability predictions for two AFSs within each of the MAGE Aptitude Areas (a) Mechanical: 411x1c - Missile Maintenance (BGM-109 GLCM), and 426x2 - Jet Engine Mechanic, (b) Administrative: 645x0 - Inventory Management, and 732x0 - Personnel, (c) General: 272x0 - Air Traffic Control Operator, and 811x0 - Security Specialist, and (d) Electrical: 305x4 - Electronic Computer and Switching Systems Specialist, and 493x0 - Communications-Computer Systems Control. Tabled estimates cannot be interpreted in any absolute sense (e.g., number of months retraining time required), but they do indicate predicted relative cross-AFS ease-of-movement (smaller numbers represent easier transitions). Also

shown in Table 5 are the AFSs' occupational learning difficulties (OLDs), and MAGE qualifying score (MAGE).

Several conclusions are suggested by results in Table 5. First, predicted ease-of-movement is generally easier within, rather than across MAGE areas. Second, movement from some AFSs tends to be generally easier (e.g. 272x0, and 493x0) or more difficult (e.g., 811x0), than from others. Third, movement into some AFSs also tends to be easier (e.g., 811x0) or more difficult (e.g., 426x2 and 305x4), than into others. Fourth, movements out of Mechanical AFSs or into Electrical AFSs tend to be more difficult. Finally, ease-of-movement predictions are similar to those which might be made on the basis of OLDs or MAGE qualifying scores, thus indicating some convergence among the three indices as indicators of job difficulty.

## V. CONCLUSIONS

1. Experienced airmen (7-skill level) were able to make reliable judgments on several task categories about the job content of 5-skill level airmen. This conclusion is supported generally by high interrater agreement indices (ICCs), and mean differences in composite RTS ratings corresponding to differences in aptitude requirements. Thus questionnaire measures such as the SKQ may be a viable means of assessing AFS task content for the purpose of cross-AFS comparisons.

2. SKQ category ratings differentiated among AFSs' task content. This was particularly clear when AFSs grouped into MAGE areas were differentiated by RTS composites defined by the PCA of SKQ items. However, the rating categories themselves should be

revised: (a) several redundant categories (e.g., Mechanical, Simple Mechanical and Complex Mechanical) should be collapsed into fewer, more distinct ones, (b) three categories' task domains are not clearly defined (Simple Nontechnical Procedures, General Tasks or Procedures, and Special Talents) and should be clarified or deleted, and (c) task categories should be defined to represent content areas in AFSs that are not currently represented in the SKQ. Mayfield and Lance (1988) make appropriate recommendations for a revised taxonomy of AFS skills.

3. A procedure for estimating cross-AFS relative ease-of-movement demonstrated interpretable predictions that paralleled AFS differences in OLDs and MAGE area qualifying scores. Predictions indicated meaningful differences in the ease-of-movement between specific AFSs, and within and between MAGE areas. This procedure, which requires general estimates of job content by SMEs, may be a cost effective method for deriving larger numbers of cross-AFS ease-of-movement predictions in the future.

## VI. RECOMMENDATIONS FOR FUTURE FUTURE RESEARCH

1. One of the most critical research needs, the development of a comprehensive task taxonomy for cross-AFS task content comparisons, is now being addressed by Mayfield and Lance (1988).

2. A second critical research need is an evaluation of the revised taxonomy proposed by Mayfield and Lance (1988), in terms of (a) the distinctness of its task categories, (b) its usefulness for discriminating among AFSs task content, and (c) its ability to generate accurate cross-AFS ease-of-movement

predictions. This could be accomplished as part of follow-on research involving larger numbers of airmen and AFSs.

3. A third critical research need is to validate relative ease-of-movement predictions derived either here, or from future studies cross-AFS skill transfer. Hook and Massar (1962) reported results of USAF Command and Staff College students' global estimates of crosstraining time required for cross-AFS transfers. However, the results they reported were limited, and the AFS structure has changed considerably since the 1960's. In one ongoing AFHRL/MOD project, SME global judgments of relative cross-AFS ease-of-movement are being obtained. Results from this study could be used as one means of corroborating ease-of-movement predictions derived here. The real need, however, is to validate relative ease-of-movement predictions, derived either from SME global judgments, or from SME judgments about task content areas, against the actual ease with which airmen were (or are) able to crosstrain and attain proficiency. This type of validation can be accomplished retrospectively, using data collected as part of an earlier series of studies (Skinner, 1983; Skinner & Alley, 1980, 1984) on the performance of retrained airmen in technical training, and their adjustment to the new assignment. Ultimately, however, a longitudinal, prospective study is needed in which airman performance, performance determinants, and adjustment to work assignments, is tracked prior to, during, and subsequent to retraining, in order to validate relative ease-of-movement predictions.

4. One research need that is as important, but less urgent

than the first three, concerns anticipated payoffs from basing reassignment decisions, in part, on predictions of the relative ease with which airmen are able to transition from various AFSs to others. Traditional approaches to utility analysis will find limited application to this problem due to its reliance on monetary payoff metrics, linearity and normality requirements, and the complexity of algebraic solutions to even static multistate job systems. However, recent work in the area of cumputer simulation modeling approaches to utility analysis, and human resource planning (HRP) in general, (Lance, 1987; Ledvinka & Ladd, 1987) have argued that payoff estimates associated with alternative personnel/human resources interventions are tractable even in dynamic, multistate, and interdependent job systems. Future work in this area could, for example, estimate differences in the overall payoffs associated with alternative personnel classification algorithms, retraining priorities, WS support structures, or airman retention programs

6. There is a continuing need for an Air Force-wide work inventory. Occupational survey data well support many USAF personnel functions, but the potential for conducting cross-AFS comparisons is limited, at best. Several general job analysis instruments exist (e.g., Cunningham, Boese, Neeb, & Pass, 1983; Jeanneret, McCormick, & Mecham, 1977; Patrick & Moore, 1985) but there are no inventories currently operational in the USAF that could be used to assess cross-AFS differences in task content. This final recommendation is for a broader, longer-term project, to develop a general Air Force work inventory for assessing AFS task content, and conducting cross-AFS comparisons.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

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FINAL REPORT


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# An Expert System Approach for Reliability Data Analysis

by

Thomas L. Landers, Ph.D., P.E.

## ABSTRACT

An expert system approach was investigated for statistical analysis of failure data in a RAMCAD environment. The research emphasized definitions of the concept, functional requirements and knowledge base. The NASA CLIPS expert system shell was selected for prototyping. The project used actual field data, from the F-16 central air data computer and radar power supply, for purposes of testing and demonstration. The expert system approach proved to be feasible for aiding engineers in failure data analysis. A rule-based expert system shell was suitable for prototyping but additional research is needed to determine resource requirements for a full-scale application.

## Acknowledgements

The SFRP Fellow is indebted to several Air Force personnel who have materially contributed to this research effort. Mr. Alan Herner (AFHRL/LRA) has been very helpful as Effort Focal Point. He has provided both support and guidance. Mr. Mark Hoffman has provided resources and assistance enabling the use of NASA CLIPS software in this research and Mr. John Ianni has made available all needed computing resources. Personnel in the F-16 System Program Office (Aeronautical Systems Division) have been very cooperative in providing case study data for use in the project. Special thanks are due Gary Arnold, Jim Bohren and Ruth Brewer, all of ASD/YP.

# I. INTRODUCTION

## A. Research Need

Engineers need to consider reliability as an objective, during the normal course of the development process. Reliability (failure) data is often available from development test, production screen and/or field operations. However, the theory and tools are not available to most engineers to effectively use this data for system design and improvement. At the same time, the theory and tools of reliability engineering, data analysis and computing continue to advance in capability. The proportional hazards theory (reference Cox [4], Lawless [8], Kalbfleisch and Prentice [7]) and the repairable systems approach (Cox and Lewis [5], Engelhardt and Bain [6], Ascher and Feingold [1]), are examples of powerful capabilities not yet widely applied by engineers.

The Air Force has taken important initiatives to promote integration of reliability, maintainability and supportability into engineering curriculum. Included within this curriculum objective is the introduction and proper use of fundamental concepts in probability and statistics. The AFIT Engineering Design Workshops for faculty and the RAMCAD Task 3 (RAMCAD curriculum [13]) are specific examples.

These programs are long-range and potentially far-reaching in impact. However, there are numerous competing pressures on engineering curriculum, including rapidly advancing technology and the humanities and social sciences. There is also a large population base of practicing engineers who are already outside of the academic environment.

The University of Maryland has developed a prototype expert system to assist engineers in RAMCAD design of electronic circuit boards [14]. This work was funded by the Institute for Defense Analysis as part of the Department of Defense (DoD) RAMCAD initiative. The Maryland system employs a component reliability data base similar to MIL-HDBK-217 [15] and permits thermal and mechanical analyses and board-level design in a stand-alone work station. This system does not currently include facilities to analyze actual test and field data on resulting or analogous designs. The Maryland system can easily interface with a system or module to perform this function and serves as an excellent model for conceptual design of an expert system for data analysis.

B. Fellow's Background and Motivation

Throughout his career the Fellow has been active in theoretical and applied work on reliability and maintainability (R&M) topics. He served as R&M engineer in the F-16 System Program Office, from prototype fly-off through full scale development. He has also been in quality assurance and warranty administration in the automotive industry. Since beginning Ph.D. work in 1983, the Fellow has focused research attention on statistical analysis of failure data and proportional hazards reliability modeling. His dissertation addressed these topics as part of the Pulsed Power Program, Strategic Defense Initiative. This background has motivated keen interest in Air Force R&M problems, and has prepared the Fellow to conduct this program of research.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The need has arisen for an expert system tool enabling the engineer to benefit from the probabilistic and statistical

approaches to reliability, without the necessity of a lengthy educational process.

The primary goal of this research was to investigate an expert system approach for reliability data analysis and modeling. Accomplishment of this goal involved definition of the knowledge base, conceptual design, requirements definition and technology assessment. In order to prove the concept, a rule-based expert system prototype was developed. There have been major recent advancements in the capabilities of discrete event simulation. A secondary goal of this research was to investigate the potential for interfacing a simulation module with the expert system. The resulting simulation module enables use of expert system outputs for purposes of modeling and trade-off studies among design or redesign alternatives. This work was discussed in a separate report, prepared by the assigned Graduate Student Research Fellow.

The research approach included a literature search within the defense documentation indexes to indentify and review relevant literature and technical reports. This step was necessary since numerous technical reports are prepared for the Department of Defense by contractors; however, many are not abstracted or indexed in the public domain.

The Fellow formulated a conceptual design and documented functional requirements, including knowledge base rules, in the form of a preliminary specification. Actual Air Force data from the F-16 was used for purposes of demonstration and validation.

## III. KNOWLEDGE BASE

The Fellow defined the relevant knowledge base through a

synthesis of personal experience and review of the literature. The concept and requirements for an exert system approach were defined and are documented in Appendix A to this report. There are two main classes of failure data: nonrepairable (throwaway) item data and repairable item data. Most of the knowledge base (theoretical and applied) pertains to nonrepairable items. However, most Air Force systems are designed to be repaired. The knowledge base is much less developed for repairable items. In the past, techniques for nonrepairable items have been applied to the analysis of repairable items, with disappointing results [1].

A major goal of the expert system is to assure that repairable item failure data is properly analyzed. For this reason, the summer research focused on the knowledge base (decision rules) necessary to identify and properly analyze the chronological pattern of failures on a repairable item.

In recent years there has been important work done in the medical field (biostatistics) which has potential application in reliability engineering. The work of Cox [4] and others [7,8] has produced reliability models accounting for the effects of explanatory variables (such as environment or application) on reliability. These techniques are referred to as covariate models. The Fellow has substantial experience applying these covariate models to engineering applications. A potential goal for the expert system is to facilitate use of the parametric (e.g., Weibull) and nonparametric (Cox) covariate models. Time during the summer research project did not permit attainment of this goal. The Fellow plans to continue work on this objective in the future.

## IV. PROTOTYPE

A prototype rule-base was developed in NASA CLIPS. Fourteen (14) major functions were implemented in 60 CLIPS rules. The rules are heavily commented with screen prompts which facilitate both validation and maintenance. The CLIPS base of 60 rules required 800 lines of code and consumed 23 kilobytes of disk storage. The CLIPS window-version executable file requires 276 kilobytes of overhead to process the rule-base. Due to time constraints, the Fellow elected to use SAS-PC to mimic several major functions of the expert system. SAS-PC requires 10 megabytes of disk storage, but provides very powerful procedures and utilities. The Fellow developed a SAS program (63 lines, 1.8 kilobytes disk storage) to invoke the SAS capabilities.

The prototype was intended as a vehicle for developing and testing rules in the knowledge base. It has an interactive, command-line interface. The user is prompted for input of qualitative data, e.g.,

"Are you analyzing a repairable item?"

Quantitative data (i.e., failure data) is read in to the system automatically from disk in ASCII sequential format. The system provides feedback to the user, in the form of screen messages, as rules fire. This feedback is essential for validation in the process of developing an expert system. The messages give insight to the flow of program control and serve as documentation of the rule-base and CLIPS code.

The rule-base includes the following functions:

1. Repairable vs. nonrepairable
2. Complete history vs. incomplete history
3. Number of units

4. Number of failures
5. File data input/output
6. Chronological time-line plot of failure data
7. Chronological plot of cumulative time versus cumulative failures
8. Visual trend detection
9. Laplace test (trend)
10. Increasing vs. decreasing trend
11. Lewis Robinson test (renewal)
12. Pearson product-moment test (serial dependence)
13. Nonhonogeneous Poisson Process, power law intensity
14. Univariate vs. covariate

In order to implement a function, the Fellow documented the relevant knowledge base, in the form of a specification. The specification included the pseudo-code for rules (if-then-else constructs). Finally, the pseudo-code was translated into CLIPS syntax and tested. Appendix B is a sample specification for the Laplace test statistic.

CLIPS is intended for pattern matching, and is not sufficiently accurate in computations. Therefore, calculations of test statistics require C programming of external functions. These functions are called from within CLIPS rules and return calculation results as parameters back to the calling rule.

The expert system functional requirements include graphical outputs of plotted data and estimation of model parameters, by the method of maximum likelihood. These facilities could require a large programming effort. As an alternative for prototyping, the built-in procedures of SAS-PC were used to demonstrate concepts and mimic expert-system outputs. A SAS-PC program implements the following functions:

1. Chronological time-line plot of failure data
2. Chronological plot of cumulative time vs. cumulative failures
3. Weibull plot
4. Weibull parameter estimates by method of maximum likelihood
5. Quantile estimates of Weibull distribution (predicted)
6. Weibull distribution (predicted) plot with 90% confidence interval.

## V. CASE STUDY

The Fellow contacted personnel in the F-16 System Program Office, to secure case study data. The F-16 CDS proved to be a useful source of data for repairable systems. The CDS implements part number and serial number (PN/SN) tracking for designated line-replaceable units (LRUs). The PN/SN history report includes several data fields, such as Julian date, how malfunctions, action taken, elapsed time indicator (ETI) reading and a free-form descriptor of fault and corrective action.

The history of EIT readings provides the best source of data. However, EIT meters are known to be unreliable themselves and are not alwavs replaced with a properly set ETI when failed. There also is inconsistency and the risk of clerical errors in the recording of ETI readings. Recently, there is an apparent trend at some bases to ignore the ETI records and even to abandon maintenance of the CDS data base. These types of problems are typical for field data and make many of the PN/SN history records unusable for the purposes of this research. The Fellow was able to identify several LRUs with adequate quality of CDS data, including the following two

units:

1. Central Air Data Computer (CADC)
   WUC: 51FA0
   Part Number: 4025116905
   Serial Number: 183

2. Radar Power Supply (RPSP)
   WUC: 74AQ0
   Part Number: 758R875G01
   Serial Number: 141

The CADC had failures at ETI readings 1644, 1785, 2532, 2637, 2641, 2643. Figure 1(a) contains the chronological time-line plot. This plot was produced using SAS-PC and clearly indicates a trend toward increasing rate of occurrence of failures (ROCOF). The successive intervals are clearly not independent and identically distributed. The Laplace test for trend supports the visual evidence. Therefore, this CADC should not be modeled with a distribution such as the exponential or Weibull.

Radar Power Supply 10141 experienced failures at ETI readings: 373, 580, 723, 1098 and 1298. Figure 1(b) depicts the chronological pattern of events. There is not a clear trend, and the statistical tests in the expert system did not detect a trend. Although the sample size in this example is somewhat small, the pattern illustrates the situation where repairable system failure intervals can be modeled as independent and identically distributed random variables.

## VI. RESULTS AND CONCLUSIONS

This research has defined the approach and a portion of the knowledge base for an expert system, to assist engineers in statistical analysis of failure data. Appendix A of this report discussses the conceptual design and functional requirements for a general system and a prototype. The major tasks in this program have been the specification of functions in the knowledge base and development of a prototype.

The task of failure data analysis is complex, with many pitfalls, and requires the involvement of specialists. This involvement can be personal consultation, capture of knowledge in an expert system, or some combination of these approaches. The thesis of this research has been that the expertise could be captured in a computer-based tool, for use by engineers. Based on progress thus far, the approach seems feasible. However, it is probably not practical or prudent to require the system to cover all possibilities. The task of developing such a system would be enormous. The preferred approach is for the system to cover the most common and important cases, and help the user to avoid common pitfalls. The system should advise the user to seek assistance of a human expert when appropriate (e.g., when beyond the scope of the system, or when results are too ambiguous).

The task under study has proven to be mostly procedural in nature. While an expert system approach is feasible, software development in a procedural language (such as FORTRAN, C or Ada) would also be appropriate, since the task is data-driven and the program flow depends upon execution of if-then-else constructs.

CLIPS has proven to be a good tool for prototyping. The

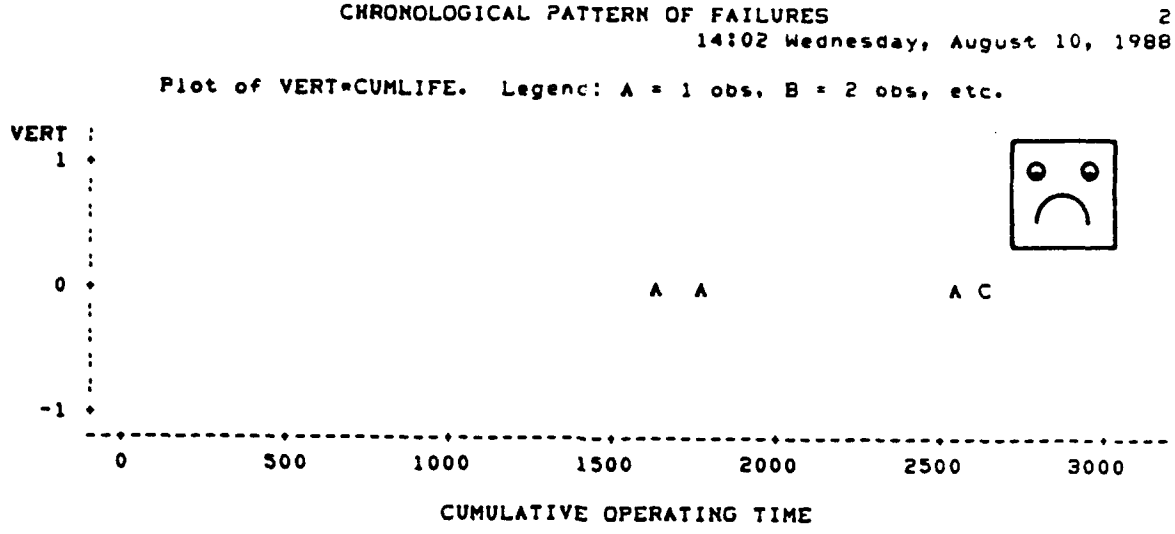
syntax is adopted from C and LISP and is reasonably easy to learn. The inference engine frees the developer of much coding burden. It is possible to develop and test logic rapidly and in a serendipitous manner, both desirable properties in the uncertain and unstructured environment of research and development. However, debugging becomes difficult as the knowledge base grows, raising questions about the suitability of CLIPS (or any other existing shell) for large applications or for systems requiring much future maintenance.

Some functions are less flexible and more difficult in CLIPS than in a procedural language. File input/output and manipulation are examples. CLIPS appears to overcome this deficiency by allowing compiling with external C functions. The Fellow plans continued research to assess the utility of this feature.

SAS-PC is an excellent tool for use by specialists in statistical analysis. It has been very useful in prototyping and demonstration. SAS includes powerful procedures needed to do analysis of failure data. SAS also includes an extensive programming language. However, it is oriented toward batch processing and includes outputs which could confuse many potential users.

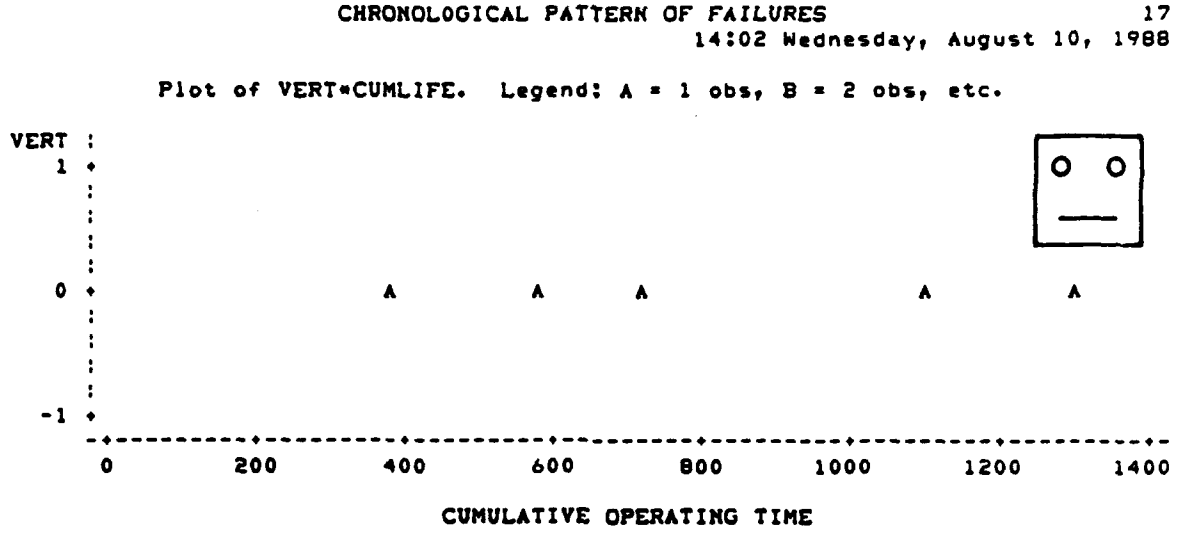
The PC version of SAS is coded in C. An interesting potential approach would be to embed the CLIPS expert system in SAS-PC and use the combined environment to develop a full-scale system. To meet the functional requirements of a full-scale developed system will likely require custom programming in a procedural language, such as C.

## CASE 1: Central Air Data Computer (CADC), 51FA□




(a)

## CASE 2: Radar Power Supply (RPSP), 74AQ□




(b)

**Figure 1. Chronological failure patterns.**

## VII. RECOMMENDATIONS:

This research relates to the Air Force initiatives in RAMCAD (Reliability and Maintainability in Computer-Aided Design) and concurrent engineering. The proposed expert system would be a valuable took for use by design and test engineers. It should be implemented as part of the RAMCAD workstation, for use in the process of weapons system development.

There is much work to be done in defining the knowledge base in a form to support further prototyping. This process involves collection of expert experience, review of literature and synthesis into specification documents, such as Appendix B. These specifications form the basis for coding of rules into the knowledge base. The Fellow encourages the AFHRL to continue this process through to complete development of a working prototype, with well implemented user interface. Such a prototype can then be tested by practicing engineers in industry, to obtain their evaluations and recommended improvements. These suggestions can then be incorporated in development of a full-scale system.

There are two alternatives for prototyping. The first alternative is to continue with the expert system approach. CLIPS is a good choice as the basis for this approach, primarily because it permits embedding and use of external functions. Alternatively, the logic could be programmed in a procedural language. Both alternatives should be investigated further, before a decision is made regarding the approach for full-scale development.

There are several related areas requiring further

research. Figure 2 illustrates the major types of failure data and the alternative models which arise from the data types. The most important distinction is that between repairable and nonrepairable items. The existing expert system prototype manages this distinction adequately, and minimizes a major pitfall in data analysis.

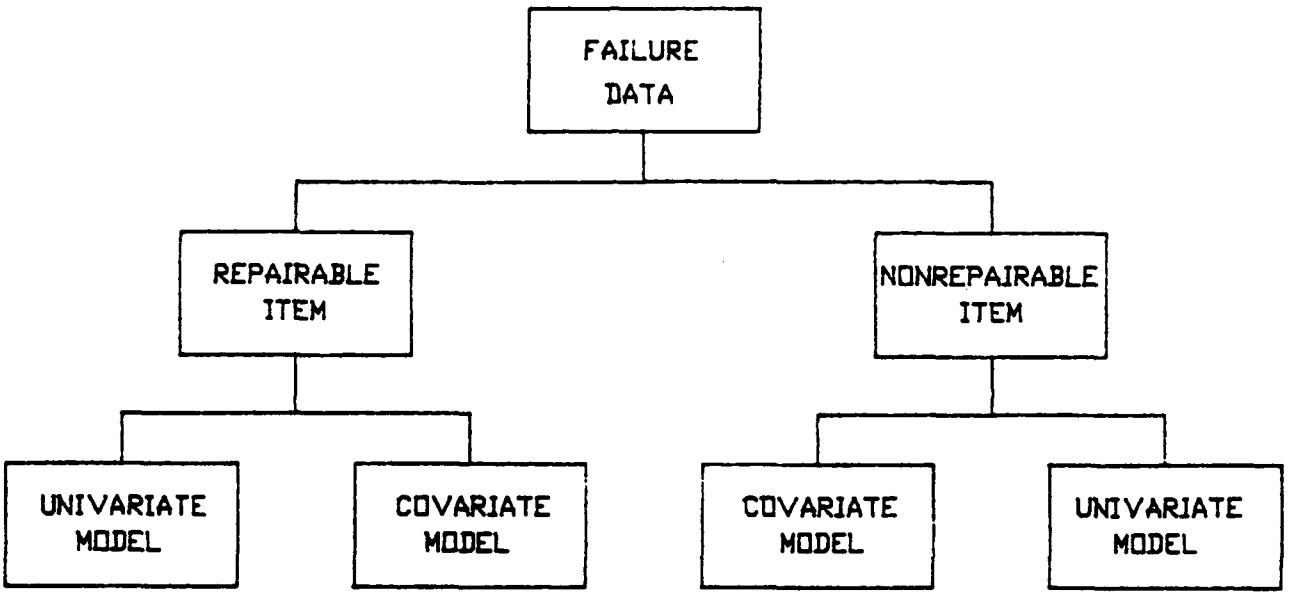



Figure 2. Classes of failure data.

The covariate models have been derived in recent years, and have received both theoretical attention, and application in the medical field, for survival studies. However, engineering application has been minimal. There is great potential application for covariate models in engineering, particularly if implemented through an expert-system approach.

Research is needed to determine adequate sample sizes, appropriate statistical tests and methods of selecting covariates.

Relatively little work has been done on repairable item reliability analysis. Most of the work thus far has dealt with the Poisson processes (NHPP). The Homogeneous Poisson Process (HPP) is a stochastic point process, with constant rate of occurrence of failures (ROCOF), whereas the Nonhomogeneous Poisson Process (NHPP) has a time-varying ROCOF. The most common parametric form of the NHPP is the power law process. The NHPP with power law intensity rate is a good model for engineering applications, for two reasons:

1. The "minimal repair" or "repaired as bad-as-old" concept leads to the NHPP [1].

2. For the power law-process, the time-to-first-failure follows a Weibull distribution.

Recently, Engelhardt and Bain proposed a general parametric NHPP model where the intensity follows a power law and the variability in intensity function, among members of the population, is Gammma distributed [6]. This work needs to be extended to the case of covariates. Lawless proposes an approach which requires further development and assessment of potential engineering applications [9]. Another important paper, by Prentice, Williams and Peterson (PWP) proposed a nonparametric covariate model for repairable item [11]. This work must be further investigated for potential engineering utility.

The Fellow plans to submit a proposal under the Research Initiation Program. The proposed research will investigate

methods of statistical analysis and modeling for repairable items. Specifically, the research will deal with extension of the Engelhardt and Bain (E&B) model, to include covariates. The research will also include size and power investigations of the E&B parametric model versus the PWP nonparametric model. For engineering applications, the PWP approach is preferred, being nonparametric, if size and power properties prove adequate and loss of information is minimal when a parametric model (such as E & B) is valid.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

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FINAL REPORT

# GRAPHICAL PROGRAMMING OF SIMULATION MODELS IN AN OBJECT-ORIENTED ENVIRONMENT


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| Contract No: | F49620-87-R-0004 |

# GRAPHICAL PROGRAMMING OF SIMULATION MODELS IN OBJECT-ORIENTED ENVIRONMENT

by

Mufit H. Ozden

## ABSTRACT

Graphical programming has been used in conjunction with conventional simulation languages via block diagrams or activity networks. Its beneficial effects on programming and modeling in simulation have been accepted by everyone involved in these languages. However, none of these conventional techniques is truely interactive. Given the level of the current hardware and software technology, it is possible to design a very good graphical programming system which supports an interactive incremental programming style in specifications of simulation models. The benefit of such a visual system would go beyond the modeling phase of a simulation study and it might as well be realized in understanding the behavior of complex problems, in being a communication and training medium for the user and developers, and finally in presenting the simulation results.

In this study, the graphical programming methodology has been investigated from the perspective of object-oriented simulation. The truely interactive and graphical orientation of some of the object-oriented languages (e.g., Smalltalk-80) has opened up new avenues of research in this very important topic. Today, the nature of this type of research will be not whether it can be done but how the known techniques should be combined to yield the highest benefit.

## Acknowledgements

I wish to thank the Air Force Systems Command and Air Force Office of Scientific Research for sponsorship of this research. I also would like to thank Universal Energy Systems for their administrative work and concern for arranging a suitable environment for the summer faculty.

I found my summer experience totally enriching and stimulating. Capt. Douglas Popken was most informative and helpful in defining the research problem area and in discussing the developing ideas. The concern of The Branch Chief Ms. Wendy Campbell is most sincerely appreciated. The family atmosphere at AFHRL/LR was most welcome, and was an enjoyable environment for my summer research for which I would like to extend my thanks and appreciation to the division commander Col. Donald C. Tetmeyer and the technical director Mr. Bert Cream.

## I. INTRODUCTION

AFHRL/LRL is currently undertaking a study that will expand the capabilities of the Air Force in analyzing logistics support systems. As a part of the Productivity Improvements in Simulation Modeling (PRISM) project, the system currently under study is an Integrated Model Development Environment (IMDE) which will create a state-of-art development and test environment for the various simulation models of capability assessment. The IMDE will consist of an integrated set of hardware and software tools which support model specifications, model development, and model verification as well as specific function such as data retrieval and update. An important feature of such an environment is the user-friendly interface programs between the user and the simulation language. To this end, the development of a graphical programming facility will be evaluated for object-oriented simulation. The graphical elements should be manipulated with friendly hardware tools, such as a mouse or touch sensitive screen. The graphical models thus created will be translated into executable simulation programs automatically. A running simulation program should be observed in several views focused on different aspects of the simulated world.

## II. OBJECTIVES OF THE RESEARCH EFFORT

The main goal of the summer research has been directed towards an exploratory investigation of graphical programming for object-oriented simulation. Graphical programming for simulation in the object-oriented environment is very new and has not been studied specifically in the research literature. At the current conceptual development stage of the PRISM project, it is

considered to be the most suitable goal to study the general interface features of the object-oriented languages and the graphical programming in the conventional simulation languages and to recommend future research directions on a promising graphical methodology for the IMDE.

In the light of the above research goal, the following activities were identified for the summer research study:

i) Review of the related literature on object-oriented programming and graphical programming of conventional simulation languages.

ii) Evaluate Smalltalk-80 for graphical programming techniques.

iii) Formulate a graphical programming methodology that will be investigated further in a future research effort.

## III. DIFFERENT SIMULATION STRATEGIES

The most important characteristic of a simulation approach is the strategy employed in selecting the next event to be executed and the time management. For this purpose, Three different types of world views have been used to model simulation problems _ event scheduling, activity scanning and process-interaction. Each world view emphasizes a different type of locality _ the property when all the relevant parts of a program are found in the same place, Overstreet, 1987. Event scheduling emphasizes locality of time. Each event routine describes a collection of actions which may all occur in one instant. Activity scanning emphasizes locality of state. Each activity routine describes a collection of actions which will occur once the certain conditions are reached. These resulting actions may occur at different time points, but they must all occur. Process

interaction emphasizes the locality of object. Each process routine describes all actions taken by one object. The conventional simulation languages use one or allow a combination of the world views. In general, the simulation programs of US origin use either the event scheduling or process interaction whereas those of British origin tend to prefer the activity scanning view. However, it has been illustrated that each world view allows simpler model specifications for some problems, no one particular view is superior to the others, Overstreet 1987, Hooper 1986, and O'Keefe 1986.

Any world view of simulation can be formulated in an object-oriented environment. However, the process interaction approach is most compatible with the object orientation where behavior patterns can be written into the object definitions as another method. This is the approach which has been employed by object-oriented simulation languages, Demos (Birtwistle, 1979) and Smalltalk-80 (Goldberg and Robson, 1983).

According to Hooper 1986, the process interaction view that is usually employed by the object-oriented simulations has the following characteristics:

* model representation is close to problem.
* straight forward model development and modification.
* greatest support from the simulation executive .
* maybe, inefficient execution time.

## IV. SIMULATION IN THE OBJECT-ORIENTED ENVIRONMENT OF SMALLTALK-80

Here in this section, we would like to briefly describe how the Smalltalk-80 environment (Goldberg and Robson, 1983) supports

discrete event simulation. Everything in Smalltalk is an object and every object is an instance of a class. Classes are arranged in a tree structure with each class having exactly one parent class. The root class of the tree structure is "Object". A subclass inherits all the variables and methods of parent class.

Simulation in Smalltalk is facilitated with the use of a small set of abstract object classes. The modeler uses some of these classes directly and/or may extend them through creating their subclasses. In a simulation study, a set of instances of these classes are formed to act according to the behavior patterns ascribed to the objects in the particular simulation situation as a combination of class methods inherited and the instance methods added during modeling. The abstract simulation classes can be grouped together into five categories: simulation executive; simulation objects; resources; statistics-gathering and monitoring classes ; basic support classes.

In summary, Smalltalk seems to provide an excellent support for discrete event simulation with its reusable classes and the graphics input and output capabilities. Smalltalk simulation environment provides the user with very powerful coding and debugging tools, leading to high productivity in writing and modifying simulation applications. This same conclusion has also been arrived by different researchers, (Knapp, 1987; Bezivin, 1987; Ulgen and Thomasma, 1986).

## V. VISUAL INTERACTIVE SIMULATION

A visual interactive simulation (VIS) is a term for a simulation which has features for specification of the model graphically, produces a dynamic display of the system model, and

allows the user to interact with the running program, ( O'Keefe, 1987; Hurrion, 1986). Thus, a VIS system typically provides facilities for:

i) Graphical Programming: where a model can be created visually on the screen in an interactive style.

ii) User Interaction: allows the user to interact with the running program. Interaction can be such that the simulation halts and requests information from the user, or the user stops the simulation at will and interacts with the program.

iii) Visual Display: portrays the dynamic behavior of the system on the screen.

The General Benefits Attributed to VIS: The research attributes various observed benefits to VIS, (Hurrion, 1986; O'Keefe, 1987; Sargent, 1986; Ozden, 1988; Browne et al. 1986). The following are the most frequently cited benefits:

a) The graphical display becomes a communication medium that provides a common base between the developer and the user for discussion on development and experimentation. It is an excellent presentation medium for the results. b) There is a lot to learn in understanding the behavior of a complex system by experimenting with the simulation model. Thus, VIS can be a teaching tool as well as an analysis tool. c) The user can be incorporated into the model with the model determined interactions. In this way, the decisions that are too difficult to be handled by the model alone can be referred to the user.

d) The graphical techniques can be a useful means of detecting faults in coding and logic. The visual monitoring of the simulated behavior makes use of the powerful ability of the human brain to recognize

logical and spacial relationships in detecting aberrant behavior. e) VIS can increase the model validity and thereby the model credibility. Especially for the unobservable systems, where comparison between the system and model behavior is impossible, VIS may play an important role in building user confidence.

## VI. GRAPHICAL PROGRAMMING

Modeling a simulation problem is a complex task demanding both the creative ability of the modeler and the support tools of the development environment . This step basically involves translation of the conceptual problem into a program which is executable by the computer. In simulation jargon, the modeler first needs to identify the temporary and permanent entities, and their data structures, as well as the behavior patterns that will closely resemble the particular problem setting under study, and then define appropriate representation forms for them in the simulation language.

Graphical programming is expected to meet the following objectives when used in a simulation environment: a) Facilitate easy use of the simulation environment. b) The graphical programming should itself be easy to use. c)Modeler's productivity should be increased. d) It should minimize programming error. e) It should facilitate easy visualization of the conceptual problem. These objectives are certainly not in conflict with each other, and an improvement of one may mean some betterment of the others.

In an object-oriented simulation where the domain independent-object and domain dependent-object classes exist in the programming environment, the modeling phase amounts to

creating the application-specific classes and the instances of all relevant classes at the proper simulated time, and defining the behavior patterns (processes) of the objects in terms of the methods that already reside within the objects. Although existence of the object classes with the proper data structures and methods for simulation is a very convenient environment for modeling, it is still a challenging job to define the application specific classes and objects with the correct processes in terms of the programming language. It requires a good deal of working knowledge with the underlying language. A better interface is a graphical programming in which the user deals with the underlying language indirectly in an easier and more natural way. By means of graphs, icons, menus, windows and forms, a graphical programming interface can lead the user to the model specification with a sequence of visual and textual cues minimizing deviations from the correct translation of the conceptual problem.

In this respect, graphical programming is an unrivaled aid for human beings to observe the spatial and logical relationships among the simulation objects. Graphical programming may take various forms depending on the domain, and the hardware and software being used. The graph of spatial symbols, icons, menus and forms, and their combination is frequently used . We will review the graphical programming approaches developed and being considered in some simulation systems currently under development below.

Ideally, a graphical programming of simulation should be performed in such a natural and simple manner with ( visual

tools and perhaps aided with a natural language processor) that the modeler will be faced with a task compatible to the human cognitive process necessary to expain the conceptual problem to another human being. At the same time, an intelligent workstation should oversee this process to catch the bugs and inconsistencies in programming. Probably, a generalized modeling environment will be realized in the late 1990's. In the near future, we have to be content with the domain specific systems where we can achieve comparibly good results.

The current graphical programming approaches can be classified in three groups: i) Network and block diagrams; ii) Icons, menus, forms and windows; iii) Dialogs and tree structured menus. We are going to describe these systems in the following sections.

Network and block diagrams have been used as a modeling and communication aid in conventional simulation languages, such as GPSS, SLAM, SIMSCRIPT, and SIMAN etc. Here, the activities which each temporary entity ( customer, transaction, or job) performs with the permanent entities ( facilities, resources or stages) are described by the use of a sequence of blocks or a network of nodes. Each block or node represents a macro-code in the host language. In this way, computer programming is facilitated in chunks of codes taken at each step in addition to the visual help. Most of these simulation languages are oriented towards simulating queueing systems and they have wide application areas. They tend to have the view of the facilities in programming rather than the individual transactions. The transactions are usually dealt with in aggregate and probabilistic manner if possible, and the average facility performance (such as the

percentage busy-time or the average waiting time at a facility etc.) is the main concern. Almost all of them now have an automatic translation feature which loads the network or block diagram as an executable code. Some even offer an interactive graphical programming feature for the restricted domains (e.g., flexible manufacturing systems) that can also display the animated view of the simulation, such as SIMAN/CINEMA and SLAM/TESS.

The number of allowable blocks or nodes may be quite large, (e.g., over 60 for GPSS). So the modeler's job is to find the right sequence of these macro elements with the correct parameter assignments. This is usually not a straight forward task and it may even require some external subroutines to be written in another programming language. SIMNET (Taha, 1987) reduces the number of these macro elements to a set of only four essential ones, and claims that this eliminates the need for external programming since it is possible to program the physically parallel processes in a parallel manner, and it is friendlier since one has to deal with only a few elements. This is a step in the right direction. But none of these simulation languages has a truly interactive graphical programming yet.

Icons, menus, forms and windows are the interface mode that has originated from the past artificial intelligence research because of the critical need for friendlier interfaces. This type of programming is the usual programming style for some modern languages, such as Smalltalk-80. But, it is better known as the MacIntosh interface mode by the general public. Especially with a pointing device such as a mouse, it is a lot friendler than

key-board entry of data. Icons facilitate easy programming for the frequently used pieces of computer codes, with a single pointing action. Menus, on the other hand, offer alternative choice of operations on fixed menu items as a pull-down menu, or as a pop-up menu on the user request for a different course of action. Windows are usually used for parallel views and programming of the different parts of a computer code. Forms are for inputing standard information in a template form. Any combination of these interface modes are possible and widely used, e.g., icon/menu or window/form. This turns out to be especially a very convenient interface mode for the object-oriented languages in which flexible, and reusable parts of codes form the main program structure of the language,(as used in the paper by Cox and Hunt 1986, these are the Software-ICs just like the silicon chips in an electronic circuitry.)

In simulation, this type of programming style has been used in specific application areas, such as computer performance evaluation or manufacturing, (e.g., Melamed and Morris, 1985; Browne, et al. 1986; Sinclair et al. 1985; Duersch and Laymon, 1985; Stanwood et al. 1986).

Dialog-based programming is new in simulation. It has been developed as a part of a simulation environment, (Unger et al. 1984; Birtwistle and Luker, 1984). It originated from the idea that all simulation programs have a structured form of specifications no matter what the application area is. Therefore, a structured dialog with the user can be prepared beforehand to obtain the necessary information for any simulation model. In the dialog, the user is first asked for the process types and other

global data and then requested to input the process details. Through the dialog, an intermediate representation of the model is built and is then run under an interpreter which may present different graphical views of the simulated world with icons and windows for verification purpose. Once the verification phase is completed the intermediate form is used to generate a compiled code for speed of execution. A different form of dialog style programming is also developed for simulation in a restricted area by Ingalls, 1986. Here, the dialog is based on a set of menus structured in the form of a tree. The user chooses a path of model specification from the root of the tree towards the lower branches pointing his choices from the menus.

## VII. GRAPHICAL PROGRAMMING FOR OBJECT-ORIENTED SIMULATION

It is important to understand that the software technology for graphical programming and object-oriented systems in general is fairly new and therefore the ideas and methodologies need to be tested in prototype systems before a full scale production system is attempted for developement. However, this orientation has many fruits to bear for the systems under development as well as for the future other systems. It has become apparent now that the software systems are the bottlenecks in modern technologies and the old paradigms do not comprise a solution to this problem. In this sense, graphical programming in the object-oriented simulation environment is future oriented and experimental in nature. In the light of all these facts, we propose a tentative graphical programming methodology below that needs to be examined with prototype problems close enough to the domain area. This methodology may have to be modified or expanded as discrepences

are observed with these experiments. For the prototyping environment, a typical object-oriented language with nice and full features of the object-oriented enviroment should be chosen so that the transfer of the methodology into the ultimate language of the IMDE will be least painful.

In an object-oriented simulation, the global simulation data (such as, the number of temporary and permanent entities involved, and the total simulation duration, etc.), the topology (objects and their relationships), and the behavior of each object have to be defined during the model specification phase. Here in this section, we will describe a possible graphical programming for an object-oriented simulation environment in a rather speculative style. The exact form of a graphical programmming application would depend upon various factors including how a set of graphical and textual features will be selected on the basis of the objectives stated in the previous section as well as the hardware and software being used. Of course, a specific application domain can be supported better than a general purpose simulation environment. Here, we assume that the IMDE will primarily be a rectricted simulation domain of capability assessment of logistics support systems.

The minimum requirements of a graphical programming for an object-oriented simulation system should cover the following:
a) A graphical programming editor to create new object classes and graphical elements (icons, menus and forms etc.) to be stored in the simulation "library" (data base for persistent objects, and to edit the old objects from the library and the simulation

applications saved in the form of graphical models. It should have a "dictionary" access to this library of objects. The dictionary could be for most part iconic and organized in some hierarchical fashion for easy access.

b) An interpreter to translate the graphical models to be created with the editor into the computer executable form .

c) View builder: the style of model development in this environment will be mostly exploratory and incremental. The objects and their relationships as created in part (a) should be able to be viewed graphically in a static manner (e.g., activity cycle diagrams). When the programs are run, the simulation with different object views should be observed possibly dynamically to facilitate verification of the model created so far. If the programs need to be modified the graphical models stored in a file should be reloaded.

A typical scenario of graphical programming in the object-oriented simulation will be depicted below. Since the global simulation data input will be conducted in a standard way for all simulation applications , this phase can be facilitated filling standard forms interactively on the screen. If the simulation has already been created or it is going to be some modified version of an old simulation program, the graphical model or the compiled code of the simulation will be reloaded from a file.

Since it is assumed that the simulation environment will have a restricted domain of application, most of the classes and subclasses of the objects needed for the particular application will be found in the objects library that can be accessed with the dictionary. Thus, when one identifies a temporary entity that

will take place in simulation ,say a particular type of airplane, the icon representing that object is looked up in the dictionary of temporary entities, and the appropriate button of the mouse is clicked on the icon. This action will load the object into the graphical programming environment from the data base and at the same time on the screen the user sees a form that prompts him to fill the related information, such as the number of airplanes of the selected type, how they will enter the simulated world (e.g., type of the interarrival distributions) and the number and types of processes in which this particular object will be involved, etc. If some particular information about an item of the object refers to some existing data in the environment, the help can be obtained by means of a menu which offers alternatives and in turn when one is selected asks for more detailed information on that particular item, e.g., the distribution and then its parameters.

All the elementary operations necessary to define a process that an object may perform will already have been defined at the creation of the object. During the process definition phase of an object in graphical programming, all the methods that may take part in process definition and the resources defined so far can be presented as menus. When a method is selected from this menu, the proper parameter settings may be asked automatically. If a new type of resource is needed from the resource dictionary it is loaded into the simulation world and an account of used resources is kept. Later on, the necessary parameter definitions of these resources will be asked from the user automatically. The simulation world thus defined is converted into an internal representation form.

The behavior pattern (processes) of object cannot be defined unless the object always behaves in the same manner in which case this can also be incorporated in the library definition. For example, the mission of an airplane will most probably change from application to application. However, as noted elsewhere ( Birtwistle and Luker, 1984 ), the structure of behavior patterns of objects is regular and is composed of a set of processes, each of which in turn demands a certain amount of a resource, holds it for some time and then returns some amount of the same resource. In fact this well structured behavior forms the basis for the graphical representation known as the activity cyle diagrams, Birtswistle,1979.

The view builder of the graphical programming should be able to display different views of the simulated world. For example, an activity cycle diagram for each primary object could display the processes and its relationship with the resources graphically on the screen ; or a resource view could present all the temporary entities that use a particular resource graphically on the screen. Such orthoganal views of a simulation application may reveal a lot for verification purpose. At the same time, these views may be used to observe the dynamic behavior of the objects. For example, the resource view may show all the objects that are using different types of resources and the objects that are blocked due to lack of resources at each discrete time as animated graphics. Once the simulation modeling is completed, the intermediate form (graphical model) would be saved and be compiled for experimental runs.

In a real-life simulation case, there may be thousands of objects that need to be defined for the simulation world. But most

of these objects will probably remain the same from one application to another and will not get involved with the other objects in complex interactions. Therefore, it may be very helpful to modify a copy of the closest simulation application stored on a file rather than creating it from scratch. This modifiability (reuseability) of the old programs is another asset of the object-oriented paradigm which will affect programmer's productivity a great deal.

## VIII. RECOMMENDATIONS

As a result of this summer research on graphical programming in object-oriented simulation, the following main points of opinion are formed:

a) Interactive graphical programming should be an integral part of the model development environment and it should support incremental programming, and load the graphical model automatically for execution. The object-oriented environment is compatible with and fully supportive of such a feature.

b) In the graphical programming, the model specification effort should be guided with icons, menus, forms and windows. There are many ways of combining these visual aids. The best design for a particular domain can be achieved through protototyping small problems in the domain.

c) Another important component of the graphical programming is a facility which will display different graphical views of the simulated "world". This will especially be helpful for verification purpose as well as reviewing typical applications saved on the files.

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# FINAL REPORT

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# A Study of Interaction Between Job Properties and Personal Characteristics in the New PACE System

by

Dharam S. Rana

## ABSTRACT

This study examined the feasibility of studying interaction between job properties and personal characteristics in the new processing and classification of nonprior-service enlistees (PACE) system. A general linear model was developed and successfully applied to assess interaction in a test example. The model was also applied to analyze interaction in the Administrative aptitude area for a particular weekly batch of the Air Force trainees. The results indicated that nearly 33 percent of the variance of the "final payoff" variable was accounted for by the interaction (residual). To investigate underlying distributions of the PACE variables, goodness-of-fit tests were performed. The findings indicated that the PACE variables with the exception of "objective interest" do not follow a normal distribution. Correlations among payoffs of different Air Force Specialties in the Administrative area were also computed for a week group of recruits.

ACKNOWLEDGEMENTS

I want to thank the Air Force Office of Scientific Research, the Air Force Systems Command and the Human Resources Laboratory at Brooks Air Force base for sponsoring this research. I would also like to thank Universal Energy Systems for their help in administrative and directional aspects of my research project.

My participation in the Summer Faculty Research Program was a rewarding experience. Dr. William E. Alley's interest and encouragement in every phase of this project served as a source of stimulation. The support provided by Dr. Manuel Pina Jr. was invaluable and was deeply appreciated. I especially wish to thank Mr. Peter Gaddis, Jr. for his help and cooperation which facilitated the completion of this research project. The concern of Colonel David E. Brown and Mr. Larry Looper was very helpful. The help of Captain Mark S. Johnson and Sargent Arthur Soria was valuable and appreciated.

# A Study of Interaction Between Job Properties and Personal Characteristics in the New PACE System

## I. INTRODUCTION

The United States Air Force (USAF) performs processing and classification of nonprior-service enlisted personnel on two computer-based systems: Procurement Management Information System (PROMIS) and Processing and Classification of Enlistees (PACE). PROMIS is a preenlistment selection and classification system managed by USAF Recruiting Service. Nonprior-service enlistees are processed by PROMIS from the time of their application to the time they enter their basic military training (BMT). PACE is a post-enlistment classification system managed by Headquarters, Air Training Command. Both PROMIS and PACE systems annually classify about 56000 enlistees into one of approximately 300 Air Force Specialties (AFSs). PROMIS classifies 50 percent of the recruits into specific AFS through the Guaranteed Training Enlistment Program (GTEP) and the remaining recruits into one of four aptitude areas: Mechanical (M), Administrative (A), General (G), and Electronics (E). The recruits in the aptitude areas are assigned to specific jobs by the PACE system according to a payoff criterion. The GTEP recruits are also screened by the PACE system to ensure their eligibility for the AFSs.

My research interests have been in the area of application of operations research and statistical techinques to solve a variety of problems. My academic credentials, especially my work on several funded projects including "Socio-economic Impacts of Large Nuclear Waste Depository", and "Oculometer Data Analysis" which required application of statistical methods, contributed to my selection by the Air Force Office of Scientific Research. My SFRP assignment to work on the Person Job Match project at the Human Resources Laboratory provided me with another opportunity to apply my quantitative expertise for analyzing interaction in the PACE system.

## II. OBJECTIVES OF THE RESEARCH EFFORT

Current processing and classification systems of the Air Force, the Army and the Navy assume that job attributes (properties) and personal attributes (characteristics) interact significantly with each other to determine the overall payoff of assigning persons to jobs. A critical review of the literature indicates that few studies have formally investigated interaction components of the selection and classification systems. This raises serval questions: How much interaction exists between the factors of job properties and personal characteristics in the new PACE system? If there is a little interaction or no interaction between these two factors, how would it affect the quality of classification of nonprior-service enlistees performed by the new PACE? Another valid concern is: Does a larger interaction mean opportunties for obtaining a larger sum of total payoffs? How are the interaction and range of total payoff

related to each other? For the case of no interaction, it could be argued that the classification of recruits done in a random manner would probably be as good as the classification of recruits performed by the new PACE system. However, the remaining questions of how much interaction and if the larger interaction means opportunties for obtaining a larger sum of total payoffs etc. remain to be examined by formal investigation. Unless these concerns are addressed, they will continue to create uncertainty about the effectiveness, validity, and superiority of the new PACE system. Thus, after consulting with my USAF research colleague, the current research was directed to address the concerns about the role of interaction in the new PACE system.

The major objective of the current study was to examine the feasibility of studying interaction between properties of Air Force specialties and the characteristics of enlisted personnel in each aptitude area. As a minor objective, the present study was also to determine correlations among payoffs of various AFSs in each aptitude area (Mechanical, Administrative, General, and Electronics).

How does the new PACE system perform with respect to the current PACE? Comparison of the two systems can be made by testing appropriate hypotheses about the population means of their variables and functions. To make a worthwhile statistical inference about the behavior of any random variable, information regarding its distribution is required. Thus, as a second minor objective, the study was to investigate the underlying

distributions of the PACE variables with special emphasis on the "final payoff" variable. Though these three objectives are apparently different, they are related to the important theme: How to maximize total payoff by manipulating interaction in the classification system?

## III. BACKGROUND

Researchers at the Air Force Human Resources Laboratory (AFHRL) have conducted many studies to develop effective and efficient classification systems. The Air Force started with a simple two-phased selection and classification system in the early 1960's. This system was inadequate to meet the Air Force needs and was replaced by a computer-based classification system called Selective, Qualitative Airman Recruiting System (SQARS). Through various stages of development, the AFHRL personnel have provided the USAF with PROMIS and PACE- highly advanced processing and classification tools. For a historical perspective on these systems, see Emerson (1987). An important aspect of all these classification methods is interaction between job requirements and personal attributes. Since the effectiveness of classification systems is based on the assumption of having substantial interaction, so significance of interaction in the assignment process should be investigated.

Since the early 1960's there has been a tremendous amount of research done on processing and classification systems. To enhance PROMIS, a Person-Job Match (PJM) program was developed. In 1976, the PJM was incorporated in PROMIS and the new system was named Advanced Personnel Data System's Procurement Management

Information System (APDS-PROMIS). Hendrix, Ward, Pina and Haney (1979) summarized the development, implementation and modification of the PJM system. Detailed aspects of this research effort are covered by Hendrix and Ward (1975), Ward (1977), and Ward and Haltman (1975).

One of the most important developments regarding selection and classification systems was the creation of mathematical models of judgment processes during mid 1970's. Ward (1977) developed general models for policy-specifying in the personnel assignment problem. These general models can be applied to many other situations by simply changing the parameters. The payoff generator in the person-job match component of APDS-PROMIS is based upon concepts developed in Ward (1977). In fact, the policy-specifying technique was later used to develop a payoff algorithm for the current Processing and Classification of Enlistees system (Pina, Emerson, Leighton and Cummings 1986). The payoff generator of both the PROMIS and the PACE systems includes consideration of interaction between personal attributes and job requirements.

The U.S. Army and the Navy have been working to develop their own selection and classification methods. Prior to implementation of the Air Force's current PROMIS, the Army was using an automated Recruit Quota System (REQUEST). For details of this system see Hawkins (1974). From 1973 to 1980, the Navy assigned recruits to jobs on a first-come, first-served basis. The Navy's computerized assignment system was adapted from Army's REQUEST and was named Personalized Recruiting for Immediate and Delayed Enlistment (PRIDE). As a result of the 1976 version of

PROMIS, the Army improved its REQUEST system by including personal charateristics in the system. In 1981, the Navy improved its PRIDE by implementing classification and assignment within PRIDE. This system was named CLASP and like PROMIS, it used information about jobs, Navy policy, and personal preferences and aptitude to provide payoff values for each possible person-job match (Kroeker and Rafacz 1983).

A major recent advance in classification techniques is the development of a new PACE Payoff algorithm (Pina, Emerson, Leighton and Cummings 1988). Implementing the payoff algorithm within the current PACE results in the new PACE system. A Payoff algorithm is a mathematical model which uses information about the individual and the Air Force Speciality to generate a payoff. Consideration of interaction between personal attributes and job requirments is included in the payoff generator. A payoff value represents the worth to the Air Force of assigning a particular recruit to a specific job. The variables in the payoff algorithm are selected to represent 10 fundamental concepts. Some of these variables are based on the findings of earlier studies. For example, job difficulty measures were developed based on detailed task analysis (Weeks 1984). Intellectual ability was based on the results of Wilbourn, Valentine and Ree (1984). For details of the variable representing probability of completing first term, see Emerson and Pina (1987), and Finstuen and Alley (1988). Full discussion of all the variables and functions of the payoff algorithm is given in Pina, Emerson, Leighton, and Cummings (1988).

## IV. RESEARCH DESIGN

### Method

1. Sample Organization: The Air Force applies the PACE system to classify week groups of nonprior-service enlistees. These weekly classifications of enlisted personnel contain data on a large number of variables including the functions and variables of the payoff algorithm. Thus, the data required in the proposed study to analyze interaction was obtained from HRL at Brooks AFB, San Antonio. The sample used in this study consisted of three conveniently chosen week groups of trainees. It is suggested that the data from a sample of at least ten weekly batches selected at random should be provided for future investigations.

2. Procedure: In a "balanced" data design, interaction can be analyzed by applying a regular factorial design. The data (payoff matrix) generated by the PACE classification process represents a special case of unbalanced data. Many cells in the payoff matrix are empty and the cells-filled have only one observation each. The use of the Cell-Means model discussed in Searle (1987) is not feasible due to the complexity of the data matrix. Techniques discussed by Marasinghe & Johnson (1981, 1982), Martin (1980), and Snee (1982) are also not suitable to study interaction in the new PACE system. Therefore, an alternative approach based on Ward & Jennings (1973) was used to develop a

general linear model to examine interaction. First, the model was developed for a test example drawn from Ward, Rocks, Powell and Dyas (1986). In this example a group of 42 pupils was assigned to three teachers in such a way that the total learning outcome for the entire group was maximized.

General Linear Model

Let E (i,j) = Expected value of predicted learning outcome of assigning j-th pupil to i-th teacher, Where j = 1, 2, ---, 42; i= 1, 2, 3.

Using a grid of 3 x 42, the hypothesis of no interaction can be written as:

$$E(11) - E(31) = E(12) - E(32) = \cdots = E(1\ 42) - E(3\ 42) \quad \text{---------- (1)}$$

$$E(11) - E(21) = E(12) - E(22) = \cdots = E(1\ 42) - E(2\ 42) \quad \text{---------- (2)}$$

For this example, the full general linear model is

$$Y = \sum_{i=1}^{3} \sum_{j=1}^{42} A_{ij} \; X^{(ij)} + E^{(1)} \quad \text{_____ (3)}$$

Where Y is a vector of interest of dimensions 126. Its elements measure learning performance of pupils when they are assigned to one of the teachers.

$X^{(ij)}$ = 1 if the element in Y is from i-th row and j-th column.
= 0 otherwise.

The terms $A_{ij}$ denote weights and $E^{(1)}$ represents a vector of random errors in the model.

Restrictions implied by the hypothesis of no interaction (equations 1 and 2) may be expressed as:

$$a_{11} = b_1 + d_1,\ a_{12} = b_1 + d_2, \cdots,\ a_{1\,42} = b_1$$

$$a_{21} = b_2 + d_1,\ a_{22} = b_2 + d_2, \cdots,\ a_{2\,42} = b_2$$

and

$$a_{31} = b_3 + d_1,\ a_{32} = b_3 + d_2, \cdots,\ a_{3\,42} = b_3$$

Imposing the above restrictions on the full model (equation 3), the restricted linear model is

$$Y = \sum_{i=1}^{3} b_i R^{(i)} + \sum_{j=1}^{41} d_j C^{(j)} + E^{(2)} \qquad (4)$$

where

$R^{(i)}$ = 1 if the element in Y is from i-th row.
= 0 otherwise.

$C^{(j)}$ = 1 if the element in Y is from j-th column.
= 0 otherwise.

The symbols $b_i$ and $d_j$ denote weights and $E^{(2)}$ represents a vector of random errors in the restricted model. The weights and error sums of squares in both models are computed by the least squares method.

## V. RESULTS

First, the restricted model was applied to assess interaction in the test example taken from Ward et al. (1986).

The results from the model's application indicated that the interaction accounted for 42.8 percent of the variability of the dependent variable Y; this compares favorably with 43.0 percent of the variance of Y explained by interaction (Ward et al 1986).

The model was also applied to determine interaction in the Administrative aptitude area. The data on week group PPJM.51 were analyzed by using SPSS/PC+ software programs on the Zenith microcomputer system. The data consisted of ten Air Force Specialities (Jobs) and 55 trainees. Two of the jobs were not filled because their eligibility requirements were not satisfied.

The results indicated that the interaction (residual) between job properties and the trainee's characteristics explained 33 percent of the variance of the "final payoff" variable. The result that the factor of residual (interaction) accounts for approximately one-third of the total variability of the "final payoff" does not answer the question: Does interaction play a significant role in the new PACE system? To determine if interaction between job properties and personal characteristics plays a significant role (in statistical sense), errors sum of squares in both models should be computed and a test of significance for interaction should be performed. Subjective assessment of interaction in the PACE system can also be made by examining the range of the "total payoff". These results will be obtained in the follow up research.

The behavior of the new PACE variables and functions can be studied by investigating their underlying distributions. The data from three weekly batches (PPJM.50, PPJM.51, PPJM.52) of Air Force recruits were analyzed by performing goodness-of-fit tests.

The Kolmogorov-Smirnov test for normal distribution was applied. The results indicated that the PACE variables and functions with the exception of "objective interest" do not follow normal distribution. The hypothesis of normal distribution was rejected at a significance level less than 0.07. The functional form of the underlying distribution of these variables and their departure from normalcy need to be further investigated. Some of the variables which seemed to follow a normal distribution for one batch, were found to have non-normal distributions for the remaining batches. This inconsistency can be attributed to several factors including large differences between batches of trainees. To identify the cause of such inconsistent behavior, additional goodness-of-fit tests would need to be performed.

The third objective involved examination of correlations among payoffs of different jobs in aptitude areas. The data from a weekly batch PPJM.51 were analyzed to obtain a 8 x 8 correlation matrix. Examination of this matrix revealed that payoffs of only one out of 8 jobs had negative intra correlations which were statistically significant with $p<0.02$. The results also showed that payoffs of six out of eight AFSs had positive intra correlations which were statistically significant with $p<0.013$. These observations perhaps reflect the limitations within the system for maximizing "total payoff" by manipulating interaction. More details of correlations results are given in the technical report of Peter Gaddis, Jr. - a UES Graduate Student Summer Fellow who worked with me during my SFRP assignment.

## VI. RECOMMENDATIONS

On the basis of this study, the folling recomendations are made:

a. First, the general linear model developed in this study should be applied separately to study interaction in each aptitude area. Procedures for testing significance of the interaction should be developed and applied.

b. Apply the general linear model to assess interaction in the combined case of all four aptitude areas. Also, test a hypothesis to determine if the interaction is significant within as well as across aptitude areas.

c. The behavior of the "final payoff" variable should be thoroughly investigated by performing additional goodness-of-fit tests. After knowing the distribution of the "final payoff", construct a confidence interval. From this confidence interval, one can obtain confidence bounds for the total payoff.

d. Generate a nearly full matrix of payoffs for AFSs in each aptitude area by moderating the restrictions of eligibility. For each area, obtain 1) a Minimum, 2) a Random, and 3) a Maximum solution. Examine the relationship between interaction and the range of total payoff by graphical and numerical methods.

e. In the new PACE, obtain minimum and maximum solutions for each aptitude area and compute the range for total payoff.

Compare this range with the range of total payoff in (d). The comparison would provide an estimate of potential gain in the total payoff to be obtained by moderating the eligibility requirements.

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FINAL REPORT


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# An Intelligent Tutor for the IBM System/360 Assembly Language: BIGBLUE

by

Sunita S. Rana &
Charles Drake

ABSTRACT

The Air Force Human Resources Laboratory (AFHRL) at Brooks Air Force Base in San Antonio, Texas is a major research center for developing Artificial Intelligence (AI) and ways in which to utilize this intelligence for the military. There is a need to supplement or replace a shrinking pool of human Air Force instructors.

Assembly language is taught at the Air Force Technical School and also as a part of the CDC 49152 for Information Systems Programming Specialist in the Air Force. The IBM system 360/370 mainframe is the hardware most commonly found at Air Force installations. During my summer research here, an attempt was made to develop an Intelligent Tutoring System (ITS) for the IBM 360/370 Assembly Language. Thus far, all ITSs in the computer programming area have been attempted for high-level languages (frequently Pascal). It was a challenge to develop one for a low-level language. Low-level languages are machine dependent and more difficult to code, as each instruction directly manipulates hardware of the machine. Assembly language is more a tool to teach computer architecture than programming style. Therefore, an ITS on assembly language requires an explanation in tandem with each instruction of the hardware basis for doing so. This is also a required language for graduate and undergraduate students at Jackson State University. The tutor was named BIGBLUE for IBM's System/360.

## Acknowledgements

We wish to thank the Air Force Office of Scientific Research, the Air Force Systems Command and the Human Resources Laboratory at Brooks Air Force Base for sponsoring this research. We would also like to thank the Universal Energy Systems, Inc. for their help during the entire period of this research project.

Thanks are due to the entire Intelligence Systems Branch unit and to the chief of this unit, Lieutenant Colonel Hugh L. Burns. Special thanks are due to Dr. Kurt Steuck for his patient guidance. His help was invaluable to this project. We are very grateful to Ms. Lillian Brooks, Administrative Assistant, Computer Science Department, JSU for helping us to complete this manuscript.

## I. INTRODUCTION:

The Air Force Human Resources Laboratory (AFHRL) at Brooks Air Force Base in San Antonio, Texas, is a major research center for Artificial Intelligence (AI) and for developing ways in which to harness this intelligence for the benefit of the military. The Intelligent Systems Branch of HRL has in its working team psychologists, education specialists and LISP programmers and is actively using this mix of experts to develop Intelligent Tutoring Systems (ITS) for military applications. A Xerox Local Area Network (LAN) provides the hardware capability to generate enough memory size for the large databases required to create an ITS. In September of 1986, this unit hosted a symposium on ITS. This was called the "1986 Research Planning Forum for Intelligent Tutoring Systems (ITS)." The Symposium was delivered by a dozen notable researchers in this field. There were 135 military academic and civilian personnel who attended this two-day meeting. All research questions in development of ITS were addressed. The proceedings of this symposium have been published by Lawrence Erlbaum Associates, 1988, under the title "Foundations of Intelligent Tutoring Systems." The editors are Martha C. Polson and J. Jeffrey Richardson of the University of Colorado and Elliot Soloway of the Artificial Intelligence Project at Yale University.

The major thrust of the research at AFHRL Intelligent Systems Branch (AFHRL/IDI) is to tackle the problem of a shrinking pool of overworked Air Force instructors by developing "Intelligent Tutors" to assist and possibly even replace human instructors.

## II. BACKGROUND:

Newell and Simon (1963) initiated early research in the development of computers that would emulate the human problem-solving and thinking processes. Hayes-Roth and Thorndyke, 1985, identified Intelligent Computer Assisted Instruction (ICAI) as an independent research and development paradigm in AI. Park, 1988, however, sees AI as at least four different paradigms that actually constitute an ICAI. According to his classification, these are:

a) The expertise module, consisting of the domain knowledge base and the criterion performance model.
b) The student model composed of the student knowledge base, learning deficiencies and learning needs catalogues and a database of individual difference variables.
c) The tutorial module consisting of a knowledge base of different types of instructional strategies, diagnostic capabilities to perceive a student's misconceptions and learning needs and how best to select an instructional treatment based on the student's learning needs.
d) An interface module consisting of a display and/or language generator and the student's input interpreter.

My area of interest in ICAIs is the learning of computers and programming. Numerous intelligent tutors have been developed in the area of computer programming. Programming languages most frequently used as teaching domains are Pascal and LISP. A "visual" programming language has been developed by Bonar and Liffick (1987). This tutor is called BridgeTalk and has some of the most extensive teaching skills of an ITS. BridgeTalk incorporates the teaching of:

a) Pseudocode
b) Pictorial representations
c) Pascal code for each language concept taught.

Any syntactic error is immediately reciprocated by helpful hints (Socratic teaching). To date, this tutor has incorporated many features of an ITS in the teaching of programming to novices.

## II. JUSTIFICATIONS AND OBJECTIVES OF PROPOSED STUDY:

The research objective of this work is to develop a tutor to teach the assembly language of the IBM 360 mainframe. This assembly language is taught at the Air Force Technical School and also as a part of the CDC 49152 for Information Systems Programming Specialist. IBM System/360 is the mainframe most widely used in the Air Force. My additional interest in this language is due to the fact that at Jackson State University, where I teach, this language

is part of the core curriculum of the undergraduate program and a required course for all graduate students. The need to teach any student of Computer Science at least one assembly language is to introduce computer architecture and machine language to the student. Assembly language is a symbolic representation of machine language, which consists of only 0's and 1's. Each assembly language instruction is equivalent to one machine language instruction (with the exception of macros). The assembly language instructions are translated into machine language by the assembler. Since this language is so close to the machine, it is also called a low-level language. Systems programmers have to code at least part of their programs in assembly language because no other language gives the programmer the ability to manipulate the hardware of the machine.

It is a challenge to develop an Intelligent Tutoring System (ITS) for a low-level language. Thus far, all ITS in the programming area deal with high-level languages, frequently Pascal. The objective of an ITS for assembly language or, for that matter, any ITS is to have, if possible, a microcomputer program that would be able to teach the subject matter independent of a human instructor and maybe just as effectively. At the very least, the ITS should be able to supplement the human instruction on this subject matter.

## IV. RESEARCH DESIGN:

### 1. Procedure

The incoming student to the Air Force training course is given instruction in Base 2 (Binary), Base 8 (Octal) and Base 16 (Hexadecimal) arithmetic and how to convert from one base to another. They also learn flowcharting. At this point, the student is introduced to assembly language. BIGBLUE will teach the language in tandem with the IBM 360/370 machine characteristics. Individual instructions and their execution by the hardware are to be illustrated graphically to the student along with a discussion. When the student sits down at the microcomputer and gets introduced to BIGBLUE, she/he will first enter their name. BIGBLUE then creates a file for that individual. This file is used to store all pertinent information the tutor can deduce about the student during their interaction. This file generates a profile of the student.

The student file that keeps evaluations of the individual student using the tutor will be based on quizzes following each section of the material taught. A student with unsatisfactory quiz scores will be given a chance to review the material again. After that, he/she will attempt to solve a quiz similar to the first one. The student who solved the first quiz will have to solve two or more like it so it becomes clear that he/she does have a complete grasp of the knowledge and is not just guessing the correct answers. At this point, the successful student will move on to new areas of learning.

The quiz has a multiple choice format. Each question on the quiz is a carefully selected one, based on general areas of vulnerability that have been repeatedly noticed by instructors of assembly language.

The bugs encountered in teaching this course are: 1) misconceptions and 2) missing conceptions. When writing programs, bugs can be in the form of syntactic errors, incorrect algorithms and misplaced logic. A sample lesson would present at least the following information on register to register (RR) instructions.

The IBM 360 Series has 16 General Purpose Hardware Registers. These registers are located in the Central Processing Unit (CPU). They are much faster memory than Main Storage. Since they are also expensive, only a limited number of registers are supplied by the manufacturer of the computer. As such, registers should therefore be used as efficiently as possible. The registers in the IBM 360 Mainframe are numbered from zero to fifteen. Of these registers, only registers two through twelve are conventionally used by programmers. The remaining are reserved for special purposes.

Registers are considered as "Scratchpad Memory" since intermediate results of operations are stored in them. Each general purpose register consists of 32 binary digits(bits) and the corresponding IBM 360 Word is also 32 bits in length. The bits are numbered from zero through thirty-one. The zero bit is the high - order and also the leftmost. The zero bit is designated as the sign bit. The largest positive value that can be represented in a register is $2^{31}-1$, the smallest negative value is $-2^{31}$.

Each IBM Assembly language instruction corresponds to exactly one machine language instruction, except macros. Macros are instructions to the assembler and consist of a dollection of instructions. Each instruction (fetched fro main memory) has the following format:

Label Opcode Operandl, Operand2 Comment

Labels are necessary for branching to instructions. Opcodes are the mnemonic (symbolic) names for machine operations in binary code. For example, hexadecimal (base 16) 5A in Assembly language (IBM 360) is an ADD instruction in mnemonic code.

Registers are more efficient because CPU cycles to fetch operands from Main Memory are not required. Another advantage is that the instruction is shorter in length since only 4 bits are needed to code any one of the 16 registers. This saves a lot of memory space. IBM uses registers in the Register to Storage (RX) instructions also by adding the contents of a specified register to the contents of a 12-bit displacement field. Since it takes $2^{24}$ bits to address all of IBM 360 Main Memory, thus method saves 8 bits (4 bits to code a register and 12 bits to code a displacement, a total of 16 bits).

IBM registers are used as index registers and base registers. Index registers can address all of main memory because of the size of the accompanying displacement field. Base registers are limited to a range of 4095 addresses, after which the current base register needs to be changed.

The IBM 360 is a 2-address machine that in most instructions contain two operands. When an operation is performed, the results are stored in operand 1 and its original contents will be destroyed.

==

operand 1, operand 2

In very few instances operand 2 will hold the result. One example is the store instruction (only used with a register):

```
ST   R,S
```

(R = register, S = address in main storage)

One major benefit of using base registers is that the loader can load the assembled object code anywhere in memory since the base register can be instructed to use the current address of the program in memory. This saves the loader a lot of time finding and changing addresses that need to be relocated every time the object code is brought into main memory, which is imperative in a timesharing environment.

Index registers are the most appropriate for writing arrays in IBM 360/370 Assembly language. If, for example, an array is set up with each element maybe 4 bytes in size, each element can be addressed in series by increasing the value of the index register by the appropriate number of bytes, which in this case would be 4. This method of accessing addresses is called "relative addressing."

An assembly language program is very structured. All subroutine calls are made using the instruction:

```
BALR  14.15
```

BALR is the opcode for Branch and Link Register. Register 14 will hold the address of the next sequential instruction. Register 15 holds the address of the subroutine.

The scarcity of registers when writing a large program is overcome by saving contents of registers in a reserved area when entering a subroutine and reloading the original contents back into the same registers in the last instruction of the subroutine. Saving is done by the instruction STM which is the opcode for Store Multiple, and reloading is accomplished by using the opcode LM; that is, Load Multiple. The subroutine returns control to the main program by using a:

```
BR   14
```

(BR = Branch to Register)

2. Materials

BIGBLUE is programmed in turbo pascal and runs on the IBM PC XT (or any compatible machine). This microcomputer is used at Jackson State University and is universally used in the Air Force also.

3. Analysis Plan

Evaluation of the tutor is important. One evaluation will be done by colleagues teaching this course. The National Science Teachers Association has a microcomputer software evaluation instrument for ITSs and will be utilized for this purpose. The critical evaluation will be that of students in CSC 216, which is the IBM 360/370 assembly language course taught at Jackson State University. This evaluation instrument will be prepared early on in the further development process of the ITS. Student evaluations will be further necessary to isolate incompetencies, inadequacies, and all possible features that would rate the ITS not intelligent enough to replace the human instructor. As the tutor is developed, all possible efforts will be made to fix these shortcomings.

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FINAL REPORT

# PRELIMINARY DESIGN CONSIDERATIONS FOR AN ADVANCED INSTRUCTIONAL DESIGN ADVISOR


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| Contract No: | F49620-87-R-0004 |

# PRELIMINARY DESIGN CONSIDERATIONS FOR AN ADVANCED INSTRUCTIONAL DESIGN ADVISOR

by

Jonathan Michael Spector

## ABSTRACT

This is a general description of an automated and intelligent tool to assist course authors in instructional design. The problem addressed by this research is the difficulty and expense of designing effective instructional materials given the complexities of advanced hardware and software technologies and the variety of instructional settings. Many automated tools to support the instructional process are being developed. However, no existing systems address the general issue of effective instructional design at the course level. An Advanced Instructional Design Advisor (AIDA) can and should be built to aid the process of course design and development. This tool should be designed within the context of a standard design philosophy for all tools developed to support the instructional process. The tool should incorporate many instructional models and prescriptions for their use at the course or course module level.

## ACKNOWLEDGEMENTS

I would like to thank the United States Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems was very helpful in providing administrative support. I am grateful to the Air Force Human Resources Laboratory for providing a stimulating environment for my research. The personnel in the Training Technology Branch of the Training Systems Division were always supportive of my efforts.

I especially appreciate the thoughtful guidance of Dr. Scott Newcomb, AFHRL/IDC Branch Chief. His ideas and suggestions always proved beneficial. In addition, I appreciate the frequent exchange of ideas with Capt. William Dimitroff, who accompanied me on two field trips and provided much needed support. I thank Dr. Newcomb, Mr. Dick Vigue, Mrs. Barbara Eaton, and Capt. Dimitroff for reviewing this manuscript. Other AFHRL/IDC members, including Dan Muraida, Bill Hawks, Lt. Walt Hodge, and Mrs. Mary Eiserer, offered much encouraging support. Members of AFHRL/IDI, especially Lt. Col. Hugh Burns and Dr. Wesley Regian, were most helpful in focusing my ideas about advanced authoring tools. I also appreciate the encouragement offered by Col. Gene Berry, Lt. Col. Roger Ballentine, and Dr. Hank Ruck.

## I. INTRODUCTION:

The instructional process involves three phases: 1) front-end analysis (FEA), 2) design, development, delivery (DDD), and 3) rear-end analysis (REA). Instructional Systems Design (ISD) has been used to guide progress in all phases, although its usefulness is somewhat limited as it provides little specific or detailed guidance at any phase. Phase 1, FEA, includes analysis of instructional requirements and the design requirements for the instructional solutions, including requirements for various courses. Phase 2, DDD, is where the primary course-level and lesson-level planning, development, and implementation occur; the work is done to comply with the instructional requirements from FEA. Phase 3, REA, is basically an analysis of how well the results of DDD met the requirements of FEA. The boundaries between these phases are not always sharply drawn, and the phases do not always occur in a linear or chronological order. Sequences are usually cyclic and somewhat irregular.

It is obvious that many persons with different talents and interests are involved in this process. If we restrict our focus to Phase 2, DDD, then we see that subject matter experts (SMEs), instructional designers, production specialists, and systems experts are typically involved. The particular contributors depend on the development and delivery system and the subject matter. As the number of specialists increases, it is reasonable to expect course development time and costs and quality to rise. As the

complexity of the development and delivery systems increases we have similar expectations. From the courseware design persepctive, the challenge is to design systems that reduce course development time and costs and yet contribute to course quality and effectiveness.

The Training Technology Branch at the Human Resources Laboratory at Brooks AFB (AFHRL/IDC) is interested in systematic improvements in the instructional process. Work Unit #49, Advanced Training Technologies, is AFHRL/IDC's vehicle for funding research and exploratory development is these related areas.

My past research efforts have dealt with the critical analysis of theories of knowledge and with software engineering and artificial intelligence (AI) projects. In addition, I have authored several courses on various authoring systems and have trained teachers in their use. As a consequence of these experiences, I have developed an interest in designing more powerful, more usable, and more effective course authoring systems.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The primary purpose of this research effort was to determine how advances in the cognitive, instructional, and computer sciences could be applied to the design of an advanced instructional design system. In order to achieve this goal, the following related questions were addressed: 1) Which theories of knowledge might be useful in the instructional

design process? 2) Which learning theories and taxonomies might be useful? 3) Which instructional design theories and models might be useful? 4) What is the potential role of AI in instructional design? 5) How is ISD useful in the process of instructional design and how can its use be automated and improved? 6) Which strategies should be followed in order to design and develop advanced authoring aids? 7) What kinds of advanced authoring aids already exist and how effective are they?

In formulating answers to these questions it became clear that a standard design philosophy to guide the development of all tools built to support the instructional process was desirable. It was also decided that an expert system to support instructional design was possible. The following sections of this report represent a preliminary specification of an Advanced Instructional Design Advisor (AIDA).

## III. PROBLEM AREAS:

The general problem with DDD is that advanced hardware and software technologies have found roosts in various instructional settings but our knowledge of how best to use such tools is limited (Montague, 1983). These technologies, including interactive video disks (IVD), speech synthesizers, networks, natural language processors, graphics editors, intelligent tutoring systems (ITSs), simulations, and sophisticated authoring systems, generally lack standards, are relatively expensive, require expertise to use

effectively, and are not always placed in appropriate settings. The basic problem, however, is that we do not know how best to design courses so as to optimize effectiveness, given that broad subject matter domains, a variety of instructional settings, diverse student populations, and new technologies are involved (Halff, 1987).

A review of the literature reveals no general consensus with regard to a theory of instructional design (Gagne, 1987). Some studies reveal that it often matters not whether certain portions of a course are omitted or whether entirely different approaches are used to teach the material (Montague, 1988).

The problem to be solved is to improve the course design process regardless of the development and delivery system (Montague, 1983). The natural setting to consider first is the most familiar and simplest: classroom lecture. Is it possible to design an automated tool to improve course design in such a setting? If the answer is positive, then we might proceed to build the tool, test and refine it, and then modify it to be more generally useful in a variety of settings. Otherwise, there is no need to attempt to build a more complex system for multiple settings.

## IV. PROPOSED SOLUTIONS:

The proposed solution to the course design problem is to develop a computer-based tool to assist in the process. The specific purpose of this tool is to reduce course development

time and costs while assisting in the production of consistently effective courses. The cost savings will come in the form of reduced needs for design experts and systems specialists as well as in time saved in course development. This means that such a system must be inexpensive and yet easy to learn and obvious to use.

In attempting to design a system to improve the course design process, two important questions will need to be resolved: 1) What are the most promising theories of instruction and how can their prescriptions be followed and tested? and 2) What are the limitations of implementations of such systems?

A review of the literature and visits to military instructional research and development centers reveal that there are no existing systems that claim to solve this fundamental problem in a general way. The Air University at Maxwell AFB has developed a tool, SOCRATES (Doucet and Ranker, 1988), that does provide lesson assistance according to Gagne's nine events of instruction and Merrill's Component Display Theory (Reigeluth, 1983). Merrill has also designed a tool (Merrill, 1986) which provides similar guidance in the lesson construction process. Such tools are significant steps forward. Their limitations are that they only provide lesson assistance for declarative knowledge. The tool to be described below is meant to be a more general solution at the course level, and it is meant to fit within the larger scheme of the instructional process.

V. SOLUTION CONTEXT:

Before describing AIDA, let's consider its context in the process of instructional design. It is reasonable to expect a set of advanced tools to be developed to automate and assist throughout the instructional process. To complement the ISD process during FEA, several programs might be useful. One might be a Training Requirements Analysis Planner that would take a roughly stated training requirement and via a query/response process develop that statement into a set of requirements and goals that are in an appropriate format to guide the course design process. Another automated aid might be a system to query a course database to find out if similar courses exist, where they are taught, etc. Yet another could be used to guide the media selection process. The FEA programs could also automate documentation.

In the DDD phase a variety of tools are possible. A program might be developed to take lesson objectives, content, and definitions as input and generate test questions, much as CBESS does for restricted domains (Wetzel, 1987). Entire systems can be built to allow authors to select and sequence lesson materials; ISS is an excellent example of such a system (Vigue, 1988). Other tools that would be useful are IVD and graphics editors and databases containing sample course and lesson templates and examples. As indicated earlier, what is especially needed in the DDD phase is a tool to assist in course design by matching instructional options, knowledge types, and settings (Madni, 1987).

It is also possible to imagine a range of tools associated with REA, including guides for establishing and conducting crucial controlled tests and access to databases containing relevant course data. A particularly difficult task in this area is testing the effectiveness of a course design process, as opposed to the effectiveness of a particular course.

## VI. FUNDAMENTAL CONCERNS:

Looking at the kinds of advanced technologies which might be developed to assist throughout the instructional process reveals two distinctions: 1) There are two different kinds of tools that can be developed -- those which automate an established process (e.g. documentation and test generators) AND those which are intended to make the process more effective (e.g. course and lesson templates, ITSs, and lesson and course design advisors), and 2) The tools that have been described can be used in two ways -- as stand-alone, special-purpose tools (e.g. practice problem generation) OR as integrated tools sharing information (e.g. a course database).

The guiding vision of this proposal is to adopt a standard design philosophy for all related programs so that as modules are developed they can be gracefully incorporated into a system of tools for the entire instructional process. Part of the standard design philosophy should be this fundamental modular concept: Tools should be developed so that they can operate as stand-alone modules or can, by way of standard

interfaces, be incorporated into larger collections of compatible modules. A standard design philosophy is needed to guide specific items, such as menu design, as well as larger concerns, such as the structure of knowledge bases. Standards for networking and graphics are also needed.

Some tools developed to make course design more effective fall into the area of AI, such as ITSs (Wenger, 1987), while some fall more clearly into the traditional disciplines of computer science, such as database design and implementation. AIDA has some aspects of both kinds of systems. A serious question is the extent that course design lends itself to AI applications (Winograd, 1986). Can an expert system for course design be developed that performs as effectively as good human designers? The initial assumption is that AI has a role to play in course design, as well as its already recognized role in ITSs (Polson, 1988). The form of such systems will be sets of production rules and inference engines to take the user from one state (e.g. specific list of course objectives and knowledge of the expected course environment) to another state (e.g. set of lesson templates outlining the course content and sequencing).

The AI questions suggest other concerns: 1) Are there existing theories of instruction design that could be incorporated as models in an expert system? 2) Do guidelines exist to suggest which models to apply in particular circumstances? and 3) What relationships exist between theories of knowledge, learning, and instruction?

The view here is that relationships between theories of knoweldge, learning, and instruction should be expected, with theories of knowledge providing links between theories of instruction and learning (Self, 1988). The theory of knowledge assumed below is a modification to the justified true belief model discussed in Griffiths (1967) and adopted by Kitcher (1983). The pragmatic modification is: Knowledge consists of well-justified and well-connected beliefs (JCB). What counts as "well" in either case depends on the particular circumstances and needs at hand.

In addition, JCB theory acknowledges the usefulness of the distinctions between declarative (knowing that), procedural (knowing how), and causal knowledge (knowing why). Each knowledge type has possible subdivisions (e.g. the distinction between physical process and mental process within procedural knowledge). Human knowledge is like a web of well-justified and well-connected beliefs (Quine, 1978); there too many threads to explore individually in order to precisely model the human belief system; but useful models can be built by emphasizing the incorporation and integration of multiple knowledge types, according to the most appropriate learning and instruction theories.

What will be carried forward are two ideas: 1) In order to establish understanding, it will be important to provide a framework or model, provide explanations within that framework, and hook that framework to other more familiar

frameworks and models, and 2) Multiple levels of understanding are possible and depth of understanding might be measured in terms of internal justifications and connections as well as external justifications and connections (e.g. meaningful analogies and metaphors).

In response to the question concerning the existence of theories of instruction, there are various theories which contain models prescribing how courses should be designed, including the Gagne/Briggs models of instruction, Landa's algo-heuristic theory of instruction, Collins-Stevens' cognitive theory of instruction, Merrill's Component Display Theory, Reigeluth's Elaboration Theory, functional decomposition and functional context theories, and scenario-based models (Reigeluth, 1983).

What are most noticeably lacking are prescriptions to follow to determine when a particular model would be most useful. Merrill has provided a few high level prescriptions for an authoring system, but they are too general to be directly helpful (Merrill, 1987). Reigeluth claims to solve this problem for three knowledge domains (conceptual, procedural, and theoretical), but his conclusions are untested and do not provide clear directions to select an organizing strategy (Reigeluth, 1983).

The strategy proposed here is to admit that much useful work has been done in theory of instruction design, select the best models and guides for their use, incorporate those

models and guidelines into course design mini-advisors, and adopt a selection strategy that recognizes that a single course will likely involve multiple types of knowledge and mastery levels. What is needed is an organizing strategy that works at the level of a group of lessons, a course module. The size of the subset may range from a single lesson to an entire course, but the typical size is expected to include several (3 to 7) lessons. Selecting an organizing strategy for lesson materials based on an appropriate instructional model for a course module has the obvious advantage of allowing the design of AIDA to be built around the best work already available.

## VII. AIDA DESIGN REQUIREMENTS:

The following requirements indicate the basic design concerns for AIDA, given the problem context, the problem areas, and the fundamental concerns just discussed:

1. Proceed according to a standard design philosophy that maintains consistency of materials and presentations. The issues with regard to standard design philosophy include but are not restricted to: standard selection processes, dynamic default settings, multiple and dynamic levels of assistance, the standard use of mini-advisors with relatively small special-purpose rule sets, standard ISD documentation and materials for students, standard design for editors, and standard interfaces to other modules and commerical systems. Because the motivation for a standard design philosophy is the integration of tools, the design of standard databases

with information about existing schools, courses, delivery systems, and students is necessary.

2. Proceed within the framework of a theory of knowledge. The suggested theory here is JCB because it is a practical modification to an established epistemological view; the modifications are intended to provide some connection to both learning and instruction theories. The emphasis on WELL-justified and WELL-connected beliefs provides a basis for guiding understanding as well as a basis for evaluating understanding. The idea of a web of beliefs and a variety of knowledge types lends itself to both instructional and learning interpretations.

3. Proceed within the framework of a modularized theory of instruction. The theory adopted initially is that Reigeluth's Elabaroation Theory, Merrill's Component Display Theory, the Gagne/Briggs models of instruction, and other theories offer meaningful, useful, and potentially effective organizing strategies for course materials. What is most needed is an algorithm to determine when to apply which model. The reason the selection algorithm has not been established is that the assumption was that the strategy would apply course-wide; this, at least, is Reigeluth's assumption. It is exactly the assumption of a single course-wide organizing strategy that this design rejects. The proposal here is that an appropriate and effective selection can be made for a course module.

4. Proceed with a design using these functional modules: a) "macro" module to assist in refining course goals into modules and objectives, b) "mini" module to assist in the selection of appropriate strategies for selecting, organizing, and sequencing course module materials , and c) "micro" module to assist in the selection and sequencing of materials within a lesson. Each module would eventually have access to databases containing course information, templates, examples, etc. Each module would also have access to a set of mini-editors to assist in the creation, alteration, and incorporation of lesson materials into a course. AIDA should be designed so that it could support a wide range of instructional settings, including the classroom lecture setting, a computerized and networked lab setting with full support for CMI, and specialized settings with ITSs and simulations. In short, the AIDA system would consist of a set of modules which all supported multiple modes of operation, multiple and dynamic levels of assistance, user-transparent interfaces, and other features prescribed by the standard design philosophy.

## VIII. RECOMMENDATIONS:

The primary conclusion of this research effort is that it is worth pursuing the design of AIDA. Significant issues need to be resolved, including the desirability, feasibility, and costs of an integrated system of modules sharing access to common databases, common selection processes, files, mini-editors and mini-advisors. If one begins with the

possibility of such a system, then one is led to the need for a standard design philosophy to guide related efforts. As a consequence, the following steps are recommended:

1. Charge a team with expertise in theory of knowledge, cognitive and behavioral theories of learning, AI, integrated computer systems, and curriculum and instructional design with the task of developing the specifications for a standard design philosophy for all tools developed to automate and assist the instructional process.

2. Charge a team with similar expertise with the task of refining the design requirements for AIDA. The design requirements need to be refined to reflect such things as the selection algorithm to be used to organize material at the course module level, the specific structure and modules required for AIDA, a minimal subset of modules to be implemented in a prototype, and initial rule sets to be used in the mini-advisors.

3. Have those documents (standard design philosophy and AIDA design requirements) critically reviewed by a team of specialists from leading academic research centers and from key military training centers.

4. Have the reviewers meet in a forum and share, clarify, and refine their critiques of the two documents. Detailed reviews of SOCRATES and similar systems should be provided so as to gain the most recent and pertinent lessons learned.

5) Produce the final standard design philosophy specifications and design requirements for AIDA.

6) Initiate work on related projects using the standard design philosophy as a guiding requirements standard.

7) Design and develop a prototype AIDA system with a minimal subset of functions and models, and test it in controlled settings.

8) Evaluate progress on AIDA and related projects.

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FINAL REPORT

# EFFECTIVENESS OF CONTRACT MONITORS IN AN AIR FORCE HUMAN RESOURCES LABORATORY: PREDICTION AND MEASUREMENT


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| Contract No: | F49620-87-R-0004 |

# EFFECTIVENESS OF CONTRACT MONITORS IN AN AIR FORCE HUMAN RESOURCES LABORATORY: PREDICTION AND MEASUREMENT

by

L. Alan Witt, Ph.D

and

Mark N. Beorkrem

## ABSTRACT

Facing decreasing operating budgets, Air Force R&D laboratories must increase the productivity of their human resources in order to maintain expected levels of performance. As a step toward that end, the multivariate study partially described in this report was designed to identify individual and situation-level factors that are related to the effectiveness of contract monitor/researchers at the Operations Training Division of the Human Resources Laboratory at Williams AFB, Arizona. Effectiveness criterion data were collected from managerial ratings, organization archives, and questionnaires administered to the contract monitors/researchers. The cognitive and affective reactions of personnel to their work environment were assessed by interviews and the above-mentioned questionnaire. Results indicated several perceptual/ affective and behavioral predictors of effectiveness.

## Acknowledgements

We wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Moreover, we acknowledge and appreciate the efficient manner in which Dr. Rodney Darrah and his staff at Universal Energy Systems handled the administrative and directional aspects of the program.

Several individuals at the Human Resources Laboratory - Operations Training Division provided us with significant assistance. Col. Michael C. Lane enthusiastically supported our research efforts and was instrumental in gaining access to division and contractor personnel. Col. Gene Jensen of HRL/HQ at Brooks AFB articulated HQ's perception of the division's operation. Lt. Col. Forrest Pate and his staff provided assistance in our compilation of archival data. Dr. Dee Andrews provided conceptual and logistical feedback as well as assistance in data collection. On a daily basis, Mrs. Eileen Evans completed a variety of clerical and logistical tasks on our behalf. Lieutenants Kevin Dixon and Pam Lasch facilitated our computer-aided data analyses. Dr. Elizabeth L. Martin provided the foundation for the study and helped us in many ways.

Furthermore, we thank the personnel who took the time to carefully and completely fill out our surveys and meet with us in formal interviews.

## I. INTRODUCTION

As noted by Engle (1977), a major challenge in today's Air Force is the problem of increasing both organization and employee effectiveness while simultaneously reducing costs. Faced with reductions in both capital and personnel budgets, Air Force units must increase the productivity of their human resources in order to maintain expected levels of unit performance. In light of a Defense Science Board panel's rather unflattering report on the state of Department of Defense research and development (R&D) organizations (Charles, 1988), this challenge is of particular concern to military R&D laboratories. Well aware of these issues, the management team of the Operations Training Division of the USAF Human Resources Laboratory (HRL/OT) at Williams Air Force Base is committed to the improvement of productivity, particularly among the division's contract monitors and researchers.

Marrow, Bowers, and Seashore (1967, pp. 233-234) discussed four approaches to improve the performance of individual personnel: (a) altering the individual's work environment, (b) altering the perceptions and cognitions of people, (c) altering the motivation of people, and (d) helping them to develop new skills and habits. In order to determine which of those approaches might be appropriate for HRL/OT, the study partially described in this report was designed to assess the factors both inhibiting and facilitating individual effectiveness in the division. It should be noted that the study was multivariate in nature, and the format limitations of this report preclude a comprehensive

discussion of the data. Additional information is available from the senior author. Our research interests and Dr. Witt's previous work in the measurement of organizational climate and organizational productivity contributed to our assignment to HRL/OT.

## II. OBJECTIVES OF THE RESEARCH EFFORT

Current evaluation of project (i.e., "work unit") performance is conducted formally on a quarterly basis by the management team. This assessment focuses on process criteria -- meeting deadlines, monies spent, logistical problems, etc. Both HQ and division-level management assume that contract monitors are using outcome criteria to evaluate project performance, and no formal, systematic method of assessing the utility or value of the projects exists. As the present study was undertaken for the purpose of identifying the individual and situation-level factors that are related to the effectiveness of HRL/OT personnel, preliminary measures of contract monitor/researcher and project performance were developed, and the cognitive and affective reactions of division personnel to their work environment were assessed. The study was designed to provide feedback to the HRL/OT management team and to contribute to the organizational behavior literature on R&D organization effectiveness.

## III. ORGANIZATIONAL EFFECTIVENESS

Organizational effectiveness in the management sciences has

a long history of intense interest but a short past of empirical study. Without a unitary conceptualization of effectiveness, a number of evaluative criteria labelled as "organizational effectiveness" have been put forth (cf. Steers, 1975; Zammuto, 1984). On the organization level, criterion measures of effective performance in R&D organizations have varied considerably; they have included reliability (Mahoney & Weitzel, 1969), value (Keller, 1986), "innovative efficiency" (Hambrick & MacMillan, 1985), and adaptability (Katz & Allen, 1985). On the individual level, McCarrey and Edwards (1973, p. 440) defined effective performance of the researcher role as "the extent to which role incumbents achieve personal, professional, and organizational goals of applied creative problem-solving and innovation." In general, researchers have employed four approaches to define and operationalize effectiveness: (a) the goal attainment approach, which assesses the extent to which the organization reaches its goals, (b) the system resource approach, which measures the degree to which the organization acquires needed resources, (c) the health systems approach, which measures the internal functioning of the organization's social system, and (d) the customer satisfaction model, which assess the degree of satisfaction among the organization's strategic multiple constituencies (cf. Cameron, 1980).

Of major concern in this investigation was the exploration of what could and should be used as appropriate measures within the unique environment of a government research and development organization. Lack of relevant outcome measures have plagued similar studies in the past. HRL/OT

personnel predominantly adopt the customer satisfaction model, emphasizing the concerns of personnel in the training directorates of the major field commands (e.g., TAC, MAC, SAC). However, others in and outside HRL use the criteria of technical or refereed publications. This presents a problem for HRL/OT: if personnel spend time composing manuscripts for publication (to satisfy command constituencies), they neglect their client base. Thus, these measures of effectiveness create two very different strategies for action. In order to examine the utility of various measures of effectiveness, we gathered several measures, some of which are described in the method section.

Empirical research has yielded a number of person and situation-level factors that are related to creative productivity and effectiveness (cf., e.g., Abbey & Dickson, 1983; Amabile, 1988; Cameron, 1978; Engle, 1977; Hall & Lawler, 1969; Hambrick & MacMillan, 1985; Hennessey & Amabile, 1988; Katz, 1982; Keller, 1986; Kozlowski & Hults, 1987; McCarrey & Edwards, 1973; Zammuto, 1984). These factors include both attitudinal and behavioral variables. We measured a number of these, which, for purposes of meeting the required format of this report, we mention in the method section below.

## IV. METHOD

### Participants

Participants were 36 contract monitors/researchers and their supervisors working at HRL/OT.

## Procedure and Measures

Personnel were sent through the inter-office mail system a letter from the division chief requesting voluntary participation in the survey. Included in the survey were several measures designed to assess the organization, some of which are described below. In addition, subjects were asked to meet with the researchers for formal interviews.

## Measures of Effectiveness

Individual perceptions of organizational effectiveness were measured by four items, two from Hendrix (1979) and two from Romzek (1985). A composite score of all four items was constructed (alpha = .69).

Several variables assessed the performance of scientists and contract monitors. Seven of the nine members of the management team rated individual personnel on four criteria: (a) significant research contributions, (b) overall usefulness to organization (adapted from McCarrey & Edwards, 1973), (c) overall productivity, and (d) clarity of oral and written communication (adapted from McCarrey & Edwards, 1973). Personnel position levels (McCarrey & Edwards, 1973) were gathered from organization archives.

In order to assess the projects on which the scientists and contract monitors were working, the seven above-mentioned members of the management team rated each of the work units on three criteria: (a)

innovative contribution to scientific knowledge, (b) overall usefulness to end-users, and (c) technical usefulness to end-users. The latter two measures were designed to assess the value of the project (Keller, 1986). In addition, the division's priority rank of the work unit was gathered from organization archives. Criterion means across the raters were calculated and, along with the priority rank, were assigned to the project contract monitor/researcher.

In order to have a single criterion of effectiveness, a composite score was calculated as the sum of the perceptions of effectiveness scale, the composite of managers' mean ratings of individual performance, and composite of managers' mean ratings of workunit performance as assigned to the individual.

## Predictors of Effectiveness

Several predictors were measured. In line with Keller (1986), tenure, educational level, and position level were included as predictors of effectiveness. Participants were asked to indicate for each person in the division (a list was provided) the extent to which they interacted with that individual on a five point scale (the higher, the more frequent interaction); this permitted assessment of the degree to which individuals' levels of interaction with other division personnel was related to effectiveness. A mean rating for each individual was then assigned to the individual as his/her mean interaction rating score.

The 39-item climate for creative productivity index (CCPI) (alpha = .85) was developed for this study to assess organization norms for creative work outcomes. Item selection was based on Amabile 's (1988; Hennessey & Amabile, 1988) discussion of the situational factors that affect creativity, Peters and O'Connor's (1980) work on situational constraints on performance, Abbey and Dickson's (1983) work on R&D work climate, and Kozlowski & Hults' (1987) work on climates for technical updating and performance. Omnibus organizational climate was measured by the frequently used 30-item revised (Schnake, 1983) Litwin and Stringer (1968) Organizational Climate Questionnaire (alpha = .89).

Commitment to the organization was measured by the four-item Hrebiniak and Alluto (1974) commitment index (alpha = .73). Assessing mood or general affective predisposition were the the Watson, Clark, and Tellegen (1988) ten-item positive (alpha = .80) and negative (alpha = .81) affect scales. Job satisfaction was measured by a 16-item index (alpha = .87) assessing satisfaction: (a) the fringe benefits you receive (Schnake, 1983); (b) the amount of freedom you have on your job; (c) the chances you have to accomplish something worthwhile (Schnake, 1983); (d) your family's attitude toward your job (Hendrix, 1979); (e) the chances you have to take part in making decisions (Schnake, 1983); (f) the first work assignment you received upon arrival here; (g) the physical layout of your work station; (h) the quality of technical equipment that you use (Keller, 1986); (i) the friendliness of the people you work with (Schnake, 1983); (j) the chances you have to learn new things (Schnake, 1983); (k) the amount of pay you get (Schnake,

1983); (l) the way you are treated by the people you work with (Schnake, 1983); (m) the amount of job security you have (Schnake, 1983); (n) the work itself (what you do); (o) the local area (weather, things to do, community size, etc.) (Hendrix, 1979); and (p) your job as a whole (Hendrix, 1979). Perceived support from the organization was measured by five items (alpha = .88) loading highest on the Eisenberger, Huntington, Hutchinson, and Sowa (1986) 36-item measure.

The personnel were requested to indicate the following: (a) number of journals subscribed to, (b) number of articles read in one month, (c) frequency of use of lab, university, and other libraries, (d) number of technical publications over the past five years, (e) number of referred journal articles published over the past five years, (f) number of conference papers published over the past five years, (g) number of scientific conferences attended over the past five years, (h) number of patents received/applied for over the past five years, (i) number of unpublished papers over the past five years, and (j) number of nonrefereed journal articles over the past five years. As they are traditional measures of scientists' performance, these were included to determine whether or not these activities would be related the criteria of effectiveness operationalized here.

Following Katz (1982), the survey requested personnel to assign percentages that reflected the extent to which the following were their sources of technical information: friends in the field but outside of lab, technical specialists in contractor of project, technical

specialists within branch, technical specialists at other USAF organization, technical specialists at a university, technical specialists in a contractor not on the project, and technical specialists at another government laboratory.

The amount of contact that scientists had with customers was measured by this item: "Please rate the extent to which you have personal contact with the clients listed below." Relevant clients were then listed. Following Miller (1967), personnel were asked to indicate the extent to which they perceived support from the organization to obtain recognition outside the division.

As one of our several measures of environmental uncertainty, personnel were asked to indicate a percentage to reflect their abilitity to predict accurately the end date of their work unit within six months. Conflict was measured by items assessing frequency of occurrence of conflict and frequency of methods used to resolve that conflict.

We mention again that this is not a comprehensive list of our criterion and predictor variables. However, we believe that this sample provides the reader with the flavor of our study.

## V. RESULTS AND DISCUSSION

Because of the space limitations of this report, results of canonical correlation and multiple regression analyses are not included here.

Zero-order correlations between predictor and criterion variables are presented in the Appendix; predictors accounting for less than 4% of the variance in the criteria were not included. It should be noted that the four sets of predictor-criterion relationships reflect different groups within the sample, because of missing data. Thus, comparisons of coefficients are inappropriate.

As shown in the Appendix, tenure in the division, positive affect, and number of journals subscribed to unexpectedly accounted for minimal amounts of variance in the criteria.

Position level, education level, and mean interaction rating accounted for moderate amounts of the variance in ratings of personnel performance. Perceptions of both organizational support and the division's climate for creative productivity acounted for weak to moderate amounts of the variance in the composite rating, perceptions of effectiveness, and ratings of work unit performance, whereas both satisfaction with initial work assignment and perceptions of the division's overall climate acounted for weak to moderate amounts of the variance in the composite rating, perceptions of effectiveness, and ratings of personnel performance. Composite satisfaction and commitment to the division were weakly to moderately related to all four criteria. Negative affect was moderately and negatively related to the composite rating. Perceptions of support from the division to gain outside recognition and ability to predict end date of work unit within six months were moderately related to the composite rating and perceptions

of effectiveness. Conflict activity moderately predicted the composite rating and perceptions of effectiveness. These results are consistent with the literature.

Surprisingly, the number of articles read per month was negatively related to ratings of personnel performance and ratings of work unit performance, while number of conference papers given was positively related to all four criteria. Use of libraries was positively related to the composite rating but negatively related to perceptions of effectiveness. Moreover, the measures of information networking -- the practices of refering with other specialists as sources of information and the frequency of contact with the various clients -- yielded mixed correlations. Thus, the nature of relationships between these typical predictors of R&D and scientific performance remains unclear.

Again, it should be recognized that space limitations preclude comprehensive discussion of the data. In general, however, the results reported here are mixed. As expected, several perceptual and affectives measures predicted the criteria of effectiveness. However, typical criterion measures of R&D performance (e.g., articles read and networking practices) yielded inconsistent relationships with the criteria operationalized here. Interview, observation, and survey data suggest a general conclusion: A major problem facing HRL/OT is the lack of a meaningful, consensually agreed-upon set of criteria for use to assess the outcome utility of work units. Further work is needed to determine which criteria will best serve the interests of HRL, HRL/OT, and AFSC.

## VI. RECOMMENDATIONS

In an atmosphere of declining funding and greater competition for research funds, the HRL/OT management team shall face two major challenges. First is increasing the productivity of their personnel. The data presented in this report have identified several factors that may be related to contract monitor/researcher effectiveness. However, further work is needed to clarify the exact nature of these relationships. Second is determining how to appropriate remaining monies to division projects and proposals. The criteria developed and reported here may provide an initial approach to assessing the value of work units, in marked contrast to the present method of assessing work units along process, finance-based criteria.

Several limitations of the present study reduce the generalizability of the findings. First, data was collected in one setting and was of a very small sample, and the problems of personnel productivity and inadequate evaluative criteria of work unit and individual effectiveness are not unique to HRL/OT. Future research is needed to both clarify and assess the validity of the findings reported here and to develop refined criterion measures which may be presented to laboratory division chiefs for consideration for adoption in their divisions.

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**Appendix. Predictors of Effectiveness Correlated with Criteria of Effectiveness**

| Predictors | Criteria of Effectiveness 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Tenure at HRL/OT | .20 | .05 | .21 | -.04 |
| Position level | .17 | .18 | .56 | .08 |
| Education level | -.10 | .07 | .43 | .08 |
| Mean interaction rating | .28 | .18 | .57 | .21 |
| Creativity climate | .23 | .48 | .09 | .34 |
| Omnibus organizational climate | .45 | .55 | .27 | .10 |
| Commitment | .31 | .21 | .37 | .39 |
| Positive Affect | -.05 | .07 | -.14 | .00 |
| Negative Affect | -.51 | -.10 | -.01 | -.10 |
| Overall Satisfaction | .27 | .44 | .22 | .24 |
| Sat w/ initial work | .38 | .35 | .35 | .11 |
| Support perceptions | .30 | .43 | .17 | .38 |
| No. of journals subscribed to | -.03 | -.08 | -.21 | -.15 |
| No. of articles read per month | -.01 | -.12 | -.42 | -.36 |
| No. of conference papers given | .25 | .30 | .22 | .35 |
| Use of libraries | .38 | -.28 | .08 | -.02 |
| Refer with | | | | |
| friends outside lab | -.03 | -.09 | -.07 | -.20 |
| contractor on project | .42 | .17 | .15 | .07 |
| specialists in branch | -.75 | -.04 | -.23 | .11 |

| | | | | |
|---|---|---|---|---|
| specialists in other branch | .23 | -.16 | .11 | .30 |
| specialists in other AF org | -.27 | -.41 | .08 | -.30 |
| specialists at university | .44 | -.01 | -.78 | .53 |
| contractor not on project | -.04 | .05 | -.03 | .27 |
| specialists at other lab | -.28 | -.14 | -.11 | -.19 |
| Perception of support from division to obtain recognition outside division | .46 | .47 | .03 | -.11 |
| Ability to predict end date of work unit within six months | .42 | .28 | -.14 | .11 |
| Frequency that conflict is: | | | | |
| Brought into open & worked out | .40 | .49 | .05 | .36 |
| Smoothed over | -.54 | -.39 | -.11 | -.12 |
| Frequency of contact with AFSC | .36 | .26 | .22 | .20 |
| Frequency of contact with MAC | -.35 | -.44 | -.12 | .02 |
| Frequency of contact with SAC | -.58 | -.65 | .24 | .34 |
| Frequency of contact with HSD | .46 | .42 | -.12 | .37 |

Note: Criterion 1 = Composite criterion; Criterion 2 = Perceptions of effectiveness; Criterion 3 = Mean of managers' composite rating of individual performance; Criterion 4 = Mean of managers' composite rating of workunit performance. Because of missing data, the N's are different across the criteria. The number of participants comprising the samples for the correlations are as follows: criterion 1 (N = 18), criterion 2 (N = 36), criterion 3 (N = 35), criterion 4 (N = 18).

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# FINAL REPORT

# COMPUTER SUPPORT of CREATIVITY in UNIFIED LIFE CYCLE ENGINEERING

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Date: Sept. 5, 1988

Contract No.: F49620-87-R-0004

# COMPUTER SUPPORT of CREATIVITY in UNIFIED LIFE CYCLE ENGINEERING

by

Lawrence F. Young, D. Sc.

## ABSTRACT

The Air Force program called Unified Life Cycle Engineering (ULCE) is concerned with more effectively designing producibility and supportability, as well as performance, cost, and scheduling, into new weapon systems. A main thrust of this program is to identify functions for a Decision Support System (DSS) for ULCE. As one aspect of DSS for ULCE, this project makes an assessment of the opportunity for computer support of creativity in the systems development process. It first examines the systems engineering and design process generically, then the specific nature of the Air Force weapons acquisition process, and thirdly, creativity support functions that might impact the problem requirements definition and systems design phases. Recommendations are made to develop computer support for requirements specification and systems design.

## Acknowledgements

Support for this research from the Air Force Systems Command and Air Force Office of Scientific Research, is gratefully acknowledged. The role of Universal Energy Systems, Inc. in research administration and in creating the linkage with the Air Force is also appreciated.

Thanks are also extended to all the men and women at the Human Resources Laboratory at Wright Patterson AFB under the command of Col. Donald C. Tetmeyer. My appreciation goes to Col. Tetmeyer and to technical director Mr. Bert Cream for the opportunity to work in their division.

I particularly wish to thank Capt. Maureen Harrington for helping me in initiating this project before she moved on to a new assignment at the Pentagon, to Capt. Rick Berry who took over from Maureen and was my guide and mentor, to Logistics Systems Branch Chief Wendy Campbell for her friendliness and extension of the resources of her unit, to Susan Stiller for helping with the literature search, and to Capt. Don Lowdermilk for letting me use his nice laser printer. Thanks are also due to ML Brei of the Institute for Defense Analysis for supplying important background and key bibliographical summary material. This work owes much to the prior and parallel work of the scientists and researchers of the Institute for Defense Analysis, the RAND Corporation, and members of other institutions comprising the ULCE DSS Task Group. Special appreciation is expressed for Sigfried Goldstein's (Sigfried Enterprises, Inc.) and David Owen's (IDA) very cogent IDA Interim Report as well as for the time that "Siggy" spent with me in illuminating the darker mysteries of the weapons acquisition and design process.

## I. INTRODUCTION

Unified Life Cycle Engineering (ULCE) is an Air Force program to develop computer support for designing weapons systems for producibility and supportability, as well as designing for performance and cost. In 1987, the Institute for Defense Analysis (IDA) organized a Working Group to identify R & D needed to achieve a decision support system (DSS) capability in an ULCE environment. Their early work did not define a prototype for a DSS, but set an analytical framework. Within this framework, "group creativity" was mentioned in the context of design negotiations, but the support of creativity in the design process was not elaborated upon. However, it is accepted in systems engineering practice that good design depends on creativity, among other skills. Thus, creativity support is a relevant and potentially important area for DSS ULCE research.

After a corporate career in the development and management of quantitative information/decision support systems, my recent interests as an academic have turned toward computer support of qualitative cognitive processes. Since 1981 I have been exploring ways computer support could enhance creativity. The ULCE DSS research project has provided me with a chance to apply what I have learned about creativity support to an important application area.

## II. RESEARCH OBJECTIVES

The goal of this Summer Research has been to complement the research framework for an ULCE DSS by considering creativity support.

Within this goal,we have attempted to:

a) Make a preliminary assessment of the "area of opportunity" for creativity support in systems engineering in general and specifically in Air Force weapons system development practice.

b) Identify potential support functions for an ULCE DSS and make recommendations for further R & D.

## III SYSTEMS ENGINEERING and CREATIVITY

### A. Survey and Assessment

Systems engineering design literature was reviewed and an assessment of the potential role of creativity in the process was made. This first assessment was made independently of the specifics of Air Force acquisition practices. The results are discussed below.

### B.Summary of Characteristics and Assessment Results

Scholars have highlighted the following characteristics of systems engineering and design practice [Christakis, Keever, & Warfield (1987) ]:

• Iterative • Hierarchical • Analytical • **Creative** • Sequential • Synthesis-oriented • Conceptual orientation • Deontic • Has a Design Target • Uses Hard &Soft Data • Group &Individual Tasks

Research in design theory and methodology has generally followed one or another of these dimensions with little effort to integrate them [Cross(1984), Nadler (1984)]. Recently, however, emphasis has grown on using more integrated frameworks. While creativity is a factor in design, little has been done to develop computer tools for creativity support. Brei (1988) concluded that "pockets of researchers throughout the world" sought to "develop (noncomputer) techniques that would help designers in certain stages of their work....[such as] brainstorming, synectics, value analysis, morphology, lateral thinking, etc.". Only recently have some of these creativity support methods emerged in DSS literature and software.

Creative thinking has been described as a divergent (non-analytical) approach in "open " problems for which objectives and criteria for "good" solutions are unclear [Guilford (1967, 1975), Fulgosi & Guilford(1968)]. A creative result is essentially a novel and meaningful synthesis arrived at through a divergent process of association. Using problem openness and the non-suitability of analytical methods as criteria, the opportunity for exercising creativity in systems development depends on the dimensions of a) Problem Definition (to what extent are objectives and requirements known, defined, and constrained, and b)Solution Methodology (to what

extent is a procedural process defined for identifying ways to attain specified objectives and requirements). Problem Definition is the central concern of the Requirements Specification phase of systems development, Solution Methodology pertains mainly to the Design phase.

We illustrate the relationship between these and opportunity space for creativity in Exhibit 1, using four categories of problem definition and five categories of solution methods..

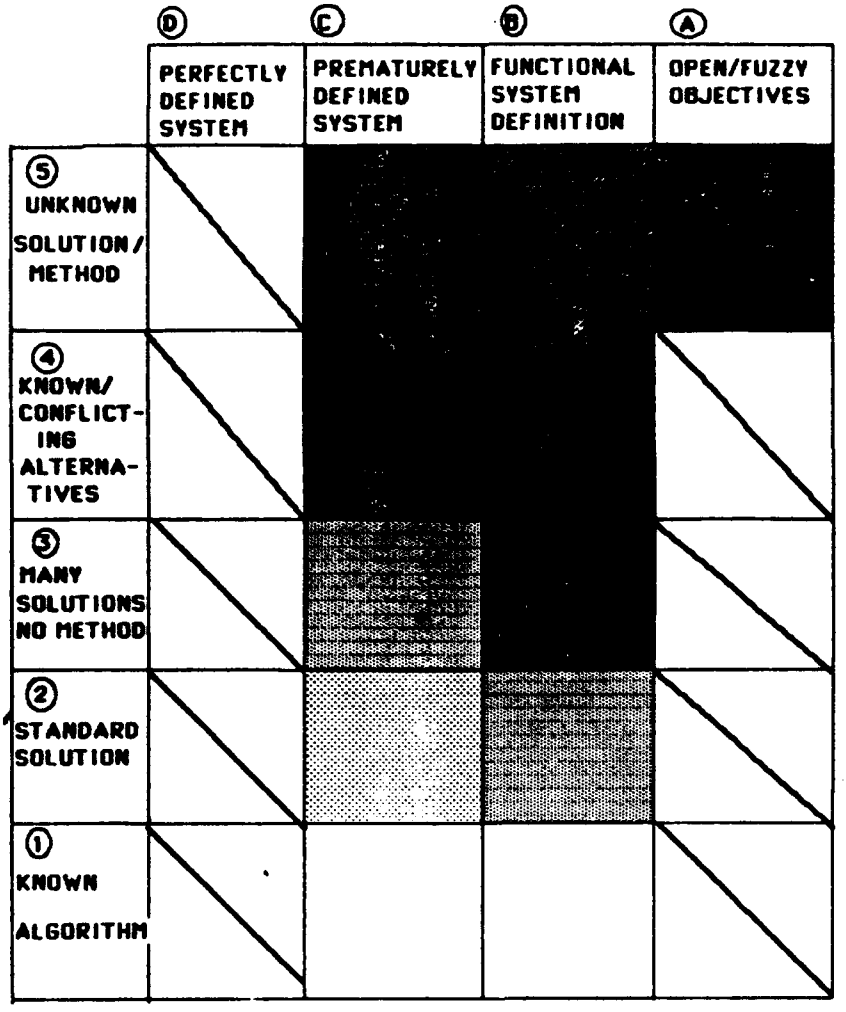



Exhibit 1 PROBLEM and SOLUTION DEFINITION vs CREATIVITY SPACE

Problem definition category A is an "open" problem with maximum opportunity for creative approaches, B and C progressively present less space for creativity, and category D leaves none. Solution definition category 1 leaves no room for creativity, while category 2 allows the possibility of seeking an innovative approach if the potential payoff warrants the effort. Categories 3 through 5 are classical situations calling for a creative approach to invent new solution approaches, to resolve conflicts in known solutions through new approaches, or to find many alternatives or new search methods. These present progressively increasing opportunities for creativity. Exhibit 1 also illustrates that several problem and solution definition categories are not compatible (those diagonally lined).

Requirements also constrain design and creativity. They are needed to provide the framework and criteria by which feasible design alternatives can be identified and assessed, and thus logically precede design. But requirements can inadvertently be stated in unnecessarily constraining ways. Requirements should only define what a system will be able to do or cope with or accomplish, and not how it is do it. The "how" questions are supposed to be answered only in the design stage. Legitimate requirements arise from ultimate ends (achievement of systems missions and objectives) or external conditions (threats, disturbances, and circumstances in the system's environment). Illegitimate requirements derive from current or assumed means (particular systems, components, processes, or operational procedures). Often, the boundary between requirements and design is inadvertently crossed because of the phrasing of requirements [Wood & Antonsson(1986), Wood, Sercel,, et al (1986) ]. Phrasing that refers to things (objects, components, equipment, etc.) instead of functions, presumes that only certain things must be used to operationalize the desired functions. Such presumptions might be correct, but it should be left up to the designer to make this determination. Hidden assumptions are the enemy of innovation.

As noted, the system development process is also both hierarchical and sequential [Hall (1962), Cross (1984), Nadler (1981) Ullman, et al (1987), Waldron & Waldron (19870]. Complexity is handled by first considering large concepts and then factoring them into progressively smaller ones in a hierarchical manner. System development is also sequentially phased (although usually iterative), moving from stating objectives, through requirements specification, into general design, followed by detailed design, and then into system building, testing, and ultimate delivery and deployment. Just as requirements can unnecessarily predetermine design, general specifications at a given phase can overly limit more detailed ones at lower hierarchical levels. This is illustrated in Exhibit 2.

Undesired inheritance of constraints can occur either while moving left to right through horizontal phases or top-down within a hierarchy. But because we are dealing with an iterative system, and also because the order of thinking of systems developers may not strictly follow the normative left to right or top to bottom paths, undesired constraints can also arise along other directions. Reverse inheritance or "reheritance" of constraints occurs when the definition or redefinition of a hierarchically lower or "later" phased component creates an inherent (not always manifest) definition or redefinition of a higher or "prior" component Exhibit 3). To guard against over-constraining design in subtle unintended ways from reheritance, monitoring of every higher level and logically (if not temporally) prior phase is needed whenever anything is changed.

Interaction or "coheritance " of constraints occurs when the definition of a component constrains the definition or creates the need to redefine other components at the same hierarchical level or in the same phase. Coheritance is a logical and necessary accompaniment to factoring (decomposing) systems into components or breaking up work phases into clusters of simultaneous tasks. But there normally are many "correct" ways to create a decomposition, one being determined in practice by a particular conceptual view. While all components need to be taken as

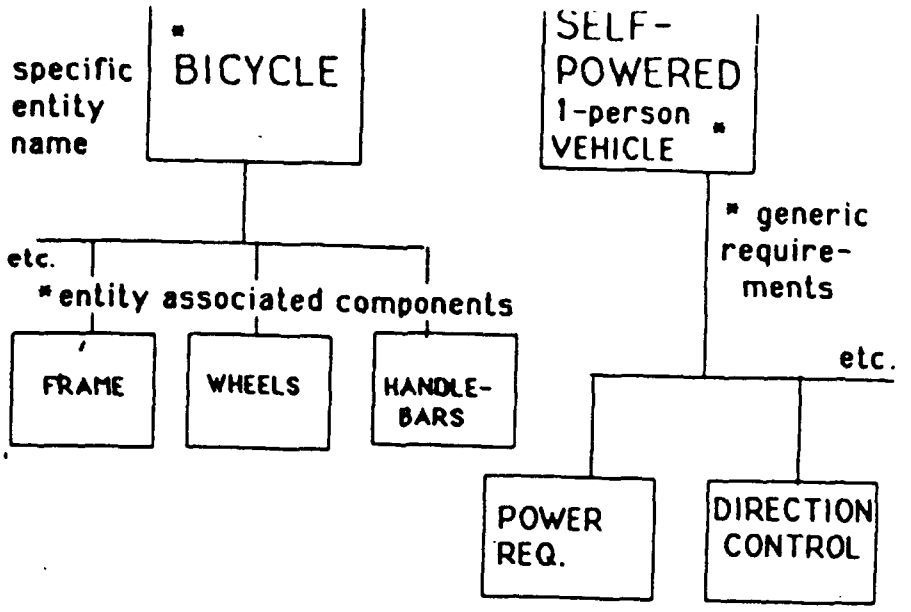



Exhibit 2

## HIERARCHICAL INHERITANCE of CONSTRAINTS

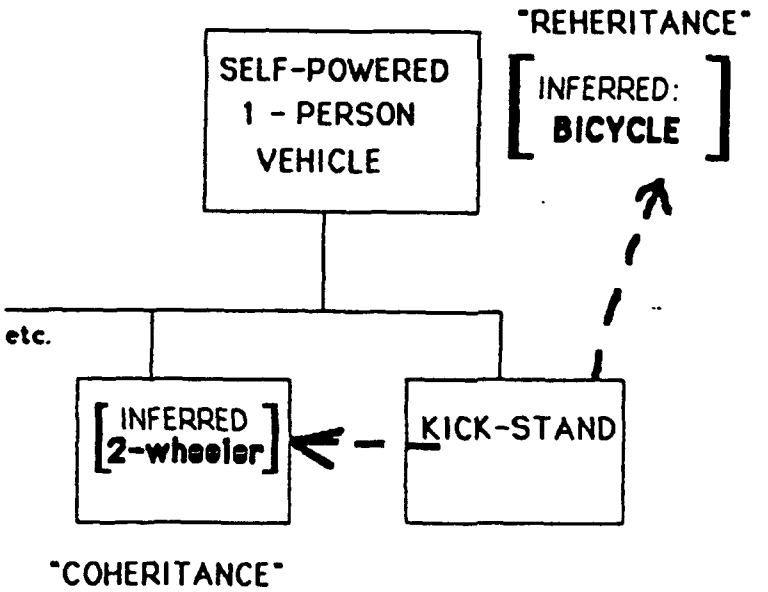



Exhibit 3

## REHERITANCE and COHERITANCE of CONSTRAINTS

a whole to verify that everything is accounted for, they are often conceived of incrementally by the analyst/designer. Constraints are placed on remaining components as soon as the first component is defined. Redefinition of any component opens new ways to define others, and an early component definition should not become fixed without trying alternatives.

## IV The Air Force Weapons Systems Acquisition Process

### a. Review and Assessment

Air Force weapons systems acquisition practices also set a boundary on the opportunity for creativity and its support. This was examined through secondary research of documents describing the process and how it was implemented in several cases, and through direct discussions with an informed expert source (S. Goldstein).

### b. Summary of Characteristics

A primary limiting factor to improvement through computer support seems to be how the first stage of the acquisition process itself is carried out. This is neither a new or unique observation. Michael Rich of the RAND Corporation recently stated that the weapons systems acquisition process is overly constraining on systems designers [Rich(1988)]. The same observation is made by Goldstein and Owen (1988), particularly with regard to the way requirements statements in RFPs affect the supportability dimension. They also point out that the recommendation that requirements should serve strictly as functional guides has already been recognized in Air Force policy as stated in OMB Circular A-109 and DoDD 5000.1.

A RAND report based on a review of 15 major systems acquisitions over a 19 year period [Stanley & Birkler (1986)] also highlights deficiencies in the statement of requirements with respect to operational suitability factors (eg availability, compatibility, transportability, reliability, maintainability, logistic supportability, etc.) It emphasizes the need for more precise expression of these in the Statement of Need (SON) that

precedes and serves as the basis for the issuance of an RFP. The report finds that operational suitability needs to be stated quantitatively, in prioritized form, along with performance factors, so that subsequent early design studies can better assess tradeoff issues as they unfold. The report also mentions (p. 12) that operational suitability and peformance might be enhanced together (without trading off one for another) if technological opportunities for accomplishing this were discussed in a more balanced way as part of needs statements. We see the resolution of this kind of apparent but resolvable conflict among design objectives as an important avenue for the potential exercise of creativity. Features that set significant bounds around the potential for creativity and variety in the design process are:

- Functional, performance, and supportability requirements are stated in a hierarchical, increasingly detailed manner, according to voluminous pre-established MIL-STDs, paralleling and often presuming the ultimate equipment design hierarchy;
- Physical sub-systems and components are physically identified unnecessarily within requirements specifications ;
- Neither trade-off criteria nor priorities between performance, supportability (including reliability), acquisition cost, and life cycle cost factors are made explicit to vendors.
- The design tasks of systems engineers concerned mainly with performance capabilities and those of supportability engineers are often first handled separately before trying to integrate design approaches and resolve conflicts.
- Unwritten, informal understanding of performance, supportability, and cost priorities exist on the part of both manufacturers and the Air Force. The understanding is that decisions are dominated by acquisition cost and performance specifications. Coupled with the effect of highly constraining requirements, this dictates that there is likely to be little variance of these dominant factors among vendors' proposals.

- Supportability and life cycle cost factors are left for vendors to attempt to independently manipulate (without affecting performance and acquisition costs) in an effort to exceed requirements as much as possible.
- Within the constraints of the current process, supportability presents the most opportunity for vendors' to exercise some degree of creativity, at least in terms of creating an argument for the advantages of their approach.

In personal discussions Goldstein also asserted that trade-offs between performance and supportability need not be made explicit by requirements statements because a "good" design approach can address these needs together, without sacrificing one in favor of the other. This echoes the RAND report statement cited above that operational suitability and peformance might be enhanced together if technological opportunities for doing so were more thoroughly explored. RAND however does emphasise a need for prioritities, not tradeoffs, to be more explicit.

Given the present procedures on most acquisitions it appears unlikely that, without basic changes, computer creativity support tools would be able to contribute much. However, certain computer support tools may be needed in order to facilitate the recommended changes in SONs and RFPs. This is discussed under the final recommendations section.

## V. CREATIVITY SUPPORT FOR PROBLEM DEFINITION AND DESIGN

### a. Review and Assessment of Creativity Enhancement Functions

Methods of creativity enhancement and idea development that have been or could be built into a computer support system were examined with respect to their application to the weapons systems acquisition process. The following section summarizes these support methods and presents an assessment of their utility for the problem definition (RFP development) and systems design phases of the weapons systems development cycle. This assessment uses the "creativity space" framework formulated by the auther and shown as Exhibit 1.

**b. Results**

Based on Brainstorming (Osborn [1963], Synectics (Gordon [1961]), and other noncomputer methods described by Rickards (1974), Young (1983,1988) identified computer functions to support creativity. This spectrum of computer support has been called Idea Processing. Idea Processing methods are briefly illustrated in Exhibit 4. Design of a particular Idea Processing support system, just as any other design, can selectively include features according to its intended use and users.

Analogies and metaphors play a key role in creativity and some Idea Processing methods. Analogical reasoning is seen as fundamental to problem solving and systems design as one kind of problem solving [Spearman (1923), Novick (1988), Findler(1981)]. The emphasis is on finding a similar problem (the "source" problem) so that its solution approach can be applied directly to the problem at hand (the "target" problem). Similarity can be based on surface features or on underlying problem structure. Experienced practitioners or experts have been found to rely on both of these in finding problem analogs. In contrast, neophytes are often ineffective because they tend to rely on surface features only and fail to recognize underlying problem structure [Novick (1988)]. Thus, one basic type of support that can augment the experience of systems engineers would be to provide a data base of problems in a form that would facilitate matching against a current case in terms of both surface characteristics as well as underlying problem structure. But problems that cannot be solved by merely applying the approach used for a similar problem call for finding less obvious, less direct, and less complete similarities that may lead to new insights and development of new approaches rather than the reuse of existing solution methods. For these cases, problem solving may depend on finding a clue in an entirely different problem realm through metaphors. Metaphors convey weaker, less direct, more symbolic connections than analogies. Their utility stems from breaking away from literal thinking along well-worn paths in order

MORPHOLOGICAL ANALYSIS (COMBINATORIAL SYNTHESIS)

*Method*: a) Dimensionalize a generic concept;
b) Generate new combinations of alternatives. c) Assess preference

*Brief Example*: Dimensions of a transportation system -

I Power Source: Coal-steam, Electric, Nuclear
II Load Container: Conveyor belt, flat car, enclosed vehicle
III Control system: Manual, automatic, mixed

Combinations of values of each dimension comprise a total design concept:
1)Coal-steam/Conveyor belt/Manual 2)Coal-steam/Conveyorbelt/automatic
3)Coal-steam/Conveyor belt/mixed 4)Electric/Conveyor belt/Manual, etc....

HIERARCHICAL OUTLINE ORGANIZATION

*Method*: Arrange and rearrange ideas, topics, in major and minor categories, with minor categories belonging to a selected larger heading or major category.

*Brief Example*

New Product Introduction Plan
  Define Market Potential
    Identify User Segments
    Estimate Usage By Segment
  Develop Promotional Plan
    Product Sampling
      Distribution Plan

SUCCESSIVE GENERALIZATION

*Method*: Enlarge or contract the boundaries of a concept by defining successively more general categories or more specific categories

*Brief Example*: Pencil - Writing Implement - Input device

SCENARIO BUILDING

*Method*: Develop a sequential history of plausibly unfolding events

*Brief Example*: The Ayatollah Dies - Civil War in Iran - The Russians Mass at the Border - The US Strengthens the Persian Gulf Fleet - etc.

IDEA TRANSFORMATION

*Methods*: Component ideas are rearranged, transformed in size or relation

*Brief Examples*: Reverse top and bottom, reverse first, second, third, etc.,make a small object large or a large object small. identify the opposite of a concept

BRAINSTORMING and SYNECTICS

*Methods*: a)Generate many ideas without judging quality (Brainstorm)
b) Use metaphorical thinking to break out of conventions and self-imposed constraints c) use verbal devices such as book titles that express a contradiction d) then examine similarities/differences to the problem.

*Brief Example*: Problem - Need to find a new kind of container that will be open to some objects and closed to others of the same size.
Generate metaphors for a containing or contained object : shackles, cells, guards, police,.....
Book Titles: "Freedom in Chains" "The Earthbound Eagle" "Floating Lead":
Possible Idea: Use a liquid to make heavier objects sink, others float out.

Exhibit 4 SELECTED IDEA PROCESSING TECHNIQUES

to gain new insights into potentially useful innovations [Gordon (1961)]. Computer support in finding such useful metaphors requires a different process than data base retrieval of functionally similar problems (See Young (1987). The synectics method uses several devices to encourage metaphorical thinking that can be computer supported.

Exhibit 5 presents conclusions on the applicability of Idea Processing methods to weapons systems definition and design. An underlying assumption is that current practices will be modified so that problem specifications in SONs and RFPs will fall into the categories of either A) Open, fuzzy objectives, or B) Functional system definitions. In these cases, each of the support methods described can apply to system design. Some of the creativity support methods, implemented in a different manner, can also support requirements definition. In this respect, a creative approach is often needed to redefine an ill-understood problem before settling on a set of requirements. In such cases, techniques such as problem boundary redefinition through successive generalization, idea transformations such as problem statement reversals, or the examination of dynamic scenarios, help to redefine the problem in a new light. As shown in Exhibit 5, more of the support function repertoire would apply for problem definition in category A problems than in category B problems, but category B support may be more important because of its relatively higher frequency of occurrence as the normative case. Exhibit 5 should be considered along with Exhibit 1 to assess the extent of space for creativity.

## VI RECOMMENDATIONS

This section presents a) recommendations flowing from this research, and b) initial follow-on research to be proposed for a mini-grant. While recommendations are listed as separate items, they are conceived of as components of an integrated support system. This support system is schematically represented in Exhibit 6.

| | Problem Definition (SON/RFP) | | Solution Definition (DESIGN) |
|---|---|---|---|
| Support Methods | Prob. Category A | Prob. Category B | Prob. Category A/B |
| Successive generalization | X | | X |
| Hierarchical outlining | X | X | X |
| Morphological analysis (combination generation) | | | X |
| Parnes transformations | X/ ? | X/ ? | X |
| Metaphorical thinking | X | | X |
| Scenario generation/ assessment | X | X | X |

KEY
X : METHOD APPLIES
X/ ? : METHOD PARTLY APPLIES
PROBLEM DEFINITION CATEGORY A and B : See Exhibit 1

Exhibit 5 Applicability of Support Methods to System Problem Definition/Design Phases

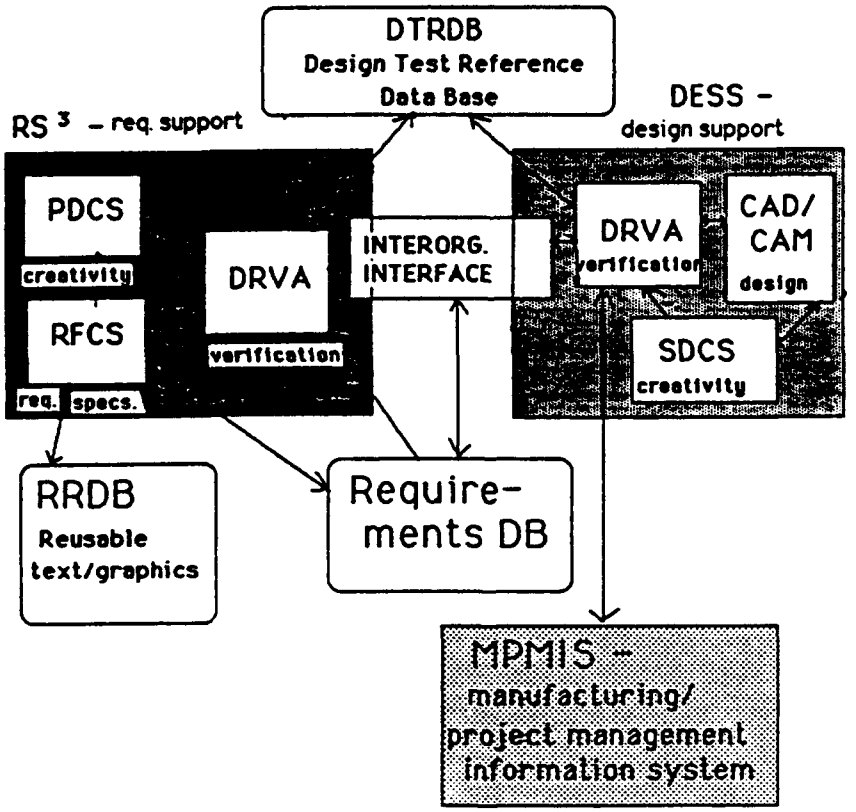



EXHIBIT 6 THE REQUIREMENTS/DESIGN SUPPORT SYSTEM

**a. Support System Recommendations**

- Replace use of detailed MIL-STDS with a computer accessed reference data base Design/Test Reference Data Base (DTRDB).
- Provide computer support for requirements specification (Requirements Specification Support System ($RS^3$) including a Requirements Frameworks and Checking Support (RFCS) component for the avoidance of various forms of "pathological coupling" among requirements and a Reusable Requirements Data Base (RRDB) providing retrieval of standard reusable requirements text and diagrammatic material.
- Provide for a future interorganizational interface between $RS^3$ and computerized proposal preparation and submission by vendors.
- Provide a common design assessment support module (Design-Requirements Verification and Assessment (DRVA)) accessible by both Air Force and manufacturers to encourage generation of alternatives for enhancing creativity as well as a common understanding of criteria.
- Selective creativity support modules should be developed for both the $RS^3$ and the Design Environment Support System (DESS) (as indicated in Exhibit 4). The design of these modules can draw upon earlier guidelines [Young(1988), Rouse (1986)].
- Existing or new CAD/CAM tools should be interfaced with other modules of a DESS so that qualitative design ideas can move from SDCS into CAD/CAM for development or so that selected CAD/CAM results can be input for qualitative idea processing within SDCS.
- A Manufacturing/Project Management Information System (MPMIS) should be provided with an interface with the DRVA component of DESS and $RS^3$ to allow for the reverification of changes.

**b) Proposed Follow-On Research**

A mini grant proposal will be submitted to further develop functional specifications for a prototype $RS^3$ as a first priority next step to remove the critical limiting barrier to improving design quality and comprehensiveness.

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# FINAL REPORT

## The Relationship Between Inspection Time and Intelligence.


| | |
|---|---|
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| Contract No: | F49620-88-C-0053 |

The Relationship between Inspection Time and Intelligence

by

Robert K. Young

## ABSTRACT

The relationship between inspection time and intelligence was investigated using a relatively homogeneous population: Air Force recruits. Inspection time is defined as the minimum time necessary to see a difference between two or more items. In the usual task, two lines are presented for an extremely short duration and the task of the subject is to indicate which of the two lines is shorter. Previous research was replicated using a relatively large sample size (N = 113).

Consistent with previously reported studies, a correlation between inspection time and the Cattell Culture Fair Test, the measure of intelligence employed, was found for the entire sample (r = 0.34). Additional analyses indicated that some subjects used an apparent movement strategy. For those subjects no relationship was found between measure of intelligence and inspection time (r = 0.21). But for those subjects who did not use a strategy, a relatively high correlation was found (r = 0.56) between inspection-time performance and our measure of intelligence. However, no mean difference in inspection time was found between the strategy and non-strategy groups. Nor was there any mean difference found between the strategy and non-strategy groups in the measure of intelligence employed.

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. Dr. Scott Chaiken provided me with support, encouragement, and a truly enjoyable working atmosphere. Help and assistance by Drs. Bill Tirre, Pat Kyllonen, and Dan Woltz was also appreciated. Mention must also be made of the outstanding work done by Mr. Rich Walker and his staff who did the programming necessary to run the subjects and gather the data. John Allison's invaluable assistance throughout my period of appointment is also appreciated. Finally, Col. Dave Brown and Dr. Joe Weeks made me feel welcome and made my stay all the more enjoyable.

## I. INTRODUCTION:

Inspection time is defined as the minimum time necessary to make a reliable judgment about two or more stimuli. For example, the standard inspection-time task is one in which two vertical lines are presented and the task of the subject is to indicate which is the shorter of the two. The inspection-time task should not be confused with the reaction-time task. Instead, in the inspection-time paradigm the subject has an unlimited amount of time to make a judgment about stimuli which are presented for a very short duration. Inspection time is then defined as the shortest presentation duration necessary for a reliable judgment.

The modern day inspection-time task was developed in 1972 (Vickers, Nettlebeck and Willson) but received little attention until a 1976 paper by Nettlebeck and Lally reported that a correlation of -0.92 was found between inspection time and intelligence with a sample of 14 subjects. (That is, shorter inspection times were associated with higher intelligence.) The implications of such a relationship were immediately seen by research workers and work has continued at a very rapid rate since then.

The work of Nettlebeck and Lally (1976) and many other studies reported in the literature have been criticized for a couple of reasons (e.g. Irwin, 1984). These include the use of an extremely small number of subjects and the use of an extremely heterogeneous subject population. The original Nettlebeck and Lally study used retardates as well as subjects who were above average in intelligence. The use of a small number of subjects who are heterogeneous in character would tend to result in relatively high

correlations.

The inspection-time task as it is generally used consists of two vertical lines connected at the top somewhat like the Greek letter Pi. The general procedure used involves presenting the test stimulus for an extremely short duration and the task of the subject is to indicate which vertical line is the shorter of the two. To prevent the use of after-images serving as cues, a backward mask is used to cover the test stimulus immediately after presentation. Unfortunately, the backward mask creates a second problem: apparent movement. Those subjects who report using apparent movement as a cue show little if any relationship between inspection-time performance and intelligence while those subjects who do not report using apparent movement as a cue show a considerably higher correlation between inspection-time performance and intelligence (Mackenzie and Cumming, 1986).

## II. OBJECTIVES OF THE RESEARCH EFFORT:

My assignment as a participant in the 1988 Summer Faculty Research Program (SFRP) was to conduct research to evaluate the relationship between the inspection-time task and intelligence. Previous studies which reported finding a relationship between inspection-time performance and intelligence often had problems of interpretation. For example, use of heterogeneous samples of small size often yielded correlations of large size that had little meaning from a scientific point of view or from an applied point of view such as would be the case for the Air Force.

Further, it was also occasionally found that some subjects were able to make use of a strategy to improve their performance on the inspection-time task. When analyses were done to look at differences between strategy users and non-users, the numbers of subjects in the resulting subgroups again became relatively small with consequent problems of interpretation.

As a consequence my assignment was to attempt to replicate these findings with a relatively large sample of Air Force recruits who, it should be noted, come from a relatively homogenous population of intelligence. That is, Air Force recruits must score in the top 60 percent on a measure of general academic ability and as a consequence there is likely to be considerable restriction in range of intelligence in this population.

My personal interest in the inspection-time topic is directed in two directions. The first is directed toward continued investigation of the relationship between inspection time and intelligence. For example, what parameters involved in the inspection-time task are also related to intelligence? Is the line length, type of mask, or distance the lines are apart critical for finding the relationship? The second is in the use of the inspection-time task as a general experimental task such as reaction time. For example, what is the relationship between inspection time and age? What is the relationship between inspection time and amount of alcohol ingested? These questions deal with cognitive functions which are generally investigated by a reaction-time paradigm. Yet reaction time, by its nature, requires a motor response which may be impaired rather than the cognitive activity inferred from the reaction times.

## III. PRESENT RESEARCH:

a. Method. A simple repeated measurement design was employed. In this experiment 113 male Air Force recruits were each given 144 trials on the inspection-time task.

Immediately following the inspection-time task, a series of 12 questions was asked which was designed to determine if the subject used an apparent movement strategy to facilitate performance. In addition, other questions designed to determine if the subject was too fatigued, stressed, or sick to participate, were employed. No subjects were dropped from the experiment.

Following the inspection-time questionnaire subjects were administered Scale 3 of the Cattell Culture Fair Test, a test similar to the Raven's progressive matrices test. Reported reliabilities of the Cattell test average around 0.60 and the test is designed to measure a general intellective factor (Anastasi, 1988)

Procedure. All testing was done on computers and subjects were tested in groups of approximately 24 subjects each. Each Subject was seated in front of a computer screen at a distance of approximately 42 cm. Subjects proceeded at their own pace. Initially, the subject read a Privacy Act statement associated with the test, and then made a decision whether to participate in the experiment.

The subject next read instructions about the ins -time task and was told what to expect. After reading the instructions, the experiment proper

began. At the beginning of each trial, an asterisk appeared for 500 msec. and served as a ready signal. The ready signal, centered on the screen, went off and after a 200 msec. interval the test figure was presented. The shorter leg was 10 mm long while the longer leg was 30 mm in length. The two vertical lines were separated by a 9 mm interval but were connected at the top by a horizontal line extending beyond the two lines both to the left and to the right. Thus the figure looked not unlike the Greek letter Pi. Following presentation of the figure a backward mask was immediately shown for 500 msec. to avoid the possibility of an after-image which could be seen and responded to by the subject. The mask consisted of two legs, each 4 mm wide, covering both the 10 and 30 mm lines of the test figure and extended from the top horizontal line downward for 50 mm. The mask was filled and was as bright, and the same color, as the test figure.

The task of the subject was to indicate which line was the shorter of the two. The subject responded to the figure by pressing "L" if he thought the shorter line was on th- and "D" if he thought the shorter line was on the left. After a 500 msec. rest interval, the ready signal was presented for the next trial.

Nine blocks of 16 trials each were given. Within each block of 16 trials, eight different exposure durations were employed: 17, 33, 50, 67, 83, 100, 117, and 133 msec. That is, the test figure was presented for those durations. In addition, the shorter leg of the test stimulus was presented on the left half the time and on the right half the time. Presentation of the various durations and positions were random within a block. To be sure the subjects knew what they were supposed to do, the subjects were told if

they were correct or wrong after each of the first 16 trials. After each block of trials, subjects were given the option of taking a short rest period and after each 48 trial block subjects were told how many times they had been correct.

b. Results. The response measure employed was number of correct responses. Correlations were found between number of correct responses at the various exposure durations and performance on the Cattell Culture Fair Test. These data are presented in Table 1. It can be seen that most of the correlations are positive with many significant. Of greatest interest is the overall correlation between inspection time and the Cattell test. This correlation was found to be equal to 0.34 which with df = 111, is significant beyond the .01 level.

The next set of analyses was between those groups identified as Users and Non-users of the apparent movement strategy. In the inspection-time task when exposure of the test figure is stopped and the mask is started, the possibility of after-images are eliminated. Unfortunately, this possibility is traded for another. Apparent movement can be seen from the shortest leg of the test figure to the bottom of the mask. Subjects can use this apparent movement to guess which line on the test figure was shorter. The use of this strategy has been reported in the past and experimenters have sought to identify those people who use this strategy and those who do not e.g. Mackenzie and Bingham (1985).

Strategy Users and Non-users were identified through the responses made to the questionnaire given to the subjects subsequent to the inspection-time

task. Comparisons of mean differences between the Users and Non-users are presented in Table 2. With the exception of the 17 and 117 msec. durations, no significant differences were found as a function of either block or presentation duration. Similarly, no mean differences were found between Users and Non-users on the Cattell Culture Fair Test ($t = 0.14$). However, differences between the two groups became apparent in the correlational analysis. These data are also presented in Table 1.

The salient correlations are as follows: for those 77 subjects who use apparent movement as a strategy to improve their performance on the inspection-time task, the correlation between intelligence and number correct on the inspection-time task does not differ significantly from zero ($r = 0.21$). On the other hand those 36 subjects who report that they did not use an apparent movement strategy showed a correlation of 0.56 ($p < 0.01$) between inspection-time performance and intelligence. These two correlations are significantly different using a directional hypothesis ($z = 1.95$, $p < 0.05$). The large correlation obtained in the Non- users analysis is apparently responsible for the overall significant relationship found earlier between intelligence and inspection time.

Discussion. Initially it should be pointed out that the negative correlations reported by other investigators (recall the -0.92 correlation reported by Nettlebeck and Lally, 1976) and the positive correlations found in the present study are not in conflict. Other research workers were using the minimum time necessary to make a discrimination and a lower score reflects better performance. In the present research our response measure was number of correct responses and the higher the score the better the

performance. In both cases better performance on the inspection-time task was associated with higher intelligence scores.

Previous studies of inspection time have been criticized because of a high degree of heterogeneity of subjects, because of small sample sizes and because of the way inspection time itself was estimated (Irwin, 1984). The present study used a large sample taken from a relatively homogeneous population and previous results were replicated. Thus, it appears that there is a robust relationship between measures of intelligence and inspection time.

Some of the implications of the results of the present experiment should be considered. The inspection-time task took only a few minutes to administer. And it probably took longer than necessary. Contrast that with paper and pencil tests which take an hour or so to administer. In addition, the inspection-time task would seem to rely less on previous learning than virtually any test of intelligence which is on the market today. Thus, this task has the possibility of being one which is closely related to intelligence on the one hand but which is relatively uninfluenced by culture, learning and practice on the other.

Consider also the possibility that the correlations which were found were reduced because of restriction of range among the Air Force recruits as compared to the general population. In addition, the validity of any test is reduced by the lack of reliability of both the test and the criterion. Thus correction for attenuation would increase the overall validity of the test from 0.56 to a considerably higher value.

Finally, there is a need for the development of an inspection time-task which does not permit use of a strategy. That is, those people who use a strategy show no relationship between inspection time and intelligence. Thus, the next task here should be to find an inspection-time task which does not allow an apparent movement strategy.

## IV. RECOMMENDATIONS:

a. Two general recommendations will be made. The first is that further research should be done on the relationship between inspection time and intelligence. Why should performance on a simple cognitive task such as this correlate highly with what has been called "the global capacity...to deal effectively" with one's environment (Wechsler, 1944)? People have no prior experience with this task but in contrast to a multitude of other tasks, practice on this task yields little improvement. Yet the Non-users' correlation of 0.56 from a homogeneous group such as was used suggests a powerful relationship between this task and intelligence measures. How does performance on this task relate to performance on other cognitive tasks? Virtually nothing is known of this task other than that it correlates with intelligence measures. In addition, research should be done with an eye to finding an inspection-time task that does not favor the use of a response strategy. The use of a response strategy obscures the relationship between inspection-time performance and intelligence. Thus, while use of a strategy provides an interesting effect, it simply makes it more difficult to investigate the variable of interest.

b. A second recommendation which has little if any relevance to applied Air

Force research concerns the inspection-time task itself. Virtually the sole interest of published inspection-time studies has been in the relationship between intelligence and inspection time. We feel that the inspection-time task itself is one which is of considerable interest.

Our second recommendation is that the task itself be studied. Such studies could proceed along two general lines of research. The first would have to do with the relationship between inspection time and demographic variables such as age, sex and socio-economic status. For example, does inspection time increase with age as do most other variables such as reaction time? The increase in reaction time may relate more to the physical problems associated with aging than to a decrease in cognitive functioning.

The second line would be research into the characteristics of the task itself. What facilitates and what decreases performance on the task? That is, such variables such as ratio of the two line lengths, distance between the lines, etc. may all relate to performance on the task. Research along these two lines of research is recommended.

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Table 1

Correlations of number correct at various exposure durations and the Cattell Culture Fair Test

All Subjects (N = 113)

Durations in Msec.

| 17 | 33 | 50 | 67 | 84 | 100 | 117 | 133 | Total |
|---|---|---|---|---|---|---|---|---|
| -.12 | .29* | .34* | .34* | .29* | .31* | .25* | .27* | .34* |

Strategy Users (N = 77)

Durations in Msec.

| 17 | 33 | 50 | 67 | 84 | 100 | 117 | 133 | Total |
|---|---|---|---|---|---|---|---|---|
| -.04 | .14 | .22 | .26* | .26* | .24* | .15 | .22 | .21 |

Non-Users (N = 36)

Durations in Msec.

| 17 | 33 | 50 | 67 | 84 | 100 | 117 | 133 | Total |
|---|---|---|---|---|---|---|---|---|
| .41* | .57** | .51** | .49** | .38* | .40* | .50** | .36* | .56** |

Significance levels:

N = 113, *r = 0.185, p = 0.05; **r = 0.241, p = 0.01

N = 77, *r = 0.225, p = 0.05; **r = 0.293, p = 0.01

N = 36, *r = 0.330, p = 0.05; **r = 0.424, p = 0.01

Table 2

Percent Correct Responses for Strategy Users and Non-Users as a Function of Trial Block and Exposure Duration

| Block | Non-Users (N = 36) | Users (N = 77) | t | Msec | Non-Users (N = 36) | Users (N = 77) | t |
|---|---|---|---|---|---|---|---|
| 1 | 82.81 | 79.87 | .95 | 17 | 63.43 | 57.00 | 2.05* |
| 2 | 84.20 | 81.25 | .85 | 33 | 83.49 | 79.08 | 1.29 |
| 3 | 85.94 | 82.63 | 1.02 | 50 | 88.43 | 86.72 | .53 |
| 4 | 89.58 | 84.42 | 1.84 | 67 | 90.90 | 86.51 | 1.68 |
| 5 | 88.37 | 84.58 | 1.43 | 84 | 91.36 | 88.82 | .97 |
| 6 | 86.11 | 84.33 | .66 | 100 | 88.73 | 88.10 | .23 |
| 7 | 87.15 | 84.17 | 1.14 | 117 | 93.83 | 87.81 | 2.35* |
| 8 | 86.28 | 82.71 | 1.30 | 133 | 92.59 | 90.91 | .70 |
| 9 | 88.89 | 84.09 | 1.89 | | | | |
| Overall | 86.59 | 83.12 | 1.53 | | | | |

* pr < .05

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FINAL REPORT

# SOLVENT EXTRACTION OF BORON FROM WASTEWATER


| | |
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| Date: | 07 October 1988 |
| Contract No: | F49629-87-R-0004 |

# Solvent Extraction of Boron from Wastewater

by

Steven C. Chiesa

## ABSTRACT

As the demand for irrigation water throughout the western portion of the United States grows, the use of reclaimed wastewater for agricultural use is being contemplated with increasingly greater frequency. The suitability of reclaimed wastewater for irrigation is strongly dependent on the post-treatment concentration of certain effluent constituents. Boron is one of these critical effluent constituents. When present in sufficiently high concentrations, boron is toxic to many forms of plant life, including many agriculturally valuable crops. Boron behaves as a conservative pollutant in conventional secondary wastewater treatment systems with little net removal normally expected or observed. Pretreatment of industrial wastewaters to remove boron may, accordingly, be necessary where locally high effluent boron concentrations limit the beneficial uses of reclaimed wastewaters and/or surface water receiving conventionally treated effluents.

This research project evaluated liquid/liquid extraction as a means of removing boron from industrial wastewaters. An Air Force photography lab wastewater was used as a test case. An aliphatic alcohol, 2-ethylhexanol, was employed in a series of batch experiments as an extractant. A mass distribution coefficient of approximately 0.3 was determined for the wastewater/organic solvent system. The distribution coefficient was independent of pH in the range of 3.4 to 7.4 and was also unaffected by the presence of compounds identifed in the literature as "salting out" agents.

Acknowledgements.

This research was sponsored by the Air Force Systems Command and the Air Force Office of Scientific Research. Universal Energy Systems, Inc. provided additional assistance in many of the logistical aspects of the research program.

The staff of the Water Quality Division of the Air Force Ocupational and Environmental Health Laboratory was also instumental in the successful completion of this project. Lt. Colonel Robert D. Binovi defined the scope of the boron problem, helped secure needed laboratory equipment and supplies and provided critical input as to the nature of site specific boron problems. Staff Seargents Mary Fields, Roberto Rolon and Robert "Pete" Davis, Tech Seargent Benjamin Hernandez and Master Seargent E. John Randall also contributed their time and effort in making sure samples were analyzed in a timely manner.

## I. INTRODUCTION:

Many communities in the western United States are contemplating the use of reclaimed wastewater for irrigation. The suitability of reclaimed wastewater for this type of disposal/resource recovery option is strongly influenced by the concentration of residual constituents in the treated effluent. Boron is one particular element which, when present in sufficiently high concentrations, can potentially render a reclaimed wastewater unfit for irrigation purposes. Boron phytotoxicity has been well documented in both agricultural and water quality oriented literature sources [1-3]. Concentrations in irrigation waters as low as 0.5 mg/L have been recommended to protect boron sensitve crops while other crops have been shown to tolerate irrigation waters containing up to 4.0 mg/L of boron [2,3]. Anticipated effluent boron concentrations should be compared against these guidelines to determine if pretreatment and/or alternative disposal options should be explored. Even where direct irrigation with reclaimed wastewater is not contemplated, receiving stream water quality standards may dictate that boron source control be considered.

The need to investigate pollution control technologies designed and operated specifically at boron removal is related to the conservative nature of this element in conventional secondary treatment facilities. Waggott [3], Dagon [4] and Lapp and Cooper [5] have all examined the fate of environmentally relevant forms of boron in a variety of secondary and tertiary treatment operations and found little net removal from aqueous carrier streams. In addition, conventional biological treatment processes have been able to tolerate/acclimate to

boron concentrations well in excess of those identified as being inhibitory to even the more resistant types of plant life [6,7].

Given the aforementioned constraints, mandated pretreatment of specific industrial wastewaters for boron removal becomes one of the few viable options available to sewerage system managers when effluent boron levels must be controlled. Ion exchange, using boron selective resins [5,8-10], and liquid/liquid extraction operations [11-16] have been successfully employed to remove boron from aqueous carrier streams. While ion exchange systems have proven to be capable of maintaining very high boron removal efficiencies, the economics of this option are strongly influenced by protective preliminary treatment requirements and resin regeneration chemical costs [5,9,10]. Applications of liquid/liquid extraction systems for boron removal have been limited to concentrated boron streams where boron recovery could be used to offset costs associated with solvent-water and boron-solvent separation operations. Few full-scale dedicated boron removal facilities have been constructed based on these technologies and fewer, if any, have been designed to operate with complex industrial wastewaters as the aqueous boron carrier phase.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

This investigation was conducted to determine the ability of a liquid/liquid extraction system to effect the removal of boron from a dilute aqueous carrier solution. The recent development and production of high quality hydrophobic and hydrophilic porous membrane systems have extended the potential concentration range of liquid/liquid

extractions to encompass relatively dilute influent carrier solutions. When employed in either flat plate or hollow fine fiber configurations, these membrane systems provide a mechanistically simple means of non-dispersive extraction of a target solute from an initial carrier phase to a second, immisible, solvent phase [17-21]. Successful application of this technology for boron removal is dependent on identifying a minimally water soluble organic solvent which is chemically compatible with the membrane material and capable of efficient extraction of boron from the aqueous carrier.

The potential efficiency of an organic solvent in a liquid/liquid boron extraction operation is reflected in its ability to partition boron between the two phases. A system distribution coefficient was used in these experiments to quantify partitioning between the two phases. The coefficient was numerically equal to the ratio of target solute equilibrium concentrations in the organic solvent and aqueous phases [22]. The influence of pH on the value of the system distribution coefficient was evaluated as were the effects of chemical agents reportedly able to "salt out" boron from aqueous solutions during organic solvent extractions. Lastly, chemical stripping of boron accumulated in the organic solvent phase was investigated as a means of regenerating the extractant for repeated use.

## III. EXPERIMENTAL METHODS:

Liquid/liquid extraction of boron was evaluated using effluent from an Air Force photo processing facility. Boron, in the form of borate, is used in photo processing operations as a component part of

photo developing and fixing solutions. At pH values realized in the field, as well as in this investigation, boron existed in solution in its boric acid form ($H_3BO_3$). Previous sampling of the waste stream serving as the source of wastewater for this investigation indicated that effluent boron concentrations ranged between 4.0 to 50.0 mg/L. Liquid/liquid extraction of boron from this base aqueous carrier stream was assessed using 2-ethylhexanol (Aldrich Chemicals, Milwaukee, WI) as the organic extractant. Aliphatic alcohols such as 2-ethylhexanol have been used to extract boron from acidic brine solutions and ion exchange chemical regenerate streams [12-16] but not from dilute industrial wastewaters.

Experimental Protocol. Experimentation first involved obtaining a 15 L sample of the photo processing wastewater and analyzing it for boron. The fortuitously low boron concentration of 4.4 mg/L found in the as-received wastewater sample allowed easy supplementation of test samples with a concentrated boron stock solution. Boron-spiked samples were thus used to investigate separation efficiency over the entire range of boron concentrations observed in the field.

Sample preparation involved taking 480 ml of the as-received wastewater and adding to this base solution three different 20 ml combinations of concentrated boron (boric acid) stock solution and distilled water. Where specified, aluminum sulfate (as alum, $Al_2(SO_4)_3;18H_2O$) was added in its salt form to the wastewater after boron supplementation. 1.0 N NaOH or $H_2SO_4$ additions were subsequently used to achieve the target solution pH. Samples used for the determination of initial boron concentration were taken after all

chemical preliminary treatment, including settling of any precipitates, was completed. The chemical dosages and associated extraction conditions provided in these experiments were designed to represent the extremes of an economically viable full-scale operating window. Chemical additions were also evaluated as to their ability to minimize operating problems conceivably affecting the performance of a membrane-based non-dispersive liquid/liquid extraction system.

Extractions were conducted by mixing and shaking equal volumes of wastewater and the organic solvent for 30 minutes. 150 ml of each of these two system components were used in all extractions to allow easy observation of emulsion formation and other potentially problematic side reactions. Separation of the two phases after mixing was carried out using a one liter separatory funnel. Re-extraction of boron from 2-ethylhexanol after initial contact with the photo processing wastewater was conducted in a similar manner to assess boron stripping efficiency from the organic phase. The only change in procedure was that 100 ml of both organic solvent and 10.0 N NaOH were used as part of these experiments. Boron stripping efficiency was evaluated by comparing the amount of boron initially removed from the waste sample with and the amount of boron recovered after contact with the caustic solution. All extractions were conducted at room temperature ($20\pm2^{o}C$).

Analytical Procedures. Boron concentrations were determined using an automated version of the carmine method [23]. pH was determined using an Orion Model 201 pH meter. All chemicals used in the experiments were reagent grade.

## IV. RESULTS AND DISCUSSION:

The photo processing wastewater as received had a yellow color and a pH of 6.4. Solution pH was reduced to 5.4 and 4.4 with the addition of 80 and 500 mg/L aluminum sulfate, respectively. With this range in alum dosage, precipitation reactions were observed at pH values of 5.4 and greater. Where precipitation did occur, there was complete removal of color from the waste sample. No color removal was recorded for solution conditions and compositions not yielding precipitates. Precipitation and removal of precipitated solids significantly increased the speed of subsequent aqueous-organic solvent phase separations. Alum-induced precipitation reactions also apparently prevented the formation of a filmy material which tended to concentrate at the aqueous phase/organic phase interface in treated and untreated wastewater samples not generating any precipitates.

Results of the 15 individual boron extraction runs are presented in Table 1. Mass distribution coefficients determined using results of each extraction were independent of aqueous solution pH for the range of pH conditions examined. This observation is consistent with experimental results reported by Grizo and Petrovska [12] for the same organic extractant. The value of the mass distribution coefficient, approximately 0.3, was considerably less than the 15.5 to 16.8 values recorded by Kristanova et al. [13] again using 2-ethylhexanol as an extractant. The lower distribution coefficient reported here is probably the result of the relatively low hydrogen ion concentrations provided when compared with those employed by others [12-16].

Organic solvents with system distribution coefficients in the

Table 1. Boron Extraction Results

| Aqueous Phase Base Solution | pH | $Al_2(SO_4)_3$ Dose (mg/L) | Boron Concentration (mg/L) Initial Aqueous | Final Aqueous | Mass Distribution Coefficient (KD) |
|---|---|---|---|---|---|
| Distilled Water | 7.4 | - | 10.2 | 7.8 | 0.31 |
| | 7.4 | - | 35.0 | 26.0 | 0.34 |
| Photo Processing Wastewater | 7.4 | - | 17.0 | 14.0 | 0.21 |
| | 7.4 | - | 42.0 | 33.0 | 0.27 |
| | 6.4 | - | 4.4 | 3.4 | 0.29 |
| | 5.4 | - | 27.0 | 21.0 | 0.29 |
| | 4.4 | - | 4.5 | 3.5 | 0.29 |
| | 3.4 | - | 26.0 | 20.0 | 0.30 |
| | 7.4 | 500 | 26.0 | 19.0 | 0.32 |
| | 6.4 | 500 | 4.4 | 3.4 | 0.29 |
| | 5.4 | 500 | 26.0 | 20.0 | 0.30 |
| | 4.4 | 500 | 27.0 | 20.0 | 0.35 |
| | 3.4 | 500 | 26.0 | 20.0 | 0.30 |

range of 0.2 to 0.6 have been sucessfully piloted for the membrane-based extraction of ethanol from fermentation broths [19]. Use of such systems for boron extraction from wastewaters (using 2-ethylhexanol or an equally effective organic solvent) would, as such, appear to be a technically feasible treatment alternative. The relative insensitivity of the wastewater/2-ethylhexanol mass distribution coefficient to variations in solution pH also provides a basis for generally expecting consistent treatment system performance. Unfortunately, a distribution coefficient of 0.3 may also necessitate recycling wastewater through an extraction system several times before sufficient net boron removal is attained. An organic solvent extractant with a numerically higher boron distribution coefficient could potentially eliminate the need for multiple waste-solvent contact phases and permit the use of single pass on-line contact systems.

For the three experimental runs where boron was re-extracted from the organic phase using a caustic solution, boron recovery was essentially complete. These results are, again, consistent with the observations of Grizo and Petrovska [12] and Eto et al. [16] for similar organic extractants. This conceptually simple, but effective, solvent regeneration technique could be easily incorporated into a continuous flow solvent loop for transfer of boron from the aqueous waste stream to an off-line caustic reservoir.

Lastly, the use of any organic extractant in a boron removal operation will be influenced by the impact of small quantities of extractant discharged with the treated waste stream. No organic solvent will be completely insoluble and potential effects on down

stream treatment operations must be identified to properly assess overall pretreatment system compatability. In this regard, 2-ethylhexanol has been shown to have fish LC50 values well below its water solubility [23], reinforcing the need for its removal during subsequent conventional treatment operations. 2-ethlyhexanol has been shown to be biologically degraded at rates which should insure its removal in conventional biological treatment processes [24].

## V. CONCLUSIONS:

The following conclusions have been reached based on the experimental results described in previous paragraphs.

* Boron, when present in an aqueous solution as boric acid, can be extracted using a suitable organic solvent.

* Alum pretreatment of the test photo processing wastewater at pH values of 5.4 and above appeared to eliminate any potential problems with interfacial film formation. Formation of stable emulsions was never observed.

* A porous hollow fine fiber membrane system should be able to effect the removal of boron from wastewaters of the type evaluated herein. Membrane systems would provide controlled contact between aqueous and organic solvent phases, thereby eliminating the need for separate phase separation operations.

## VI. RECOMMENDATIONS:

The results of this investigation strongly imply that a microporous membrane system should be able to effect the removal of boron from aqueous wastewater carrier streams. In particular, a hydrophobic membrane material could be used to prevent mixing of the aqueous and organic solvent phases while still providing the necessary contact between phases. A diagram conceptually depicting a membrane assisted boron extraction operation is depicted in Figure 1. The hydrophobic nature of the membrane prevents water from occupying any of the membrance void space at normal operating pressures. Appropriate organic solvents will, however, wet the membrane pores and completely fill all voids. Where dissolved species are to be extracted from aqueous carriers, the organic solvent must necessarily have a low water solubility. By maintaining aqueous phase operating pressures slightly greater than organic solvent phase operating pressures, organic solvent flow through the membrane is prevented and an aqueous phase-solvent phase interface is continuously maintained at the membrane surface. Any ionic or molecular form soluble in both phases will diffuse across the interface until equilibrium conditions are achieved.

The membrane surface area needed to achieve satisfactory boron removal will be dictated by boron diffusion rates and distribution coefficients. To optimize available surface area and boron removal rates, a thin walled hydrophobic hollow fine fiber membrane system can be used to define the boundary between solvent and aqueous phases. Hollow fine fiber modules would be employed for both boron extraction and subsequent boron stripping from the solvent.

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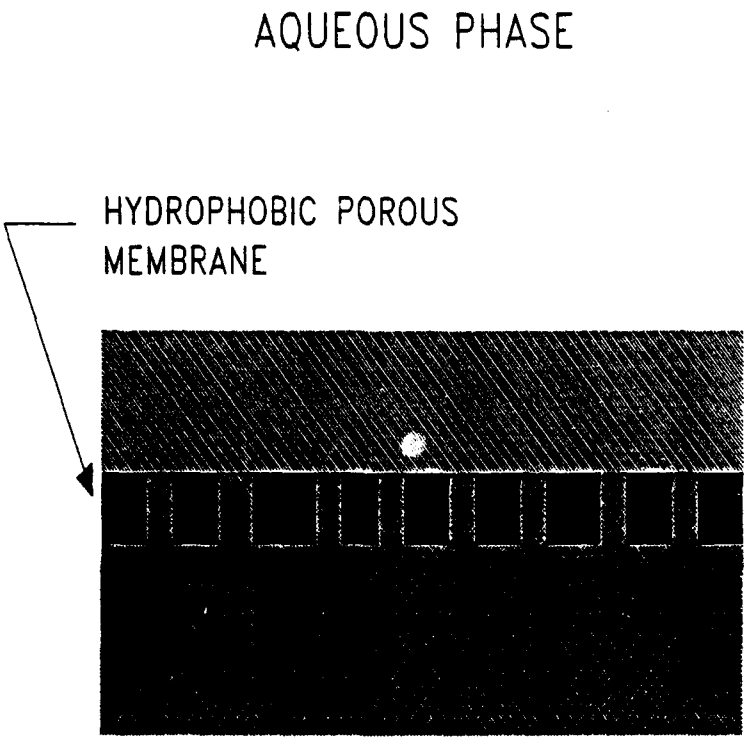



Figure 1. Separation of Aqueous and Organic Solvent Phases Using A Microporous Hydrophobic Membrane

In addition to the aforementioned physical and chemical characteristics, full-scale system configuration will be a function of wastewater flow patterns. Where intermittent high rate, short duration discharges are expected, a collection, storage and treatment system may be appropriate. As shown in Figure 2a, wastewater would be circulated through the boron extraction module where boron would accumulate in the solvent phase. The solvent would be stripped of boron as it passes through the second hollow fine fiber module in its internal loop. Effluent would be continuous circulated through the primary extraction module for an amount of time sufficient to insure necessary boron removal efficiencies. Boron would accumulate as borate ion in the caustic solution until a reduction in the boron stripping driving force dictated the need for fresh caustic.

Wastewater streams characterized by fairly continous, uniform flow rates could, as indicated in Figure 2b, be piped directly into the primary hollow fine fiber extraction module. The system would operate in a manner similar to that described above except the effluent would receive a only single pass through the primary module and would then directed elsewhere for further treatment. Use of a single pass system, however, whould require the use of an organic extractant with a high boron distribution coefficient.

Additional research is needed to verify the ability of the modular hollow fine fiber solvent extraction/solvent regeneration system to reliably remove boron. As opposed to the equilibrium studies conducted as part of this research, studies investigating mass transfer rates through hydrophobic membranes systems are required before full-

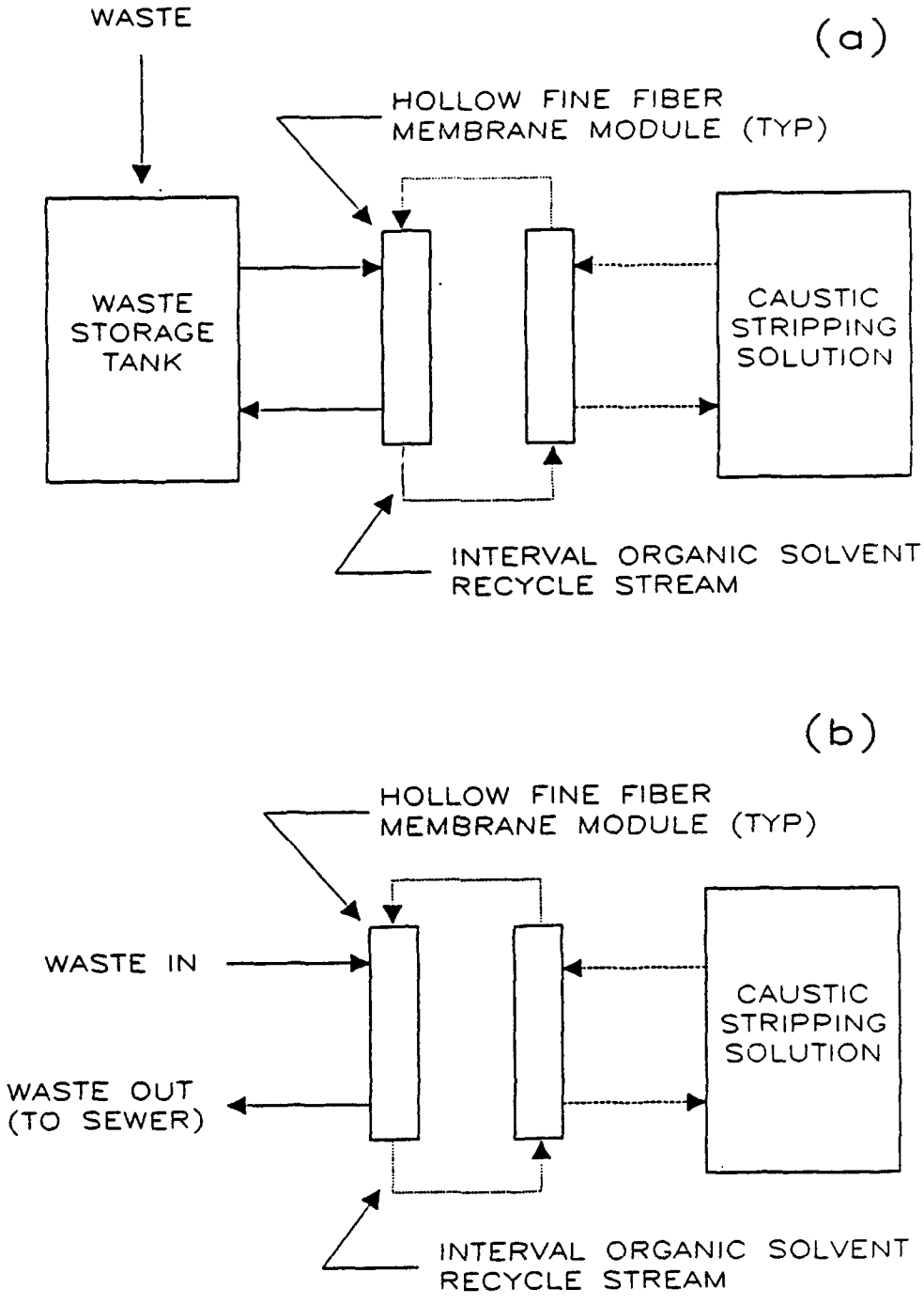



**Figure 2.** **Membrane-Based Boron Extraction Systems Incorporating (a) Multiple Pass and (b) Single Pass Waste/Solvent Contact Provisions.**

scale application of this technology is attempted. Such a study should also evaluate the efficiencies of alternative extractants and establish general operating strategies. Evalation of the caustic borate solution in regard to its effective removal capacity is also necessary as is an examination of final disposal/recovery options.

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FINAL REPORT

# Extention of the Detection Limits of Arsenic and Selenium in Solid Samples by ICP/AES Utilizing Preconcentration Techniques


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| Date: | 31 Aug 88 |
| Contract No: | F49620-87-R-0004 |

# Extention of the Detection Limits of Arsenic and Selenium in Solid Samples by ICP/AES Utilizing Preconcentration Techniques

by

Randolph B. Huff

## ABSTRACT

The extention of the detection limit for arsenic by ICP/AES analysis was extended from 0.5 ppm to 0.1 ppm by selecting a different emission line for analysis other than the one normally used for arsenic determination by ICP/AES. The preconcentration of selenium by extraction as the diethyldithiocarbamate, prior to ICP/AES analysis, was proven to be a feasible route for extending the detection limit of selenium by ICP/AES.

## Acknowledgements

I wish to thank the Air Force Systems Command and the United States Air Force Office of Scientific Research for sponsorship of this project, as well as Universal Energy Systems for their administrative direction.

The experience I had in the Metals Analysis Section of OEHL at Brooks Air Force Base was enriching and rewarding. I enjoyed observing the atmosphere that exists in a production analysis laboratory.

Many thanks and kind appreciation are extended to Leo J. Jehl, Jr., Chief of the Metals Analysis Section, USAFOEHL/SAO, and to Ed Hrna, Metals Analysis Section, USAFOEHL/SAO for their support and helpful suggestions. Without their assistance, this work could not have been accomplished.

# I. INTRODUCTION

As of this summer, the detection limits for arsenic and selenium utilizing the Perkin Elmer ICP/6500 instrument employed in the Metals Section at OEHL at Brooks Air Force Base were 0.5 ppm and 1.0 ppm, respectively, when utilizing the wavelength emission line (usually the most intense) recommended by the manufacturer. Many of the solid samples routinely analysed by this laboratory require that both arsenic and selenium be determined down to 0.1 ppm. For this reason I was assigned the problem of extending the detection limits of both arsenic and selenium utilizing ICP/AES (Inductively Coupled Plasma/Atomic Emission Spectroscopy).

Some of my modest research efforts at Presbyterian College have included a study of manganese in roadside soils (Huff, Sollee, Carter, 1980) utilizing atomic absorbance analysis for manganese detection. During that period, a manganese compound was substituted for tetraethyl lead in unleaded gasoline, and the combustion of such gasoline resulted in the release and distribution of manganese into the environment. I assume that I was assigned to the metals section at OEHL because of the above decribed work, and also because of experience gained in metal complexation chemistry which involved the use of dithiocarbamate as a coordinating ligand (Manoussakis, Lalia-Kantouri, Huff, 1975).

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The overall goal of my research effort was to find methods of increasing the sensitivity of detecting arsenic and selenium in solid waste utilizing ICP/AES techniques. The current method employed at Brooks Air Force Base using this technique, employing a Perkin Elmer ICP/6500 instrument, has a detection limit of 0.5 ppm for arsenic and only 1.0 ppm for selenium. The major thrust of my work was to extend the detection of both arsenic and selenium down to a limit of 0.1 ppm utilizing ICP/AES analysis. Although detection limits extending into the part per billion range were accessible by AA (Atomic Absorbance) employing a Perkin Elmer 5500 instrument equipped with a graphite furnace, extending the detection limits of these elements by ICP/AES would be advantageous because of increased accuracy (Beer's law obeyed over a greater range for ICP/AES compared to AA), and analyses can be performed more quickly.

My primary assigned goal was to establish a way of extending the detection limit of arsenic to 0.1 ppm by ICP/AES. A secondary assigned goal was to also establish a method for extending the detection limit of selenium to 0.1 ppm by ICP/AES.

III.

a. In order to increase the detection limits of arsenic and selenium to 0.1 ppm by ICP/AES analysis using the Perkin Elmer 6500 instrument, one must find a way to preconcentrate the sample before ICP/AES analysis. Otherwise, the detection limits imposed by the instrument can not be exceeded.

It has been reported (Huang and Wai, 1986) that preconcentration of arsenic in soil digests can be accomplished by extraction of As(III) quantitatively as the pyrrolidinedithiocarbamate complex. The first part of their method involved adjusting the acidity of the acid digest of a given soil sample to pH 1 by addition of sodium hydroxide solution. The digest was then treated with sodium sulfite solution in order to reduce any As(V) to As(III). After reduction with sulfite solution, the resulting solution was treated with sufficient ammonium pyrrolidinedithiocarbamate (APDTC) solution to complex the As(III) present. The As(III) complex was extracted into a known volume of chloroform and back-extracted into a known volume of 25% nitric acid solution. Analysis of the preconcentrated arsenic in the nitric acid was finally carried out by ICP/AES. Samples treated in the manner just described were reported to have extended detection limits for arsenic.

In an attempt to capitalize on the reported procedure (Huang and Wai, 1986), my initial approach for extending the detection limit for arsenic was to employ their method for preconcentrating arsenic by extraction with pyrrolidinedithiocarbamate.

My approach to extend the detection limit for selenium was to extract selenium with diethyldithiocarbamate, which was reported (Bachmann, 1981) to be a very efficient complexing agent for selenium. Again, the approach was preconcentration of the metal before analysis by ICP/AES.

b. All attempts to extract As(III) quantitatively as the pyrrolidinedithiocarbamate complex following the reported procedure (Huang and Wai, 1986) failed. While they reported that the most efficient extraction of As(III) by pyrrolidinedithiocarbamate occurs at a pH of 1, and that they carried out their extractions at that pH value, this investigator questions how this could be true. On all samples studied by me, in an effort to utilize their procedure, the addition of the sodium sulfite solution before extraction always changed the pH of the solution from the preadjusted value of pH 1 to a pH value between 6 and 8. Obviously, any subsequent extraction of As(III) from the solution will not be carried out at pH 1 if their procedure is followed.

Preconcentration of As(III) by extraction with pyrrolidinedithiocarbamate was abandoned after discovering that the goal of extending the detection limit of arsenic to 0.1 ppm using ICP/AES could be obtained by utilizing a different arsenic emission wavelength other than the one normally used. While many investigators, including those in the Metals Section at OEHL at Brooks Air Force Base, have employed the most intense line of arsenic (193.7 nm) in the ICP/AES determination of arsenic, several (Liversage, R., et. al., 1984; Huang and Wai, 1986) have used the 189.0 nm emmission line in order to minimize spectral interference by other elements in the sample matrix. While trying to evaluate the preconcentration of As(III) by extraction with pyrrolidinedithiocarbamate, it was discovered that arsenic could be detected in standard arsenic solutions down to 0.1 ppm using ICP/AES if the 189.0 nm emission line was used rather than the 193.7 nm line. For some unknown reason, the Perkin Elmer 6500 instrument was much more sensitive using this wavelength than it was using the 193.7 emission line, where a detection limit of only 0.5 ppm was possible.

While the goal of extending the detection limit of selenium by ICP/AES was not completely realized because of lack of time, the concept of extending the detection limit by preconcentration prior to ICP/AES analysis was demonstrated. Selenium was quantitatively recovered from 2 ppm selenium solutions and preconcentrated to 10 ppm by extraction as the diethyldithiocarbamate into chloroform and back-extraction

into 25% nitric acid. A solution of 2 ppm selenium concentration was prepared from a Fisher certified selenium reference standard by taking an appropriate amount of the standard and diluting with 25% nitric acid. Nitric acid was used for dilution to approximate the acidic nature of a soil digest. For the extraction procedure, 50 ml of the 2 selenium solution was taken and the pH adjusted to 1.5 by the addition of 6M sodium hydroxide (it had previously been determined that extraction of selenium as the diethyldithiocarbamate was most efficient at a pH of 1.5). After the adjustment of the pH to 1.5, 20 ml of 4% sodium diethyldithiocarbamate solution was added, which caused the pH to increase to between 5 and 6. The pH was again adjusted to 1.5 by the addition of 25% nitric acid and the solution then placed into a separatory funnel containing 40 ml of chloroform. The mixture was shaken for 1 to 2 minutes and the chloroform layer quickly removed and placed into another separatory funnel containing 10 ml of 25% nitric acid. This mixture was shaken for 10 minutes and the acid layer removed and saved for ICP/AES analysis for selenium.

Three different runs, after preconcentration of selenium by extraction from solutions initially containing 2 ppm selenium, gave final concentrations in the acid extract of 10.4, 10.1, and 10.6 ppm selenium, as determined by ICP/AES analysis, using the 196.0 nm emission line. Theoretically, if 100% of the selenium was recovered, the selenium concentration would have been 10.0 ppm.

IV. RECOMMENDATIONS:

a. To extend the detection limit for arsenic to 0.1 ppm utilizing ICP/AES, use the 189.0 nm emission line rather than the 193.7 nm line.

b. The preconcentration of selenium prior to ICP/AES analysis as a means of extending the detection limit down to 0.1 ppm requires further study. While extraction of selenium as the diethyldithiocarbamate was shown to be quantitatively possible starting with selenium concentrations of 2 ppm, initial concentrations of 0.1 ppm selenium need to be studied to determine if the extraction process works equally as well at this lower concentration.

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FINAL REPORT

# Determination of Asbestos Fibers in Environmental Samples Using Scanning Electron Microscopy and Energy Dispersive X-ray Analyses (SEM-EDXA)


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| Date | August 23, 1988 |
| Contract # | F49620-87-R-0004 |

# Determination of Asbestos Fibers in Environmental Samples Using Scanning Electron Microscopy and Energy Dispersive X-ray Analyses (SEM-EDXA)

by

Larry R. Sherman

## ABSTRACT

Large numbers of environmental samples are submitted to the OEHL/SA laboratory for fiber counting. The fibers often need to be identified by a rapid inexpensive method to supply information to the people who make health hazard decisions. SEM-EDXA was selected to provide this information using IUCC, NIOSH and NBS reference materials.

Field samples were mounted on SEM studs, coated with a Au/Pd coating, scanned at 600x and 2000x with an Amray 1820 SEM to determine if the morphology of the fibers warranted analyses. EDXA analysis was performed with a Tracor Northern Series II X-ray analyzer (TN-II). An SQ software package (© Tracor Northern) was used to acquire data and quantitate the X-ray data for Si,Mg,Fe,Ca,Mn and Na using atomic number (Z), absorption (A) and fluorescent (F) corrections. The element mass ratios (Mg/Si, Fe/Si, Ca/Si, Mn/Si and Na/Si) were calculated along with the cation/anion ratios for the fibers. The stoichiometric Si content was normalized to 8 and used as the anion factor. The stoichiometric sum of the other five metals normalization was used as the cation ratio. Element mass ratios and cation/anion ratios along with morphology were

compared with empirical data derived from reference standards to make judgments as to the asbestos type.

In blind tests, the correct assignments were made for more than 95% of the fibers. Computer programs have been prepared for a semi-routine analyses of fibers. A single fiber analysis requires approximately 10 minutes, after mounting the sample in the SEM chamber.

## I. INTRODUCTION:

Asbestos fibers are ubiguous (1,2) and have been linked to a series of lung and gastrointestinal diseases (3,4,5,6). They are or have been used in industrial, commercial and residential areas for pipe insulation, building insulation, concrete pipe reinforcement and auto brake lining. They are a minor constituent of most rocks and are found as a man induced and natural contaminant of water and air.

Polarized light microscopy (PLM) is often used for analyzing asbestos but only electron microscopy offers a more definitive identification of the fibers.

TEM has been strongly advocated for asbestos work (2) even though TEM has the following disadvantages: a) few facilities have a TEM; b) analyses are costly, costing about $500 per sample during preparation of this report; c) procedures are operator and instrumentally variable and can rarely be reproduced by different operators or by the same operator on different instruments; d) only a small portion of a sample can be analyzed (2). Furthermore, TEM requires highly skilled technicians and does not lend itself to routine analyses.

In the past, SEMs have not generally been used for asbestos work because their resolving power was insufficient to characterized fibers <0.5 um and the EDXA system was incapable of differentiating between asbestos and non-asbestos mineral fibers (1). Because of improvement

in the "state-of-the-art" and new discoveries a revaluation of SEM procedures became the purpose of this project.

The author has a strong research background in environmental analytical chemistry, has published more than twenty papers on pesticides or heavy metals in the environment and has published a book on computer programming. These backgrounds, plus a strong instrumental background developed through twenty years of teaching instrumental analysis, made an ideal combination for this SEM-EDXA project.

## II. OBJECTIVES OF THE RESEARCH EFFORT.

Prior to this study, OEHL/SA possessed no means of qualitatively identifying the fibers found in the routine air and water samples submitted to the laboratory. When excessive fiber counts were determined an appraisal of the fibers was required to assess the environmental health hazards. Outside contract laboratories were used for this work. The cost was significantly greater than an "in-house" analyses and took several weeks to produce an answer. To adequately serve the bases submitting samples, the SEM-EDXA method was selected as a possible means to fulfill the laboratory's analytical requirements.

The summer fellow was assigned the duty of setting up a workable analytical method, modify the software purchased from Tracor Northern and to write new software for performing the analyses. He was also asked to develop sample pre-treatment procedures to enhance the analyses.

Initial screening of select samples for morphology was completed by the mentor, K.T. Roberson, prior to the arrival of the summer fellow. Comparison of these initial samples against standard reference materials did not yield satisfactory results. A search of the literature indicated that ZAF corrections were essential and that the addition of two possible modifications, the element mass ratio and cation/anion ratio methods, might solve the problem. A large number of instrumental and computer modifications

were tried before a definitive analytical method was developed.

## III. EXPERIMENTAL

Instrumentation:

An Amray 1820 scanning electron microscope (SEM) equipped with an energy dispersive spectrometer (EDS) and a Tracor Northern Series II X-ray Analyzer (TN-II) were used for this study. The best parameters for the accuracy and precision of the SEM were: 20 kev at 90 ma, $38^{o}$ horizonal tilt angle, 12 mm working distance, 45 mm detector slide distance, and a 14 mm vertical distance from the pole piece to the crystal center line.

The TN-II SQ[1] x-ray data acquisition and analyzer program were used. The X-ray spectrum was collected from 0-10.24 kev using 10 ev pulse channels. In quantitatizing the data, SQ applied ZAF and background corrections.

Sample preparation:

*Union Internationalle Contre le Cancer* (UICC), NBS and NIOSH asbestos standards were used for establishing the element mass ratio and cation/anion ratios (Table 1 & 2).

Four types of fiber samples were used in this study:

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1. Copywrited software package of Tracor Northern, Middleton WS 53562 for performing quantitative analysis on X-ray Spectra using a library of references stored on disk. The program uses Multiple Least Squares Analyses and ZAF matrix correction procedures to calculate elemental concentration results. ZAF corrects for atomic number (Z), absorption (A) and fluorescence (F) effects. It is a theoretical correction procedure based on excitation conditions, geometry of the sample and intensity ratios contructed from pure element samples. The package also makes non-linear background corrections on a channel by channel basis but is dependent upon the sample to detector geometry.

(a) Bulk asbestos samples collected as insulation, steam pipe coverings, floor tile, etc. These samples were dirty and could not be satisfactorily analyzed as received. They were cleaned using a modification of the method suggested by Plowman and Hobson (7). Discretionary selection of samples for cleaning minimized adverse difficulties inherent in the cleaning. (b) NIOSH air standards sent to OEHL by NIOSH for training technicians or for certifying individuals for fiber counting. These fiber samples were received on filters and contained a known concentration of chrysotile or amosite fibers. (c) Air samples submitted to the OEHL laboratory for routi .. fiber counting. The samples were obtained on cellulose acetate filters. (d) Two liter samples of San Antonio water were filtered through a 0.8 um millipore filter, washed with distilled $H_2O$ and dried in a descicator.

The bulk samples were initially analyzed by PLM to determine the asbestos type in the sample. The NIOSH samples, as indicated above, were known to contain either amosite or chrysotile. The air and water samples were unknowns with no knowledge that any asbestos fibers were present.

All samples were mounted on standard SEM aluminum studs after coating the stud with Amray carbon paint. Loose samples (e.g. untreated bulk samples) were pressed into the wet carbon paint. Approximately 5 mm x 5 mm representative portions of the filter samples were cut from the filters

and pressed into the wet paint. When dry, the samples were vacuum coated with a 10 nm Au/Pd deposit using an Anatech Ltd. vacuum metal coating apparatus. The metal thickness was monitored with an Anatech DTRM-S plating monitor. All samples except the UICC standards, which were already mounted and carbon coated, were coated with Au/Pd.

The samples were scanned at 600x and a representative fiber selected for analysis. At 2000x, the morphology of the selected fiber was examined to assure that it appeared to be an asbestos fiber (e.g. serpentine fiber for chrysotile, rod like for amphiboles or twisted fibers for non-asbestos organic fibers). After a visual inspection at 2000x, the magnification was increased until the SEM partial field (3cm x 2cm display on the VDT) fitted entirely within the fiber image and the EDXA obtained for the fiber. During spectrum acquisition, the TN II cursor was set at the 1.720 kev silicon X-ray detection channel and data was collected for 90 seconds or until the 1.720 kev channel exceeded 200 counts; whichever was greater. The accumulated counts in the entire silicon region needed to be > 2400, which constitutes the minimum number of counts required for good data processing with the TN II. The higher the magnification the longer the data acquisition time and also the greater number of background counts. All spectra was recorded on floppy disks using arbitrary file numbers.

EDXA data were collected for Si, Mg, Fe, Ca, Mn, and

Na. Although Al can replace Si in some asbestos crystals (8), the fibers were not analyzed for Al because of the high background produced by the studs. Other elements were not usually present in the fibers and were not monitored. When large extraneous peaks appeared in the spectrum, the EDXA spectrum data were not processed.

IV. RESULTS:

The TN-II SQ program listed intensity for all channels in the six elemental region. The intensity is dependent upon the acquisition time, the magnification, electron scanning rate and thickness of the fiber. The number of counts were difficult to reproduce for different portions of the same fiber and impossible to duplicate for different asbestos fibers in the same sample. Quantitatized datum (normalized % composition for the six elements) using ZAF corrections yielded information which was reproducible to a minimum of two significant figure for replicated analyses.

The actual percentage composition of the fibers was of little use since minor components (e.g. Ca,Mn and Na) distorted the datum. Useful X-ray identification was possible from the element/silicon ratios ($\%_{metal}/\%_{Si}$)(9). A table of these ratios was constructed from standard reference materials (Table 1). The ratios obtained from the unknown fibers were compared with the values in Table 1. Differences were observed in the EDXA analyses for clean and dirty samples. However, fiber analysis of the same

sample yielded insignificant variation and no separate criteria were established for clean and dirty asbestos fibers.

Champnes et al. (8) recommended using the gram atom composition for silicates and aluminates as the anions and the other metals (Mg, Fe, Ca, Mn and Na) as the cations for identifying asbestos fibers. Dividing the ZAF corrected elemental % by the atomic mass of the element yields the gram atoms. The silicon gram atom (anion) was normalized to eight, the number of silicon atoms found in many asbestos formulae. The atomic mass for the cations (Mg, Fe, Mn, Ca & Na) was divided into the ZAF corrected % and normalized using the silicon correction factor. The sums of the normalized gram atoms of the cations for the seven asbestos types studied are presented in Table 2. However, since aluminum studs were used, aluminum could not be included in this study and led to some discrepancy in these cation/anion values with those published by Champnes et al. (8). Champnes et al. (8) reported that the cation/anion ratio was 7/8 for amphiboles and 8/8 for pyroxenes. They published no data on other materials. Different values were obtained in this study, but in all cases were reproducible and yielded correct asbestos types. Examples from this project are presented in Table 3. Computer programs have been written to perform the routine computation and are available on the USAFOEHL.LAS computer system.

Malami et al. (9) reported that the intensity ratios,

$I_{me}/I_{Si}$, for chrysotile increased as the fiber thickness increased but became a constant when the thickness of the fiber >0.2 um. Analyzing a series of tremolite fibers, whose thickness ranged from 0.4-27.0 um (Table 4) verified Malami et al. (9) conclusions.

V. DISCUSSION:

Early attempts to assign asbestos types from EDXA element mass ratios alone, especially when the $\%_{me}/\%_{Si}$ were not within the standard deviation for the asbestos type, met with 100% judgment errors. As such, a conservative approach was adopted. If the element % ratios and cation/anion atomic ratios were not within the standard range for the suspected asbestos type, no assignment was made. This approach may produce low results but, when compared with known samples, produced correct answers for more than 94% of the fibers analyzed.

Using the morphology of the fibers also reduced the number of incorrect assignments. The analyzed fiber must conform to the morphology for an asbestos fiber, e.g. have a length >3 times the width; have either a serpentine or rod like fiber structure; the surface of the fiber must be smooth and show few if any fracture planes. Indiscriminate X-ray analyses of material, which did not fit the morphology criteria, usually yielded erroneous results. As shown by Ruud et al. (2), SEM results can be confusing for asbestos analyses; however, SEM morphology along with EDXA

datum yielded extremely accurate results and in a single trial yielded positive identifications for more than 95% of the samples analyzed. A trained technician can identify an asbestos fiber, using the computer software, in about 10 minutes once the sample is positioned in the SEM.

## VI. CONCLUSIONS:

Although SEM-EDXA analyses may still be inferior to TEM-SAED, (this has yet to be determined), it is considerable faster, is less capital costly, requires less sample preparation, and is much less costly per sample.

The project certainly enhanced the researcher's understanding and practical application of X-ray analyses and opened up a new field of research, SEM. Hopefully, he will continued the work through a mini-grant.

Table 1
Composition Ratios for Asbestos Standards
Elemental Mass Ratios(std. dev.)

| Material | Si/Si | Mg/Si | Fe/Si | Ca/Si | Mn/Si | Na/Si |
|---|---|---|---|---|---|---|
| Chrysotile | 1.0 | 0.73(0.045) | .051 | .0060 | .014 | .0059 |
| Amosite | 1.0 | 0.23(0.035 | .87(.084) | .0093 | .027 | .023 |
| Crocidolite | 1.0 | 0.081 | .67(.092) | .0019 | .015 | .27(.050) |
| Anthophollite | 1.0 | 0.45(.031) | .14(.044) | .0039 | .0067 | .023 |
| Tremolite | 1.0 | 0.37(.026) | .080 | .30(.039) | .0044 | .0082 |
| Actinolite | 1.0 | 0.35(.034) | .11(.031) | .27(.038) | .0030 | .0065 |
| Ferroactinolite | 1.0 | 0.35(.022) | .16(.033) | .30(.030) | .0071 | .031 |

Table 2
Cation/Anion Ratios

| Fiber | Si | Cations |
|---|---|---|
| Chrysotile | 8 | $7.4 \pm 0.5$ |
| Amosite | 8 | $6.6 \pm 0.6$ |
| Crocidolite | 8 | $7.2 \pm 0.5$ |
| Anthophollite | 8 | 4.9 |
| Tremolite | 8 | 5.7 |
| Actinolite | 8 | 5.6 |
| Ferroactinolite | 8 | 5.7 |

Table 3
Test for Cation/Anion Ratios

| Sample | Si | Mg | Fe | Ca | Mn | Na | Sum | Type | Ans. |
|---|---|---|---|---|---|---|---|---|---|
| 74D | 1.0 | 0.30 | -- | 0.39 | -- | 0.94 | 14.24 | Tremolite? | No |
| 68A | 1.0 | 0.35 | 0.86 | 0.001 | 0.003 | 0.36 | 6.49 | Amosite | Yes |
| 69C | 1.0 | 0.36 | 1.14 | 0.035 | 0.20 | 0.04 | 8.62 | Amosite? | No |

Table 4
Effect of Thickness upon element/Si ratio for Tremolite

| um | Mg/Si | Fe/Si | Ca/Si | Mn/Si | Na/Si |
|---|---|---|---|---|---|
| 0.40 | .34 | .10 | .28 | .01 | ---- |
| 0.64 | .42 | .07 | .31 | .009 | .023 |
| 0.74 | .40 | .06 | .27 | .004 | .007 |
| 0.84 | .38 | .07 | .32 | .006 | .02 |
| 0.99 | .37 | .04 | .27 | .009 | .007 |
| 1.18 | .42 | .04 | .44 | .01 | .01 |
| 1.20 | .36 | .09 | .33 | .01 | .01 |
| 1.50 | .37 | .07 | .28 | .002 | .009 |
| 1.68 | .39 | .06 | .24 | .006 | .006 |
| 1.83 | .38 | .04 | .37 | --- | .01 |
| 2.00 | .32 | .06 | .42 | -- | .002 |
| 2.30 | .33 | .09 | .35 | .023 | .0004 |
| 3.00 | .38 | .07 | .35 | .008 | .005 |
| 3.50 | .38 | .06 | .28 | .01 | ---- |
| 3.90 | .35 | .09 | .30 | .006 | .009 |
| 9.80 | .36 | .12 | .32 | .007 | --- |
| 27.00 | .38 | .04 | .27 | .0007 | .0002 |
| AVE. | .37 | .07 | .32 | .02 | .007 |

## VI. ACKNOWLEDGMENTS:

The author wishes to acknowledge the Air Force System Command, the Air Force Office of Scientific Research and the Occupational and Environmental Health Laboratory at Brooks AFB, TX for sponsorship of this research. He also wishes to acknowledge administrative and logistic support by Universal Energy Systems, Inc.

On the personal level, he wishes to thank his mentor, Mr. Kenneth T. Roberson, for selecting him for the project, for encouragement and technical assistance, especially during the periods of frustration when "nothing would work!" He wishes to thank Mr. T. Thomas, Lt. Col. M. Anderson and all the personnel at OEHL/SA for their encouragement and inclusion in the technical, administrative and social functions of the section. Because of their help and acceptance, the project was extremely rewarding.

## VII. RECOMMENDATIONS:

1. It is recommended that the SEM-EDXA method developed by the summer fellow be adopted by USAFOEHL on a limited basis to identify fibers in air samples which have a high count (more than 30 fibers/100 fields). When more information is available, the method can be expanded to yield greater technical service information.
2. The objectives of the USAFOEHL/SA laboratory have been

achieved by the summer fellow, but some question exists as to the general applicability of the SEM-EDXA method. It is recommended that split samples be analyzed at USAFOEHL and by TEM in another laboratory. A mini-grant will be submitted by the summer fellow to perform the latter part of this work at his home institution.

3. A technician is needed to perform the routine work associated with this analytical method. Either an entry level military or civilian employee can perform this time consuming work.

4. After the standard deviations have been assigned to all calculations, these results should be published in a peer reviewed journal to lend credibility to the study. Hopefully, the summer fellow and his mentor will accomplish this in the next year.

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**APPENDIX**

**SAMPLE INTERNAL REPORT**

20.0 kV 2.4 Degrees

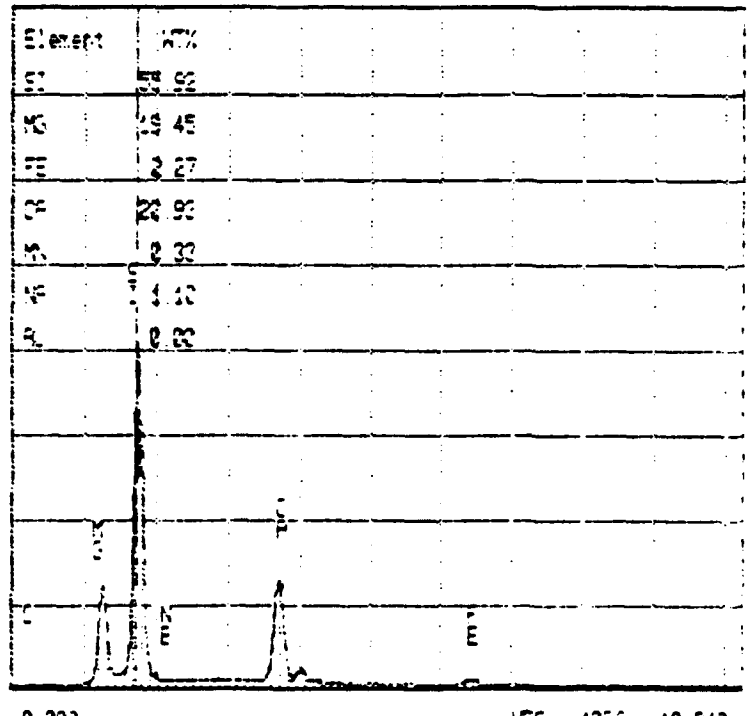
Chi-sqr = 5.71

26D

| Element | k-ratio | Net Counts |
|---|---|---|
| SI | 0.51715 +/- 0.00461 | 30051 +/- 268 |
| MG | 0.19297 +/- 0.00410 | 7022 +/- 166 |
| FE | 0.02159 +/- 0.00465 | 747 +/- 111 |
| CA | 0.23505 +/- 0.00467 | 11661 +/- 231 |
| MN | 0.00071 +/- 0.00172 | 29 +/- 48 |
| NA | 0.00307 +/- 0.00172 | 161 +/- 45 |
| AL | 0.01786 +/- 0.00412 | 425 +/- 98 |

ZAF Correction 20.00 kV 27.40 deg
No. of Iterations = 2

| Element | k-ratio | Z | A | F | Atom% | Wt% |
|---|---|---|---|---|---|---|
| SI-I | 0.517 | 0.994 | 1.519 | 0.998 | 56.32 | 55.59 |
| MG-I | 0.193 | 0.998 | 1.581 | 0.989 | 24.60 | 20.41 |
| FE-I | 0.022 | 1.114 | 1.046 | 1.000 | 1.39 | 2.67 |
| CA-I | 0.235 | 1.016 | 1.109 | 0.999 | 14.95 | 20.45 |
| MN-I | 0.001 | 1.135 | 1.066 | 1.000 | 0.03 | 0.06 |
| NA-I | 0.004 | 1.018 | 1.084 | 0.996 | 0.67 | 0.52 |
| | | | | | Total= | 100.00% |



**SAMPLE INTRENAL REPORT**

```
**********************************************************
*                                                        *
*  ANALYSES OF THE X-RAY DATA FROM THE TRACOR NORTHERN   *
*              SERIES II XRAY ANALYZER FOR               *
*              DETERMINING  ASBESTOS FIBERS              *
*                                                        *
**********************************************************
```

SAMPLE # FOR THIS DATA IS

| STUD # | SAMPLE # | TYPE OF MATERIAL |
|---|---|---|
| 27 | NBS | BULK |

THE FILE NUMBER IS 14-27

THE % COMPOSITION FOR THE SIX ELEMENTS ARE:

| | |
|---|---|
| SI= 55.89 | MG= 20.43 |
| Fe= 2.64 | CA= 20.45 |
| MN= .06 | Na= .53 |

THE MASS ELEMENT RATIOS ARE GIVEN BELOW

| | |
|---|---|
| Mg/Si IS | .365539 |
| Fe/Si IS | 4.72356E-02 |
| Ca/Si IS | .365897 |
| Mn/Si IS | 1.07354E-03 |
| Na/Si IS | 9.48291E-03 |

THE MASS RATIO TABLE

| | Mg/Si | Fe/Si | Ca/Si | Mn/Si | Na/Si |
|---|---|---|---|---|---|
| CHRYSOTILE | 0.73 | 0.051 | 0.0060 | 0.014 | 0.0059 |
| AMOSITE | 0.23 | 0.87 | 0.0093 | 0.027 | 0.023 |
| CROCIDOLITE | .081 | 0.67 | 0.0019 | 0.015 | 0.27 |
| ANTHOPHOL | 0.45 | 0.14 | 0.0039 | 0.0067 | 0.023 |
| TREMOLITE | 0.37 | 0.080 | 0.30 | 0.0044 | 0.0082 |
| ACTINOLITE | 0.357 | 0.11 | 0.293 | 0.0073 | 0.0109 |
| FERROACTIN | 0.35 | 0.16 | 0.30 | 0.0071 | 0.031 |

THIS FIBER COULD BE ACTINOLITE OR TREMOLITE

THE ANION/CATION RATIOS FOR THIS FIBER ARE:
ANION -Si- IS 8.0  CATION IS =  5.71547

THE CATION DATA IS:

| Mg | Fe | Ca | Mn | Na |
|---|---|---|---|---|
| 3.37715 | .190032 | 2.05122 | 4.39044E-03 | 9.26793E-02 |

THE ANION/CATION TABLE IS:

| | CATION | | CATION | | CATION |
|---|---|---|---|---|---|
| CHRYSOTILE | 7.4(.5) | AMOSITE | 6.6(.6) | CROCIDOLITE | 7.2(.5) |
| ANTHOPHOLI | 4.9 | TREMOLIT | 5.7( | ACTINOLITE | 5.63(.6) |
| FERROACTIN | 5.7 | | | | |

THIS FIBER COULD BE ACTINOLITE OR TREMOLITE
CHECK ALL YOUR DATA BEFORE MAKING A DECISION!

1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

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FINAL REPORT


| | |
|---|---|
| Prepared by: | Gary R. Stevens,Ph.D. |
| Academic Rank: | Assistant Professor |
| Department and University: | Statistics Department<br>Oklahoma State University |
| Research Location: | USAFOEHL/TSS<br>Brooks AFB<br>San Antonio, TX 78235 |
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| | |
|---|---|
| Date: | 29 July 1988 |
| Contract No: | F49620-87-R-0004 |

# Analysis of Contaminated Ground Water Using Kriging Methods

by

Gary R. Stevens

## ABSTRACT

Two plumes of contaminated ground water at Wurtsmith Air Force Base in Michigan are analyzed using Kriging, a stochastic method for interpolation of spatially correlated data. The methodology is extended to create zones of influence for the monitoring wells in the contamination plumes. These zones are then used to help determine a sampling plan for future monitoring of the ground water in these plumes. This new methodology is then verified by the use of cross validation and prediction intervals for five data sets from two plumes at the Air Force Base.

A more standard statistical analysis involving tests of hypotheses is used to establish the frequency of sampling for the wells used in future monitoring of the plumes.

## ACKNOWLEDGMENTS

I would like to thank the Air Force Systems Command, the Air Force Office of Scientific Research, and the Air Force Occupational and Health Laboratory for their sponsorship.

Personally, I would like to thank Philip Hunter for teaching me the basics of hydrology, supporting the new ideas developed and working closely with me. The help of Mr. Richard Anderson and Captain Art Kaminski with the VAX was appreciated. Mr. Earl O'Carrol's logistic support was always well timed. Col. R.C. Wooten provided a good working enviroment. Finally, I would like to thank Col. Warren Hull for handling the administrative details of this program.

# I. INTRODUCTION

In recent years the analysis of contaminated ground water has received considerable attention in both statistical and hydrogeological literature. Much emphasis has been focused on mapping the plumes of contaminated ground water and predicting the contamination levels at unsampled locations based on the sampled locations. Several methods have been used to map the plumes. Splining [Ripley(1981) and Meinguet(1979)] and Kriging [Journel and Huijbregts(1979)] have been used successfully. Kriging has been useful in other situations because it also measures the spatial variation and correlation of the process with the variogram. It also has the advantage that one can obtain estimates of the standard errors associated with the predicted grid points.

The USAF has been involved in litigation concerning the release of contamination into ground water surrounding Wurthsmith AFB. The analysis of the data involves the use of spatial distributions and of spatial correlation and trends. Splining techniques have been used in the past, however, the investigation of Kriging was warranted because of its ability to measure the spatial correlation and to give standard errors of the estimates.

My research interests have been in the area of spatial times series analysis and spatial distributions. My work has been involved in the properties of spatial spectral densities

and estimation of important frequencies. Recently, my research has been in the area of Kriging because of its flexibility and wide range of applications.

This report outlines the methology used in analyzing the data from Wurtsmith AFB and gives the foundation for determining a sampling scheme for the future monitoring of ground water and for determining the spatial migration of contamination associated with the plumes.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

Since the Air Force anticipates having to analyze more spatial data in the future, there was a need to know the advantages and disadvantages of both Kriging and splining and their realtionship to each other. Since there is no inexpensive, user friendly commercial software for Kriging, a general easy to understand methodology and software needed to be developed so that the analysis could continue in the future.

During my tenure at Brooks AFB, the court rendered its preliminary deceision on the Wurtsmith AFB case. The magistate proposed an expensive and time consuming sampling plan for the future monitoring of ground water contamination in the two plumes. The plan essentially involved sampling all the wells that have ever been sampled in the area, on a monthly basis. Therefore, my new objective was to develop a new sampling plan

that would be less expensive and less time consuming and still accurately monitor the contamination in the plumes.

## III. RELATIONSHIP BETWEEN KRIGING AND SPLINING:

The general theory of Kriging involves the well developed area of mathematics called intrinsic random functions. One nice property of these intrinsic random functions is that they possess a generalized covariance function. This generalized covariance is the basis for Kriging. It was shown [Cressie(1988)] that spline interpolation is the same as Kriging when the generalized covariance of intrinsic random functions takes on a certain form.

Since Kriging is a more general procedure and therefore applicable to a wider variety of situations, we will restrict our attention to the development of Kriging in the analysis of the data from Wurtsmith AFB. To help in the analysis of the data, an interactive program that is user friendly was developed. The program, SGSKRG, and documentation was designed for use in future analysis of contaminated ground water.

## IV. DEVELOPMENT OF KRIGING FOR GROUND WATER ANALYSIS:

### A: Data Base

The data used in this study consists of measurements

taken on the 47 shallow wells in the Alert Apron Plume and the 24 shallow wells in the Northern Landfill Plume. Due to the large number of non-detects the data sets used were reduced to 24 wells in the Alert Apron Plume and 14 wells in the Northern Landfill Plume. There are five data sets from the two plumes. These data sets represent the levels of trichloroethylene in the Alert Apron in 1980 and 1987, and the levels of benzene, dichloroethylene, and vinyl chloride in the Northern Landfill in 1987.

B: Development of Kriging

Kriging is an optimal linear interpolation method for data that is spatially correlated [Cressie(1988)]. There are two basic areas of Kriging. The first is the determination and estimation of the variogram. The variogram is a general measure of the relationship between observations. When the correlation function exists, the variogram is equal to one minus the correlation. The second is the use of the estimated variogram to determine the optimal weights for prediction and interpolation of the process at some unsampled location.

The usual methods of estimating the parameters of the variogram, generalized least squares and weighted least squares, did not perform well on the data sets. This was brought about because of the unstable behavior of the point variogram. The unstable behavior was caused by large variation in the point

variogram which was a result of having so few data points. Therefore, several reasonable assumptions were made to allow the use of the correlation function instead of the variogram. This is expedient since the correlation function has only one parameter to estimate. To estimate this parameter we used the method of cross validation, which does not require the calculation of a point correlation function and thus avoids the problem of unstable point estimates of the function.

We used the exponential correaltion function, given by

$$\rho(h) = \exp(-h/a)$$

where (h) is the distance between wells and (a) is the range or zone of influence parameter. The exponential model was chosen for two reasons. The first is that the dispersion of contaminants through the aquifer is believed to be exponential. Secondly, the exponential model yielded the best fit to the data using the minimum residual variance criterion for the cross validation procedure.

C: Development of the Sampling Plan

Since the sampling of all the wells in both plumes is expensive and not necessary because of the correlation between wells, a sampling plan was devised in which the number of wells sampled was reduced yet, the levels of contamination in the plumes could still be monitored accurately.

A basic assumption in devising the sampling plan is that

wells close together are correlated. Therefore, given the level of contamination in one well, the contamination level of wells close to it can be predicted. Kriging provides a way to measure the correlation of the wells as well as a measure of closeness. The parameter (a) in the correlation function is called the range. This value is the distance beyond which wells are no longer correlated. Kriging is also an optimal linear prediction method that provides an estimate of the accuracy of the prediction with the prediction variance.

Therefore, the proposed sampling plan is as follows:

1) estimate the range (a) of the correlation function
2) use the estimated range to jacknife krige the data points, i.e. remove each datum point in turn and obtain its predicted value and its prediction variance ( the idea being that wells with high prediction variance are sampled and wells with low prediction variance are predicted ).
3) the number of sampling points is chosen in a manner similar to backwards selection in regression, with the selection criterion being a significant reduction in the prediction variance.
4) each prediction point should have at least two sampling wells in it's zone of influence [Cooper and Istok(1988)].

To validate this procedure, we performed it on the five

data sets used in this study. We constructed 95% prediction intervals for the predicted values. For all predicted points the measured level of contamination was inside the prediction interval, indicating there is no statistical difference between the observed contamination level and the predicted contamination level.

As a result of this sampling plan the total number of wells sampled was reduced from 99 to 32. The number of shallow wells sampled in the two plumes was reduced from 71 to 23.

D: Frequency of Sampling

The court proposed sampling plan called for monthly sampling of the wells. From a logistic point of view this is difficult and needless to say expensive. In order to counter the court's sampling plan with a more practical sampling arrangement, statistical evidence was needed to determine and justify a new proposed frequency for sampling the wells. A reasonable assumption here is that wells are correlated over time [Verducci and Nabor (1987)], i.e. the level of contamination in a well in one month is related to the level of contamination in the same well in the following months. Therefore, a period of time had to be determined where a statistical difference could be detected in the mean level of contaminants.

There were three wells that had been monitored monthly for

at least five years starting in 1980. These wells were 04S from the Northern Landfill and R34S and R35S from the Alert Apron. To establish the frequency of sampling, the mean of the first through twelfth differences was examined. The hypothesis that the mean difference, $\mu_d$ d=1,...,12, is equal to zero was tested. The idea being that if $\mu_d$=0 then the mean level of contaminants is the same for months seperated by d-1 months. This approach was applied to the data from the three wells. The first statistical difference occured at quarterly intervals for well 04S. Therefore, sampling the wells at quarterly intervals was warranted.

E: Cost

The overall impact of the proposed procedure can best be visualized in cost reduction. The original sampling plan proposed by the court had an estimated cost of about $1,800,000. After applying the proposed procedure to the wells at Wurtsmith AFB the estimated cost was reduced to about $230,000, a reduction in cost of about 87%.

V. RECOMENDATIONS:

A: Publication

The details and theory of the analysis of Wurtsmith AFB

should be written for publication in an applied statistics or hydrogeological journal. This would not only give credence to the work being done at the OEHL at Brooks AFB but also present these new ideas to other researchers for possible improvement.

B: Implementation and Investigation

Because of the general nature of the sampling plan, it is believed that it can be implemented at other Air Force Bases to set up a ground water monitoring program. However, further investigation should be done to study the limitations of the procedure with regard to sample size, number of sampling and prediction wells, and variability of the data. The results of new research should be used to upgrade and improve the theory and methodology of the procedure.

The results from this investigation are encouraging. Most of the published results have data sets with more than 150 observations [Cressie(1986), Zirschy and Harris(1986), Myers et al. (1982)]. However, here we were able to successfully analyze spatial data with less than 30 observations. This is something that, to the author's knowledge, has not yet been published.

C: Future Monitoring

Since Kriging is a spatial prediction method, research should be done on its application to the use of experimental

design in determining the location of new monitoring wells. Some work has been done in this area [Yfantis,Flatman,and Behar(1987)] but the results are limited and generally not applicable to ground water monitoring. Barnes (1978) used a site specific design to conduct a geostatistical analysis of radiological data from a Nevada test site. This design has not been generalized for use at other sites.

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1986 USAF-UES SUMMER FACULTY RESEARCH PROGRAM

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FINAL REPORT


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|---|---|
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# Blood Flow Distribution In The Non-Working Forearm During Exercise

by

Ronald Bulbulian

## ABSTRACT

A non-invasive method for determining forearm blood flow was investigated by combining Laser doppler velocimetry and strain gauge plethysmography. Procedures were developed for deriving absolute muscle blood flow in non-working musculature during leg exercise on a cycle ergometer. Experimental artifact associated with heart rate, exercise (movement) dehydration, and instrumentation calibration were thoroughly investigated and identified. Equipment was designed and manufactured to control motion artifact and non-experimental (error) signals associated with skin (Lazer) and forearm (plethysmography) blood flow. It is determined from the pilot data completed that the procedures and equipment developed is appropriate for quantitative fractionation of forearm blood flow into skin and muscle blood flow in non-working muscle when an accurate assumption or measurement of resting skin blood flow is available.

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 accomodating individuals who helped in providing technical support, and a truly enjoyable working atmosphere. The help of Don Tucker in overcomming many technical roadblocks, and Dr. Loren Myhre in providing encouragement and timely help was greatly appreciated.

Professional interaction with Susan Bomalaski, Dr. Sally Nunnley, Dr. S. Constable, and Mr. John Garza were very helpful and provided added enlightenment.

Exercise induced redistribution of visceral blood flow to supply the needs of working muscles is well established. However, the redistribution of cardiac output (Q) from non-working muscle to the metabolically active tissues is less established. Much of the literature reporting on this important area of research is anchored by studies which attempt to fraction the skin and muscle blood flow (SBF, MBF) in the non-working forearm. One group has measured the absence of SBF through epinephrine iontophoresis which is less than totally effective (9) and, the second report has predicted SBF from oxygen saturation of deep and superficial veins (2). Neither of the preceding two reports measured blood pressure or cardiac output and the exercise parameters are ill-defined and inadequate. Nevertheless, the current research continues to buttress its case on these reports and the belief that non-working MBF is unchanged or decreased during lower body leg exercise and that forearm blood flow (FBF) evaluation can be interpreted as elevation of SBF (6,8).

Greenleaf et al. (4) have recently shown an increased FBF in the resting muscle during supine and sitting ergometer exercise. This report has been interpreted by Lamb (7) to provide evidence for increase in MBF to non-exercising muscle during exercise. This interpretation may be partly due to reports of skin vasoconstriction (i.e., lower finger blood flow) in the hand during bicycle work (3) or similar vasoconstriction during rest to exercise transition at the onset of work (1,2), but it is certainly misinterpretation of Greenleaf s data which was taken 1 minute post maximal exercise and most likely represents a hyperemic response following work cessation (1,2).

Given present concerns with cardiovascular drift (CVD) during prolonged exercise and the need to better understand circulatory adjustments to accommodate metabolic and thermoregulatory needs (8), the methodological shortcomings (i.e., exercise quantitation, Q measurement, assumptions) in the landmark exercise-SBF studies of Wells et al. (9) and Bevegard and Shepherd (2) make a reexamination of FBF distribution necessary.

## METHODS

### Subjects

Moderate to well conditioned subjects 18-30 years of age will be recruited for participation in the study. Procedures of the experiment will be made available in writing and also full verbal explanation before obtaining informed consent.

### Research Design

All subjects will participate in each experimental trial in a counterbalanced one x four (subject x treatment) design. ANOVA will be used to compare workload effects, and correlation analyses to study the relationship of cardiac parameters and blood data.

Subjects will be tested at the same time of day at least 3 days apart to minimize fatigue and training. Subjects will be fully hydrated before the test, but no fluids will be provided during exercise or before completion of the recovery period.

### Experimental Protocol

(1) Subjects will exercise on a mechanically braked cycle ergometer in the sitting position. They will be dressed in shorts and

running shoes and will be exposed to rapid air flow (fan) to facilitate convective and evaporative heat loss and effective thermoregulation.

(2) Nude body weights will be taken to measure evaporative heat loss (sweat) before and after the experimental period and will be adjusted for carbon exchange, respiratory water loss, and non-exercise sweat loss before and after weighing.

(3) Following a 20-minute rest period, seated on the cycle, exercise will then be introduced at 30, 50, or 70% of $VO_2$ max on the three separate test days and will continue until a steady state core temperature (Tre) has been attained. This period will vary with intensity of exercise ranging to 80 minutes for the 70% $VO_2$ max load. For comparison with relative loading responses, an absolute workload of 100 watts will also be performed by all subjects. Following the exercise, a recovery period will be monitored for an additional 20 minutes.

(4) 5 ml of blood for Hb and Hct to estimate changes in plasma volume will be drawn from an antecubital vein before and during the last minutes of cycle exercise.

(5) All testing will be performed in a temperature regulated environment (22 ± 1.0 C and 30-35% relative humidity).

(6) All measures with the exception of $VO_2$, Q, and blood samples will be made at 5-minute intervals throughout the rest, exercise, and recovery periods. The order of measurement will be as follows: HR, BP, Tre, Tsk, FBF, SBF, $VO_2$, and Q. Q is measured last in order to allow sufficient time to reach CO2 equilibrium (CO2 rebreathing method for Q) prior to $VCO_2$ and SBF measurement and the

following period (at least 5 minutes).

(7) Blood pressure will be measured in both arms to initially determine differences between arms in all subjects. Subsequently, BP will be measured in the left arm and adjusted to appropriately reflect the values in the right arm on which FBF and SBF will be measured. The BP and FBF measures will be taken within a 1-minute period (temporal shift).

(8) To appropriately evaluate the effect of sweating on the SBF relationship to exercise, a hygrometer will also be used to locate and identify in time the onset of sweating.

Experimental Assessments

All volunteer subjects will be cleared for exercise stress experiments by the usual procedures. Aerobic capacity will be determined by a treadmill and/or cycle ergometer graded exercise protocol. All other experiments will use submaximal exercise on the cycle ergometer.

Briefly, each subject will be asked to report to the laboratory in the morning before eating breakfast or drinking caffeinated beverages. He/she will be monitored for the parameters discussed above before, during, and immediately following exercise sessions of up to 90 minutes in length. All experiments will be completed prior to 1200 hrs (noon).

Experimental procedures include the following:

a. FBF: Measured by a Whitney strain gauge which consists of a small-diameter surgical tube filled with a column of mercury. The tube encircles the arm while maintaining a very light tension.

Increases or decreases in arm blood flow are detected by expansion or contraction of the tube.

b. SBF: Non-invasive Doppler sensor in contact with a specific area on the skin and held in place by an adhesive disk.

c. Heart Rate: Standard electrocardiogram and/or chest lead cardiotachometer (Exersentry).

d. Tre and Tsk: Standard YSI thermistors: Tre probe is sterile and disposable.

e. Oxygen Consumption ($VO_2$): One to 5-minute collections of expired air (mouthpiece, 2-way valve, and nose-clip) at appropriate intervals during the experiment.

f. Cardiac Output (Q): $CO_2$ rebreathing method which involves 5 to 8 deep breaths through a mouthpiece in and out of a 7-liter anesthesia bag containing approximately 94% $O_2$ and 6% $CO_2$. Samples of end expiratory air are taken from a tap in the mouthpiece at the end of each breath: gas is analyzed for CO2.

g. Blood pressure and blood samples are standard laboratory procedures. BP will be measured with an automated BP monitor.

Human Subjects Issues

The potential discomforts and risks of the experiment are listed below:

(1) Treadmill Test for Aerobic Capacity: An exhaustive bout of treadmill and/or cycle exercise may be hazardous for individuals who have been previously cleared by a physician but may still have some form of silent heart disease. Standard precautions will be employed by the attending physician who will continuously monitor the subject s

EKG during all maximal exercise tests.

(2) Core Temperature: Sterile, disposable rectal thermistors fulfill the hygienic requirements of this procedure. Still, some apprehension and/or slight irritation may be expected to occur initially.

(3) Blood drawing is subject to bruising or minor hematoma. Normal sterile procedures will be used to minimize the possibility of infection.

## Results

Not withstanding delays in manufacturing and delivery of research equipment necessary for the project, the progress made was satisfactory and paved the way for completion of the proposed project and subsequent work in blood flow and thermoregulation.

### Arm Support Assembly

Figure 1 shows the pilot exercise skin blood flow (SBF) tracing from the laser Doppler instrument during a graded exercise trial. The arm was supported in a sling wrapped around the neck and motion artifact was amply evident. However, the exercise response in non-working forearm SBF is discernable and encouraging. An improvement for forearm mobilization during exercise was designed and manufactured. The completion date did not allow time for subsequent data collection. That process is ongoing.

### Laser Doppler (LD)

The SBF measurement were undertaken with a new LD instrument whose reproducibility, reliability, and performance characteristics had to be evaluated under exercise conditions. Methodological shortcomings in the original protocol were revised to improve data acquisition. The arm support assembly was one of the several modifications necessary to optimize the LD data acquisition process.

### Venous Occulusion Plethysmography (VOP)

VOP is a technique not idealy suited for exercise research. However, through pilot research investigations, the method was refined for use with the inactive forearm to produce reproducible and reliable

measures of forearm blood flow during leg exercise. The physiograph recording during pilot studies is presented in Figure 2 and the plethysmograph is currently being fitted for digital computer recording of the output signal to be undertaken during the study.

Summary

Due to equipment manufacturing and acquisition delays, the summer research work focused on learning to operate new equipment, design appropriate modifications to existing or new equipment, calibration procedures, and research protocol refinements which allows a more stringent test of the proposed research hypotheses.

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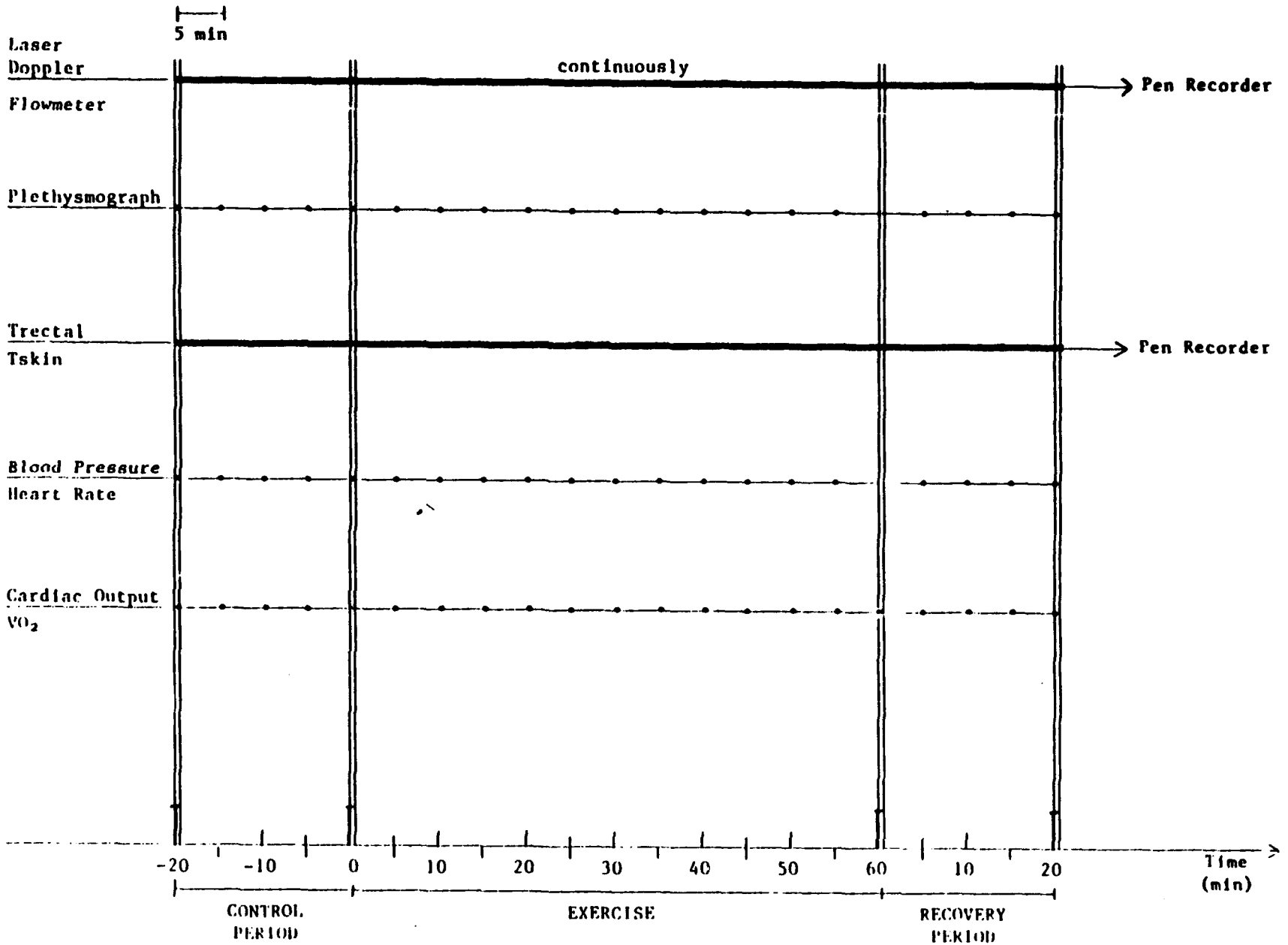

5 min
Laser Doppler Flowmeter
continuously
Pen Recorder
Plethysmograph
Trectal
Tskin
Pen Recorder
Blood Pressure
Heart Rate
Cardiac Output
$VO_2$
-20
-10
0
10
20
30
40
50
60
10
20
Time (min)
CONTROL PERIOD
EXERCISE
RECOVERY PERIOD

FIGURE 1. Laser Doppler skin blood flow during various phases of graded exercise. Blood pressure and heart rate recordings are made on the paper skip chart on random basis. Irregularities in the thermosensitive record are due to blood flow pulsations and motion artifact.

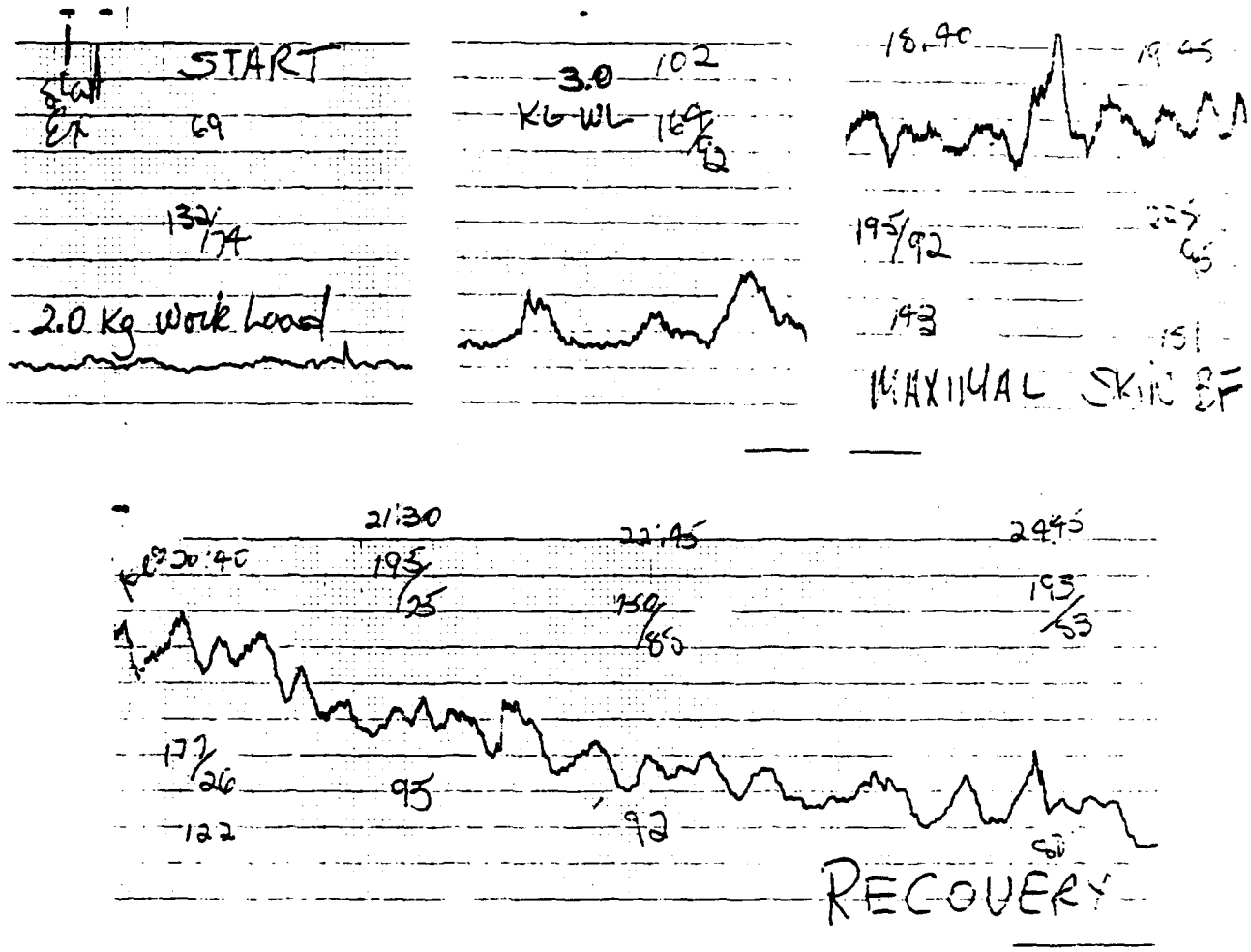
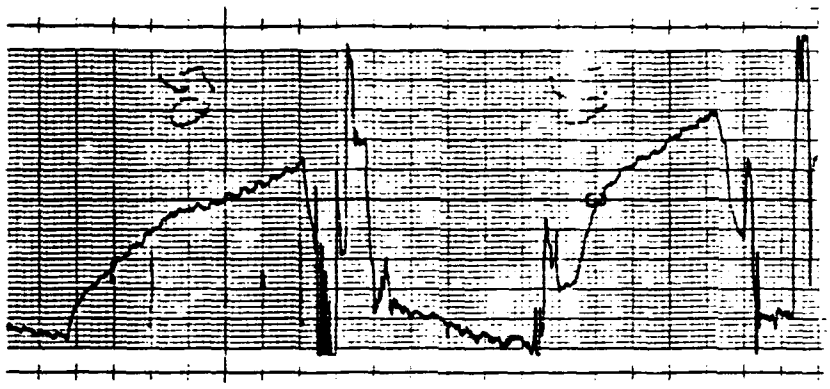


FIGURE 2. Venous occlusion plethysmography with 50 mmHg venous occlusion. Initial non-linear increase in forearm volume represents compression artifact related to the pressure cuff inflation. Subsequent linear increases are due to blood flow (pooling .



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Final Report

# PHOTOPHYSICS AND PHOTOCHEMISTRY OF TRANSITION METAL COMPLEXES OF 8-QUINOLINAMINE SCHIFF BASES


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Date: July 22, 1988

Contract No: F49620-87-R-0004

# PHOTOPHYSICS AND PHOTOCHEMISTRY OF TRANSITION METAL COMPLEXES OF 8-QUINOLINAMINE SCHIFF BASES

by

John A. Burke, Jr.

## ABSTRACT

Interaction between 633 and 532 nm laser beams has been observed when these beams intersect orthogonally in a solution of a cobalt(II) Schiff base complex derived from 8-quinolinamine and pyridine-2-carboxaldehyde. The effect observed is dependent on the power density of the 532 irradiation and the alignment of the two intersecting beams. The power density dependence follows that observed for standand saturation phenomena and has been fitted to the expression

$$f(P) = \frac{96.6P^2}{100P^2 - 0.097} \qquad (1)$$

where P is the relative power of the 532 laser beam and the function, f(P), is the change in optical density observed when the sample is irradiated. Other complexes have been prepared and investigated for beam interaction effects but none have been observed.

ACKNOWLEDGEMENTS

Support for this work provided by the Air Force Systems Command and the Air Force Office of Scientific Research is greatly appreciated. Administration of the Summer Faculty Research Program by Universal Energy Systems is also acknowledge with apprecation.

The technical and intellectual support given to this effort by John Taboada, PhD, and his able staff have added significantly to the work reported here. Chemical laboratory space made available for the preparative chemistry by Dale Clark, PhD, was invaluable during this stay at Brooks AFB. Mr. Leo Mosser has been particularly helpful by identifying and providing ancillary chemicals and items needed for experiments.

Trinity University has provided chemicals and laboratory glassware needed to perform the synthetic chemistry done on the complexes. Continuing support for this work by Trinity is very helpful.

## I. INTRODUCTION

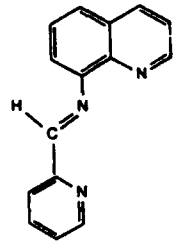
Aromatic nitrogen donor transition metal complexes, particularly those of polypyridines, have been thoroughly investigated and continue to reveal exciting new results (1-6). Photophysical and photochemical properties of iron(II), chromium(III), and ruthenium(II) complexes are interesting because they provide access to knowledge about excited states in transition metals. Tris(2,2'-bipyridine)ruthenium(II) when in its excited state is a powerful reducing agent. Attempts to utilize this property to photochemically liberate hydrogen from water with visible light have been reported (22 and references therein).

Accessibility of reasonable excited state concentrations by laser irradiation is rapidly expanding the knowledge about processes that occur when transition metal complexes absorb light. Luminescence (7), electron transfer (8), excited state absorption spectra (1,9), and solvatochromism (10) are being investigated in systems that involve transition metals bound to aromatic nitrogen donor ligands.

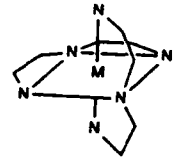
## II. OBJECTIVES

A recent disclosure has reported a facile route to the transition metal complexes of the Schiff base derived from 8-quinolinamine and pyridine-2-carboxaldehyde (11). The present study expands this route to other complex ions and

initiates the investigation of their photochemical and photophysical properties. Similarity between the Schiff base ligand, 8-((pyridine-2-methylene)amino)quinoline and 2,2'-bipyridine prompted interest in these complexes. Bis-(8-((pyridine-2-methylene)amino)quinoline)iron(II) iodide is well known and has been charaterized, but other complexes of this Schiff base have not been investigated. Significant improvement on the synthesis of bis(8-((pyridine-2-methylene)amino)quinoline)iron(II) iodide, as well as new synthetic routes to bis(8-((pyridine-2-methylene)-amino)quinoline)cobalt(II) chloride and bis(8-((pyridine-2-methylene)amino)quinoline)ruthenium(II) hexafluorophosphate have been developed. The structures of the Schiff base ligand (I) and the metal complexes (II) are shown below. The structure of the ligand in the complex has been simplified for clarity in the diagram by illustrating only the chelate rings binding the metal ions.




I




II

The scheme utilized in all of these syntheses involves complexing 8-quinolinamine to the metal ion of interest before reaction with pyridine-2-carboxaldehyde to form the Schiff base complex. Subsequent steps are then taken to oxidize or reduce the metal ion, to purify the product and to isolate the desired form of the complex salt. Characterization of the isolated materials is by infrared spectroscopy and, when available, metal ion and anion analysis. Ultraviolet and visible spectra are used to guide solution preparation prior to experiments with laser techniques. Preliminary results of the iron and cobalt complexes reveal that both exhibit fluorescence in the visible upon laser irradiation with 532 nm light. Niether of the materials exhibit processes by which orthogonal beams of 1064 nm and 532 nm light interact when the time of arrival at the sample for the 532 nm pulse is swept through the time of ar of the 1064 nm pulse. Timing for the 532 nm pulse is varied with a Dove prism delay line mounted on an optical track.

Beam interaction has been achieved when a helium-neon laser beam (633 nm) and the second harmonic beam of the YAG laser (532 nm) intersect orthogonally in the cobalt Schiff base solution. The effect of the 532 beam on the intensity of the 633 beam has been investigated and is discussed.

## III. EXPERIMENTAL, RESULTS AND DISCUSSION

### Experimental

Materials and chemicals were all either reagent grade or research grade. Ligand reactants were supplied by Sigma-Aldrich and were used without further purification. Solvents were also used as supplied by their source. Analytical services were provided by the Occupational and Environmental Health Laboratories (OEHL) at Brooks AFB. Infrared spectra were obtained in KBr disks with a Perkin-Elmer 1600 FTIR. Spectra of solutions in the near IR, visible and ultraviolet regions were measured with either Cary 2400 or Cary 2315 spectrophotometers.

Cobalt(II) Schiff base complex synthesis - Cobalt(II) chloride hexahydrate (0.531 g, 4.71 mmol) and 8-quinolinamine (0.678 g, 4.71 mmol) are dissolved in 65 ml of 85% aqueous methanol by the addition of 1 ml (10.1 mmol) of pyridine-2-carboxaldehyde. A catalytic amount of charcoal is added followed by the dropwise addition of 25 ml of 3% hydrogen peroxide. Hydrogen peroxide decomposition is accomplished by heating the reaction mixture for 4 hr. Removal of the catalysts by filtering yields about 50 ml of red-orange filtrate. Dropwise addition of 25 ml of concentrated hyrochloric acid is followed by evaporation to induce crystallization. At a volume of 50 ml, cooling the solution in an ice bath and filtration isolates the crude product. Recrystallization first from 25 ml of water and

then from 20 ml of 5% isopropyl alcohol in water produces a bright red-orange hydrated solid. Drying this solid initially over a steam bath and then in a dessicator over magnesium perchlorate darkens the color of the product. Chemical analysis reveals Co, 7.28 and Cl, 9.1. The calculated atom ratio of Cl:Co is 2.07 indicating a cobalt(II) complex. Bands in the IR spectrum are consistent with those of other Schiff base complexes in this series. This sample will be referred to as CoSB1 in the discussion section.

Pulling air through a reaction solution containing cobalt(II), 8-quinolinamine and pyridine-2-carboxaldehyde also yields the cobalt(II) Schiff base complex. Charcoal catalysis of this reaction does not alter the nature of the isolated product. Approximately 0.1 g of cobalt(II) chloride hexahydrate and 0.14 g of 8-quinolinamine are dissolved in methanol to which 15 drops (0.75 ml) of pyridine-2-carboxaldehyde are added. Air is drawn through the solution in a reaction flask that is assembled from a side arm filter flask and a pipet in a stopper inserted from the top to extend into the solution. Air is pulled through the solution with an aspirator for about 3 hr. The crude product is isolated by addition of saturated aqueous KI and recrystallized in hot water by the addition of saturated KI.

The reaction when repeated in the presence of

catalytic amouts of charcoal shows no significant differences in the product. These products have been partially characterized by infrared spectra and visible spectra in several solvents. Yields of the crude products are 57% for the first and 42% for the second reaction.

Time domain laser experiment - The two beams of frequency doubled output from a YAG mode locked, Q-switched laser were used in the time delay studies. The 532 nm and the 1064 nm beams were diverted and aligned to intersect orthoganally in the sample cell holder. Timing is varied by changing the pathlength of the 532 nm beam from 75.2 to 95.2 cm using an optical trombone, a Dove prism mounted on an optical track equipped with a metric ruler and pointer for distance measurements. Infrared reflective mirrors were adjusted in the 1064 nm beam path to set the point of intersection of the two beams at a pathlength of 85.4 cm. With this arrangement the difference in the arrival time of the two beams at the sample could be set and measured up to 3.3 nsec both positive and negative. Intensity of the 532 beam is measure with either a photomultiplier or a silicon photodiode. That of the 1064 beam is measured with a calorimeter. Beams are attenuated with 0.1-2 OD neutral density filters chosen for the level required by the detector for each experiment. Solutions of appropriate concentration for each sample were chosen by obtaining the absorbance spectra. Fluorometric cells with 1 cm path were

used to irradiate the samples in the cell holder. The beams were aligned visually utilizing an IR fluorescent card to illuminate the path of the 1064 beam.

Helium-neon 633 cm beam and YAG 532 nm beam interaction experiment- A similar arrangement is used to align these two beams to intersect at right angles in the cell holder. For these experiments the 1064 nm beam is blocked at a short distance from the source for safety. A He/Ne laser is mounted in line with, but in the opposite direction to, the 1064 path and beyond the cell position. This allows the use of the 532 beam without disturbing the set up for the time domain experiments. Beam intersection is done visually by adjusting the position of the He/Ne laser with thumb screws in its mounting device. Either a 1 cm or a 0.2 cm fluorometer cell were used in experiments with this optical arrangement so that the intensity of the 633 beam exiting the cell had sufficient intensity for precise measurements.

Detection - Visible light intensities are monitored with a Thorn EMI, Inc. C-10 Photon Counter system. Cooling the photomulti- plier tube is done electronically and is monitored by an EMI Gencom Inc FACT 50 MKIII device. Temperatures of the tube were maintained between -23 and -26C during the measurements of changes in intensity. Uncertainity in the intensity measurements as estimated from standard deviations from the average of repeated

measurements are less than 2%. Each reading is corrected for dark counts obtained before, during and after each experiment. Beam intensities are adjusted for each experiment with neutral density filters to read at least a ten-fold increase over the dark count readings.

For experiments that monitored the intensity of the 1064 nm beam the intensity is measured with a Scientech 3600 Laser Power Meter equipped with a digital voltmeter readout. Differences in the intensity of 0.2% are detectable with this device while readings of 50 to 65 mv are maintained.

Results and Discussion

Beam interaction has been detected by monitoring the intensity of the 633 beam while switching the 532 nm beam on and off as they intersect in a methanolic solution of CoSBl. The magnitude and the sign of the interaction depends on the power density of the 532 beam, the effectivness of the beam overlap and the concentration of CoSBl. The visible spectrum of the solutions used in these experiments reveals that the solution has an absorbance of 0.19 at 633 nm in the absence of 532 irradiation. Tables I and II summarize the Interaction and spectral data, respectively.

Analysis of the data in Table I reveals that the effect being observed obeys standard saturation behavior at high 532 irradiation power densities. Also for the range

of power densities explored the effect can either increase or decrease the intensity of the 633 light passing through the irradiated solution. The data may be fitted by Equation 1, a normal saturation function. Similar experi-

$$f(P) = \frac{96.6P^2}{100P^2 - 0.097} \qquad (1)$$

ments with bis(8-((pyridine-2-methylene)amino)quinoline)-iron(II) iodide have shown no detectable changes in the 633 beam on 532 irradiation of the aqueous solution. The fit of the data to this expression is illustrated in Figure 1.

Several attempts to reproduce and quantitate the effect have been thwarted by difficulties in controlling the power of the 532 beam and alignment of the two beams to maximize their intersection. When either of these problems arise the intensity of the 633 beam is not changed by irradiation of the sample with 532 light. Tuning the YAG laser to increase its efficiency and aligning the 633 nm beam restore the effect, but its magnitude and its sign are often changed relative to previous experiments.

Table III contains data for the time domain experiments on solutions of CoSB1 and bis(8-((pyridine-2-methylene)amino)quinoline)iron(II) iodide. Sweeping the time of arrival of the 532 nm beam at the cell across that of the 1064 beam exhibits no detectable effect on the

intensity of either the 532 or the 1064 nm radiation. The iron complex absorbs strongly in the visible at 532 nm, whereas the CoSB1 absorbs light of this wavelength moderately. Both samples absorb 1064 light to less than 0.020 absorbance units.

The difficulty in oxidizing cobalt(II) to cobalt(III) in the presence of the Schiff base ligand is unexpected. This inertness to oxidation may indicate that the ligand is strongly bound to the cobalt(II) ion protecting it from oxidation in solution with oxygen tension as high as that produced in a few percent of hydrogen peroxide.

Solutions of these air oxidized samples exhibit a shift in the lowest energy absorbance band as a function of the solvent. Wavelength maxima are observed at 419, 460, 479 and 470 nm in water, ethanol, DMF and acetone, respectivly. Shifts such as this have recently been reported in dicyanobis(1,10-phenanthroline)ruthenium(II) (12). These shifts are correlated with the acceptor number for the solvents proposed by Gutman.

The infrared spectra of the all of the cobalt(II) complexes discussed here are similar, however, there are notable differences in the relative intensities of the peaks. The three strong bands at 1500, 1470, and 1390 $cm^{-1}$ present in each of these spectra are particularly indicative that these samples are mixtures of at least two products. The IR spectrum of CoSB1 is quite well resolved

and has its most intense peak at 1654 $cm^{-1}$. This strong peak agrees with the C=N stretching region for these Schiff base complexes (2).For example, bis(8-((pyridine-2-methylene)amino)quinoline)cobalt(II) iodide is reported to show this peak at 1649 $cm^{-1}$.

IV. RECOMMENDATIONS

The excited state spectra for these complexes should be explored further. Only one wavelength has been examined in this study. Dye laser radiation can extend the spectra over a wider region in the 500 to 600 nm range. Excitation of the samples with a shorter wavelength while monitoring the 400 to 800 nm region should also be explore since all these sample absorb strongly in the ultraviolet. Potential optical switches are going to be needed throughout the visible and near infrared region in the near future. These complexes have versatility in that they absorb strongly in the visible region and the position of the wavelength maximum can be adjusted by suitable substituents on the aromatic ligands and by the proper selection of the central metal ion.

More efficient control of the beam alignment in the sample should be developed. Now that it has been demonstrated that the observed effects are so sensitive to these variables, optimum control of the YAG laser power can be improved by more careful monitoring of the mode of the pulses and the power density with available devices.

Additional examples of transition metal complexes of these and similar Schiff bases need to be synthesized and characterized. This will include a complete characterization of CoSBl. Elemental analysis for carbon, hydrogen and nitrogen are needed to confirm its formulation and chemical nature. Electrochemical investigations such as cyclic voltametry on the cobalt complexes will elucidate the redox couple that needs to be overcome to synthesize the cobalt(III) species. The same data is required to characterize the chromium and ruthenium complexes that have been investigated under my direction by C. M. Nelson, USAF-UES Graduate Student Fellow 1988.

TABLE I. DATA FOR CoSBl OPTICAL DENSITY CHANGE AT 633 nm VS POWER OF 532 nm BEAM

| N.D. FILTER OD | POWER 10exp-OD | EXPERIMENTAL Io/Ii CORR. | LOG Io/Ii | 1+LOG Io/Ii | EQUATION 1. 1+LOG Io/Ii | LOG Io/Ii |
|---|---|---|---|---|---|---|
| 0 | 1.000 | 1.018 | 0.0077 | 1.0077 | 0.9707 | -0.0293 |
| 0 | 1.000 | 0.939 | -0.0273 | 0.9727 | 0.9707 | -0.0293 |
| 0 | 1.000 | 0.921 | -0.0357 | 0.9643 | 0.9707 | -0.0293 |
| 0 | 1.000 | 0.934 | -0.0297 | 0.9703 | 0.9707 | -0.0293 |
| 0.1 | 0.794 | 1.082 | 0.0342 | 1.0342 | 0.9713 | -0.0287 |
| 0.2 | 0.631 | 0.924 | -0.0343 | 0.9657 | 0.9722 | -0.0278 |
| 0.3 | 0.501 | 0.929 | -0.0320 | 0.9680 | 0.9736 | -0.0264 |
| 0.4 | 0.398 | 0.912 | -0.0400 | 0.9600 | 0.9758 | -0.0242 |
| 0.4 | 0.398 | 0.950 | -0.0223 | 0.9777 | 0.9758 | -0.0242 |
| 0.6 | 0.251 | 0.970 | -0.0132 | 0.9868 | 0.9850 | -0.0150 |
| 0.7 | 0.200 | 0.992 | -0.0035 | 0.9965 | 0.9941 | -0.0059 |
| 0.7 | 0.200 | 1.015 | 0.0065 | 1.0065 | 0.9941 | -0.0059 |
| 0.8 | 0.158 | 1.040 | 0.0170 | 1.0170 | 1.0089 | 0.0089 |
| 0.9 | 0.126 | 1.059 | 0.0249 | 1.0249 | 1.0332 | 0.0332 |
| 0.9 | 0.126 | 1.034 | 0.0145 | 1.0145 | 1.0332 | 0.0332 |

The fit of the data by Equation 1 is obtained by calculating one plus the power function, F(p). Intensities of the 633 beam with the 532 beam off and on are represented by Io and Ii, respectively. For this fit:

$$1 + f(P) = 1 + \mathrm{LOG}(Io/Ii)$$

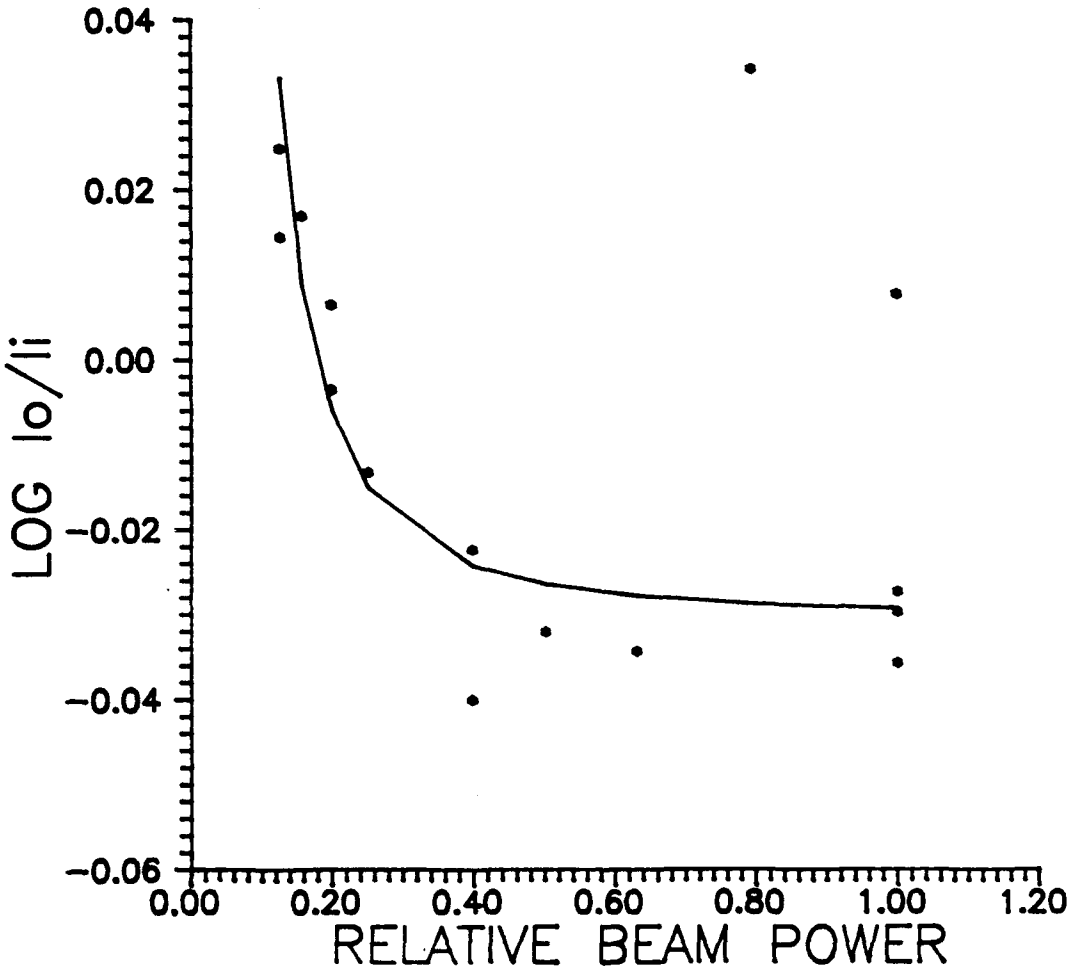
TABLE II. SPECTRAL DATA FOR CoSBl

| Wavelength (nm) | Absorbance | Solvent | Concentration (M) | Extinction Coefficient |
|---|---|---|---|---|
| 633 | 0.009 | water | 0.000186 | 43 |
| 500 | 0.190 | water | 0.000015 | 1022 |
| 386 | 0.225 | water | 0.000015 | 15100 |
| 259 | 0.699 | water | 0.000015 | 46900 |
| 219 | 0.759 | water | 0.000015 | 50900 |
| 633 | 0.020 | methanol | 0.000800 | 25 |
| 532 | 0.580 | methanol | 0.000800 | 725 |
| 500 | 0.770 | methanol | 0.000800 | 962 |
| 395 | 0.246 | methanol | 0.000016 | 15400 |
| 262 | 0.829 | methanol | 0.000016 | 51800 |

TABLE III. TIME DOMAIN STUDY FOR IRON(II) COMPLEX INTENSITY RATIO AS A FUNCTION OF RADIATION BY 532 OR 1064 LASER BEAM AND TIME OF ARRIVAL AT THE CELL

| | Monitoring 532 | | Monitoring 1064 | |
|---|---|---|---|---|
| Track | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Scale (cm) | Io/Ii | Io/Ii | Io/Ii | Io/Ii |
| 2 | 0.9968 | 1.0175 | 1.0000 | 0.9965 |
| 3 | 0.9893 | 1.0365 | 0.9961 | 0.9965 |
| 4 | 0.9744 | 1.0365 | 1.0019 | 0.9983 |
| 5 | 0.9893 | 0.9951 | 0.9962 | 0.9966 |
| 6 | 0.9934 | 1.0032 | 1.0000 | 0.9983 |
| 7 | 0.9995 | 1.0124 | 1.0038 | 0.9966 |
| 8 | 0.9882 | 1.0197 | 1.0000 | 0.9983 |
| 9 | 0.9963 | 0.9901 | 1.0019 | 0.9966 |
| 10 | 1.0054 | 1.0084 | 1.0093 | 0.9983 |
| 11 | 1.0150 | 1.0044 | 1.0128 | 0.9917 |
| 12 | 0.9881 | 0.9546 | 1.0000 | 0.9967 |
| 13 | 1.0107 | 1.0026 | 1.0053 | 1.0016 |

FIGURE 1. PLOT OF DATA FROM TABLE I.



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FINAL REPORT

# Immunocytochemical Localization of Vasoactive Intestinal Peptide, Neuropeptide Y and Arginine Vasopressin within the Suprachiasmatic Nuclei of the Rat


| | |
|---|---|
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| Research Location: | USAF School of Aerospace Medicine<br>Brooks Air Force Base |
| USAF Researcher: | Dr. Michael Rea |
| Date: | September 26, 1988 |
| Contract No. | F49620-87-R-0004 |

# Immunocytochemical Localization of Vasoactive Intestinal Peptide, Neuropeptide Y and Arginine Vasopressin within the Suprachiasmatic Nuclei of the Rat

by

Bennye S. Henderson

## ABSTRACT

Neurons that secrete vasoactive intestinal peptide (VIP), neuropeptide Y (NPY) and arginine vasopressin (AVP) were localized within the rat suprachiasmatic nuclei (SCN) using an indirect immunofluorescence technique. VIP-immunoreactive cell bodies were observed only along the ventrolateral border of the nucleus in close association with the optic chiasm. VIP-containing fibers were distributed throughout the nucleus and extended into the surrounding hypothalamus. In contrast, AVP-containing cell bodies were concentrated in the dorsal half of the nucleus. AVP-immunoreactive fibers innervated the dorsal aspect of the SCN and many projections were observed ascending in the direction of the paraventricular nucleus. NPY-positive fibers were concentrated in the ventral half of the SCN. NPY-containing cell bodies were not observed.

## Acknowledgments

I take this opportunity to express my thanks to the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this program. For the competent administration of the program and for my acceptance as a participant, I am grateful to Universal Energy Systems, Inc. I'm indeed appreciative to Dr. Michael Rea for personal support and provision of all necessary supplies. The interest and concern in this project shown by Dr. Robert Gannon and Dr. David Terrian served as sources of encouragement. For her assistance in helping me find my way around and making the working atmosphere so pleasant, I thank Ms Heather Alexander. The technical advice and assistance extended to me by Sgt Froilan Ramos helped make this project a success. This entire experience has been enlightening and enjoyable.

## I. Introduction

Individuals who adhere to irregular work/rest schedules or are frequently involved in transmeridian flight experience desynchronization of circadian rhythms resulting in compromised mental performance. Thus, Air Force personnel are perhaps uniquely vulnerable to the performance limitations imposed upon them by the circadian timekeeping system. For this reason, the Air Force Office of Scientific Research has committed support to investigators seeking to acquire an understanding of the cellular and molecular bases of circadian rhythmicity.

Drs. Michael Rea and David Terrian have undertaken an investigation of the neurochemistry of the light-entrainable circadian pacemaker located in the suprachiasmatic nuclei (SCN). A characterization of the neurochemistry of the SCN should contribute to our understanding of circadian physiological and behavioral rhythms and provide a knowledge base for the development of pharmaceuticals with predictable effects on the circadian system. It is apparent from the work of several investigators that certain neuropeptides located within the SCN

function as neuromodulators of the circadian pacemaker. These include vasoactive intestinal peptide (VIP), neuropeptide Y and arginine vasopressin (AVP). My responsibility was to assist in the establishment of immunohistochemical methods for the detection of VIP, NPY and AVP, and to describe the distribution of these peptides within the rat SCN.

## II. Objectives of the Research Effort

The overall objective of the research project was to determine the distribution of neuronal cell bodies and fibers within the SCN of the rat which are immunoreactive for VIP, NPY or AVP.

The specific goals were:

1. To establish indirect immunofluorescence methods for the detection of VIP, NPY and AVP in histological sections of rat brain.
2. To describe the distribution of neuronal cell bodies and fibers within the SCN which contain VIP, NPY or AVP.
3. To prepare protocols for these methods with sufficient detail to permit the routine application of the methods in support of the ongoing investigation of the role of these peptides in SCN pacemaker function.

III. Establishment of Indirect Immunofluorescence Methods

Adult, male Sprague-Dawley rats were subjected to transcardiac perfusion under Ketamine/Xylazine anesthesia. After the animals had attained a deep state of anesthesia, they received a bolus transcardiac injection of 1000 units of heparin, followed by 150 ml of 10 mM phosphate buffered saline (PBS; pH 7.4) and 200 ml of fixative at a perfusion pressure of 40 - 50 psi. Several fixatives were screened for effectiveness, including (a) 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PBP; pH 7.4), (b) 4% paraformaldehyde in 0.1 M potassium borate buffer (pH 10.2), and (c) PBP fixative containing 0.08% glutaraldehyde. Of these, PBP fixative was most effective in preserving tissue integrity and peptide immunoreactivity. After perfusion, the brains were removed and cut in the coronal plane at the level of the anterior commissure and just anterior to the mammilary bodies. The tissue block bounded by these cuts was incubated overnight in fixative at $4^{o}$ C followed by incubation in 10% buffered sucrose (pH 7.4) for at least 12 hours at $4^{o}$ C. The blocks were frozen on microtome chucks at $-20^{o}$ C and 20 um-thick coronal sections were prepared and collected in ice cold

10 mM PBS. Selected sections were washed in PBS and incubated 48 - 72 hours at 4° C in primary antisera raised against VIP, NPY or AVP at a final dilution of 1:500 (dilution which yielded best results). Control sections were incubated in antiserum solution containing the appropriate synthetic peptide at a concentration of 10 mM. After incubation, the sections were washed twice with 2 - 5 ml of ice cold PBS followed by incubation with fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit IgG (1:10 final dilution) for 30 minutes at room temperature. The sections were washed thoroughly with PBS, mounted on acid-washed microscope slides with 1% p-phenylenediamine in phosphate-buffered glycerol and examined using an Olympus Model MTS1 fluorescence microscope. Photographs of selected sections were made using an Olympus OM-4 SLR camera onto Kodak Ektachrome 200 film.

IV. Distribution of Immunoreactive Neuronal Cell Bodies and Fibers

A) Vasoactive Intestinal Peptide

VIP-containing cell bodies were observed throughout the rostro-caudal extent of the nucleus. Fluorescent multipolar perikarya were localized to the ventro-lateral

border of the SCN, in close association with the optic chiasm. A few cell bodies were actually embedded within the optic chiasm. In general, VIP-immunoreactive cell bodies were present at highest concentration in the middle third of the nucleus. No immunoreactive cells were observed outside the SCN.

VIP-containing fibers appeared as fine varicosities throughout the SCN, although the highest density was observed in the ventral half of the nucleus. Immunoreactive fibers were observed at all rostro-caudal levels and projections could be traced both dorsally and caudally from the SCN, extending beyond the anatomical borders of the nucleus.

B) Neuropeptide Y

No NPY-positive cell bodies were observed in the SCN or surrounding hypothalamus. NPY-containing fibers were observed at all rostro-caudal levels of the SCN. These fibers were concentrated along the ventral aspect of the nucleus, overlapping the distribution of VIP-containing cell bodies. Some fibers were observed extending laterally into the hypothalamus in sections cut along the caudal third of the nucleus.

C) Arginine Vasopressin

Brilliantly-stained AVP-containing perikarya were densely concentrated in the dorsal half of the SCN throughout the caudal two-thirds of the nucleus. In addition, in the middle third of the SCN a few immunoreactive cell bodies were observed along the medial aspect of the nucleus, with some immunopositive neurons scattered along the ventral border of the nucleus, distributed among VIP-containing cells. Scattered AVP-positive cell bodies were present along a line extending dorsally from the SCN to the paraventricular nucleus which contained a large number of AVP-containing cells. AVP-fluorescent neurons appeared as large multipolar perikarya with one or two primary dendrites and a single, unbranched axon which often could be observed projecting a considerable distance within a given section.

A dense plexus of AVP-positive fibers was present in the dorsal half of the SCN. The fine immunoreactive varicosities were observed ascending into the hypothalamus and projecting as far as the paraventricular nuclei. A few immunoreactive fibers were present in the ventral half

of the nucleus; however, the source of these fibers and their direction of propagation could not be determined.

## V. Recommendations

Abundant evidence in the literature indicates that a major circadian pacemaker is located in the SCN of mammals. Furthermore, the SCN pacemaker is perhaps unique in that it is entrainable to photic cues conveyed to the pacemaker via direct retino-hypothalamic fibers. Within the SCN, distinct neuronal populations and projections are thought to influence pacemaker function through the release of peptide neuromodulators, notably VIP, AVP and NPY. The indirect immunofluorescence technique provides an important tool with which to identify, localize and monitor the activity of neurons which synthesize and, presumably, release these neuropeptides. It is recommended that these procedures be incorporated into the routine methodology of the host laboratory to complement state-of-the-art neurochemical procedures currently in use.

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FINAL REPORT

# Development of Improved Assays for Cholesterol and Major Lipoprotein Fractions


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| Date: | 3 August 1988 |
| Contract No: | F49620-87-R-0004 |

ABSTRACT

Development of Improved Assays for Cholesterol and Major Lipoprotein Fractions

by

Eric R. Johnson, Ph.D., and Thomas E. Lane

A sensitive method suitable for the analysis of subnanogram amounts of cholesterol by electron capture gas chromatography has been developed. The method involves extraction of cholesterol and cholesterol esters from saliva or urine followed by hydrolysis and derivatization with 2,3,4,5,6-pentafluorobenzoyl (PFB-) chloride. The yield of the PFB-esters of cholesterol and the internal standard epicoprostanol was in excess of 99%. The method has a lower limit of sensitivity for cholesterol of approximately 500 pg injected, which corresponds to 250 ng per mL of saliva or urine. The coefficient of variation for the extraction and analysis of cholesterol from urine samples was found to be 4.2% (cholesterol concentration 570 ng/mL). This method, which is approximately 1000 times more sensitive than gas chromatographic methods utilizing flame ionization detection, is applicable to the analysis of non-esterified cholesterol and total cholesterol (cholesterol plus cholesterol esters) in saliva and urine.

Development of an improved high performance liquid chromatographic assay for the major lipoprotein fractions in serum was also initiated. A system comprised of three size exclusion columns linked in series was found to give good resolution between low density lipoprotein, high density lipoprotein, and albumin.

## Acknowledgements

We are grateful to the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research project. We thank the USAF School of Aerospace Medicine, Brooks AFB, for providing the facilities and the instrumentation used in this research project. We also thank Universal Energy Systems, Inc., for their administrative support of the 1988 Summer Faculty and Graduate Summer Research Programs.

In the accomplishment of our research objectives during this very productive and rewarding summer project, we are indebted to a number of people. Dr. Harvey A. Schwertner contributed enthusiastic support and encouragement, expert technical advice, and excellent ideas to this project. Dr. Dale Clark and Dr. Joe Ross, an FSRP colleague, graciously provided us with ultracentrifuged serum samples from their own projects. SSgt Clifford Sage contributed technical advice on the operation of the HPLC and GC instruments. SSgt Sage and Mr. Leo Mosser successfully kept us stocked with supplies for the project. The clinical laboratory section under the direction of Cpt. Paul Barnicott provided additional patient samples and performed cholesterol and HDL-cholesterol analyses on a selected number of serum samples.

## 1. INTRODUCTION

Coronary artery disease (CAD) is a leading cause of non-accidental death in the United States. In 1985, over 500,000 deaths were attributable to CAD (1). In addition to the immeasurable pain and suffering of the individual victims and their families, CAD is very costly to society with an estimated annual cost of over $60 billion (1,2). A portion of this financial burden falls upon the Air Force as CAD is also the leading cause of non-accidental deaths among active duty Air Force personnel. A 1981 study (3) estimated that the cost to the Air Force in lost training, hospitalization, and disability retirement of a rated pilot affected by CAD could exceed $500,000, which translates to an annual cost of over $40 million.

Although still a serious problem, the incidence of CAD is declining, due at least in part to the identification and popularization of risk factors associated with the development of CAD (4). While these results are encouraging, further decline in CAD incidence depends on the earlier detection of CAD in people at risk. At present, the earliest CAD symptoms appear too late for many individuals, leaving coronary bypass surgery or balloon angioplasty as the only alternatives to prolong and improve the quality of life. Sensitive and specific methods must be developed to identify those persons at risk at an earlier stage, when dietary and lifestyle modifications can be used to better advantage.

Several potential markers could prove to be useful in predicting risk levels in CAD. One such marker is the fraction of cholesterol in serum that is not bound to protein. This small but important pool of nonprotein-bound cholesterol could mirror physiological events occurring in the cardiovascular system that reflect the extent and severity of CAD. A method to detect this admittedly low concentration of nonprotein-bound cholesterol in serum would be valuable for the evaluation of this marker as a predictor of CAD.

Since salivary fluid has been suggested to be a membrane ultrafiltrate of serum (5), cholesterol levels in salivary fluid may correlate with the nonprotein-bound cholesterol levels in serum. Measurement of salivary cholesterol levels is attractive in that samples can be taken from patients by non-invasive means. Salivary cholesterol could be useful in predicting risk levels in CAD.

Detection of salivary cholesterol or nonprotein-bound serum cholesterol requires the development of a sensitive assay for the relatively low cholesterol levels that will be encountered in these samples.

High density lipoprotein (HDL) subfractions, particularly $HDL_2$, may also be indicators of CAD risk (6). Development of a convenient and reliable assay for $HDL_2$ would allow the assessment of this subfraction as a potential CAD indicator. Since HDL subfractions are generally regarded to be heterogeneous populations of molecules that are grouped together due to similar densities, a separation technique based on density or a related property such as molecular size will likely be successful in quantitating $HDL_2$. High performance liquid chromatography using molecular exclusion column technology is most promising for this type of analysis.

In this research project, an assay for trace quantities of cholesterol in physiological fluids was developed. In addition, some preliminary work on the separation of lipoprotein fractions was accomplished.

My research interests include chromatographic separations using gas-liquid and high performance liquid chromatography. My recent research has been focused on the specificity of denaturant-stable proteolytic enzymes. I would suspect that my experience in chromatographic separations and protein chemistry were major factors in my assignment to USAF SAM/NGIL.

## 2. OBJECTIVES OF THE RESEARCH EFFORT

In a short-term research situation such as the FSRP, it is desirable to have two potential projects in place. If one project stalls or hits a dead end, there is another project ready to go. For this reason, we formulated objectives that included the development of an ultrasensitive cholesterol assay in physiological fluids as well as the assessment of size exclusion HPLC columns for the separation of HDL subfractions. The objectives established at the beginning of the research period are outlined as follows:

I. Develop a sensitive analytical method for the analysis of submicrogram quantities of non-protein-bound cholesterol and cholesterol esters.

A. Develop and optimize procedures for the separation of non-protein-bound cholesterol and cholesterol esters from biological samples using ultrafiltration devices.

B. Evaluate methods for extracting cholesterol and cholesterol esters from these ultrafiltrates. Non-esterified cholesterol will be assayed on extracts before hydrolysis and cholesterol esters will be determined after hydrolysis of the lipid extracts.

C. Evaluate 2,3,4,5,6-pentafluorobenzoyl chloride and other compounds reactive with alcohols for use as derivatizing agents for the analysis of submicrogram quantities of cholesterol by electron capture gas chromatography.

D. Evaluate several cholesterol analogues for use as an internal standard for this assay.

E. Determine the linear response range, lower limit of sensitivity, and the accuracy and reproducibility for this cholesterol assay.

II. Develop an improved high performance liquid chromatographic (HPLC) method for the analysis of high density lipoprotein subfractions ($HDL_2$ and $HDL_3$).

A. Evaluate several new molecular exclusion HPLC columns for use in the analysis of high density lipoprotein subfractions ($HDL_2$ and $HDL_3$).

B. Evaluate various chromatographic parameters (flow rate, ionic strength, pH, etc.) to optimize resolution of $HDL_2$ and $HDL_3$ on these columns.

III. Use the above methods to analyze the concentration of unbound cholesterol, $HDL_2$ and $HDL_3$ in serum of ten patients with proven coronary artery disease and ten age-matched individuals without coronary artery disease. Determine if the measured cholesterol, $HDL_2$ and $HDL_3$ levels are significantly different in the two populations.

3. METHODS AND RESULTS

I. Development of sensitive assay for cholesterol

A. Separation of non-protein-bound cholesterol

Approach - An Amicon MPS-1 ultrafiltration device with 30,000 molecular weight cutoff membrane was evaluated for use in the separation of non-protein-bound cholesterol and cholesterol esters. This system was chosen as a result of its routine use in the analysis of

non-protein-bound testosterone (7), a compound which is similar to cholesterol in structure. Samples of ultrafiltered serum were spiked with various detectable concentrations of free cholesterol and passed through a fresh ultrafiltration membrane. Samples were assayed for cholesterol using the assay method developed in this project, which is described below.

Results - While cholesterol could easily be detected in the spiked sample before ultrafiltration, no cholesterol was detected in the spiked sample after ultrafiltration. This indicates that the added cholesterol was not passing through the membrane. While a strong interaction of cholesterol with the membrane would explain this observation, it seems unlikely that cholesterol would bind tightly to a membrane that has been designed to eliminate testosterone binding. Perhaps the particular lot of membranes that we were using was defective. Based on published results with testosterone, we believe that the MPS-1 system will work in this application and merits further study with different lots and perhaps types of membranes.

B. Extraction and hydrolysis method

Approach - Several different organic solvent systems were tested for the extraction of cholesterol and cholesterol esters from saliva and urine. A portion of the extract was then treated with methanolic KOH at elevated temperature to hydrolyze the cholesterol esters to free cholesterol. Both the hydrolyzed and unhydrolyzed extracts were then assayed for cholesterol using the assay method described below.

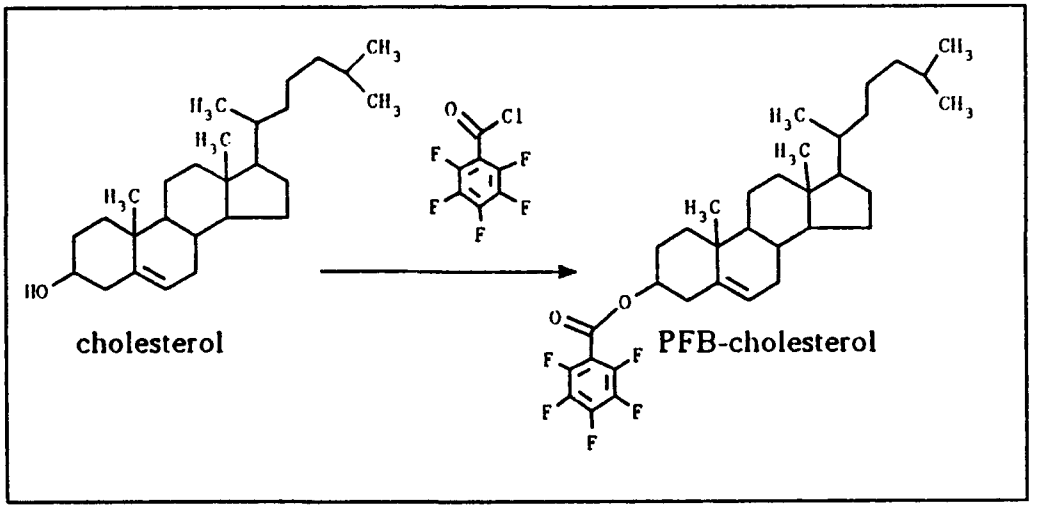
Results - Extraction with chloroform-methanol (2:1, v/v) followed by hydrolysis with methanolic KOH and extraction of the hydrolyzate with hexane was found to give good recoveries of cholesterol from saliva and urine. Analysis of the hexane extract using our sensitive cholesterol assay gave a value for the total cholesterol (cholesterol plus cholesterol esters) in saliva or urine samples. Good values for non-esterified cholesterol in saliva or urine were obtained by omitting the hydrolysis step.

C. Optimization of derivatization reaction

Approach - To gain sensitivity in developing this gas chromatographic-based cholesterol assay, electron capture detection was selected. As cholesterol is not sufficiently electronegative to be

detected by electron capture methods, conversion of cholesterol to a more electronegative derivative was required. Based on literature reports on its reaction with amines and alcohols, 2,3,4,5,6-pentafluorobenzoyl (PFB) chloride was selected as the derivatizing agent for the hydroxyl group of cholesterol. The PFB-ester of cholesterol would be very electronegative and easily detected by electron capture. The effects of temperature, time, and solvent were systematically varied to optimize the conversion of cholesterol to the PFB-ester.

Results - Optimal conversion of cholesterol to the PFB-ester was obtained by reacting 25 microliters of PFB-chloride with a dried film of cholesterol (no added cholesterol) in a capped test tube at 85 $^{o}$C for 60 minutes. The reaction shown below was determined to be over 99% complete under these conditions using gas chromatography with flame ionization detection to separate unreacted cholesterol from PFB-cholesterol.




D. Internal standard

Approach - Internal standards for such an assay must behave in a fashion similar to cholesterol in the extraction, derivatization, and chromatographic phases of the assay. In addition, the internal standard should not be normally found in samples for assay. Several sterols were considered before epicoprostanol (5-beta-cholestan-3-alpha-ol) was selected for testing. Epicoprostanol was subjected to extraction and derivatization with PFB-chloride.

Results - Epicoprostanol, when added to urine or saliva samples, was extracted in good yield using the procedure described in section B

results. Epicoprostanol was converted to its PFB-ester by reaction with PFB-chloride in excess of 99% yield using the conditions described in section C under Results.

E. Sensitivity and reproducibility

Approach - Chromatographic parameters (temperatures of the injector, column, and detector, as well as carrier gas flow rates) were systematically varied to give optimal separation of standard samples of PFB-cholesterol and PFB-epicoprostanol from each other and other interfering substances. These parameters were also optimized to give maximum sensitivity while maintaining chromatographic resolution. The linear response range and lower limit of sensitivity were determined by variation of the amount of cholesterol while maintaining the amount of epicoprostanol at a constant value. The reproducibility of the entire extraction and assay process was determined by repetitive assay of different aliquots of a single urine sample.

Results - The chromatogram of a standard sample containing 4.0 ng each of PFB-cholesterol and PFB-epicoprostanol is shown in Figure 1. This chromatogram shows good resolution between the PFB-cholesterol eluting at 12.14 min and the PFB-epicoprostanol eluting at 10.22 min. As shown in Figure 2, an excellent linear correlation (correlation coefficient = 0.9992) between the cholesterol/epicoprostanol detector response ratio and cholesterol concentration was obtained.

The following procedure was devised for the assay of total cholesterol (cholesterol plus cholesterol esters) and non-esterified cholesterol in saliva and urine samples.

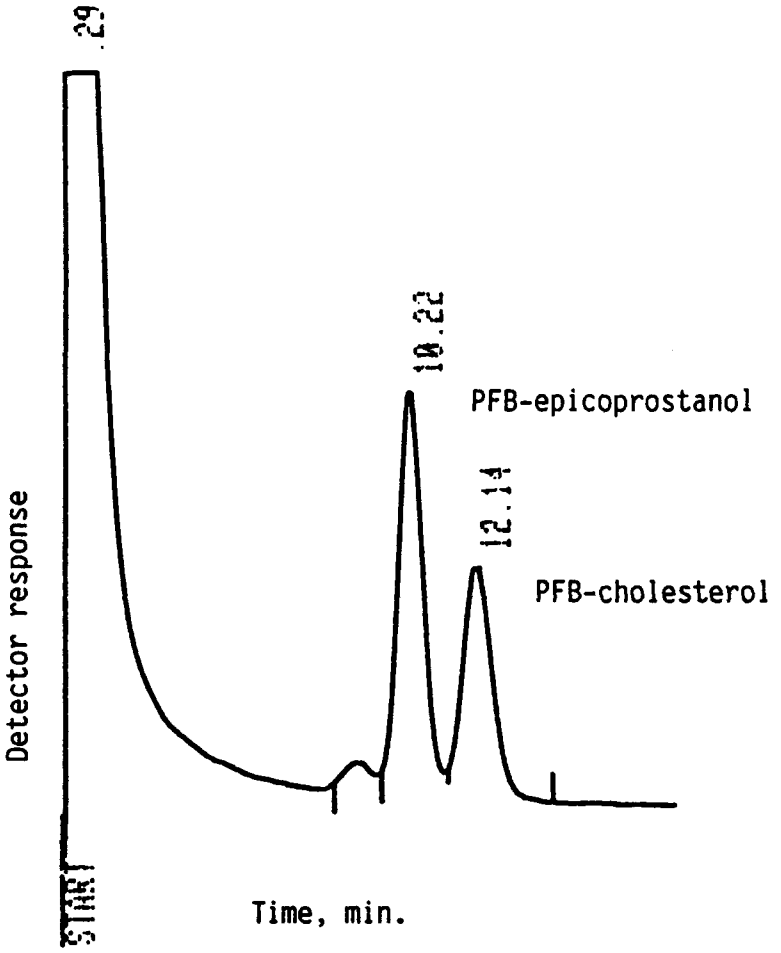
1. Centrifuge freshly collected urine or saliva at 3500 x g for 20 min. Place 2.0 mL of the urine or saliva supernatant and 2.0 mL distilled water in a 25 mm x 150 mm screw-capped culture tube.

2. Add 100 microliters of the internal standard solution (epicoprostanol, 0.03 mg/mL in 100% ethanol).

3. Add 15 mL chloroform-methanol (2:1, v/v), cap, and shake for 10 min on an Eberbach shaker at 4 linear cycles per sec. Centrifuge the sample for 5 min at 1500 x g to separate the phases.

4. Transfer approximately 9 mL of the chloroform layer (lower phase) to a 16 mm x 125 mm screw-capped culture tube. Evaporate the chloroform with a gentle stream of nitrogen at 60 $^{o}$C in a heating block.

Figure 1. Electron capture gas chromatographic analysis of standard samples of PFB-cholesterol and PFB-epicoprostanol




Instrument: Hewlett-Packard 5890A gas chromatograph equipped with a $^{63}Ni$ electron capture detector and autosampler

Column: 0.53 mm x 5 m HP-1

Injector temperature: 280 °C

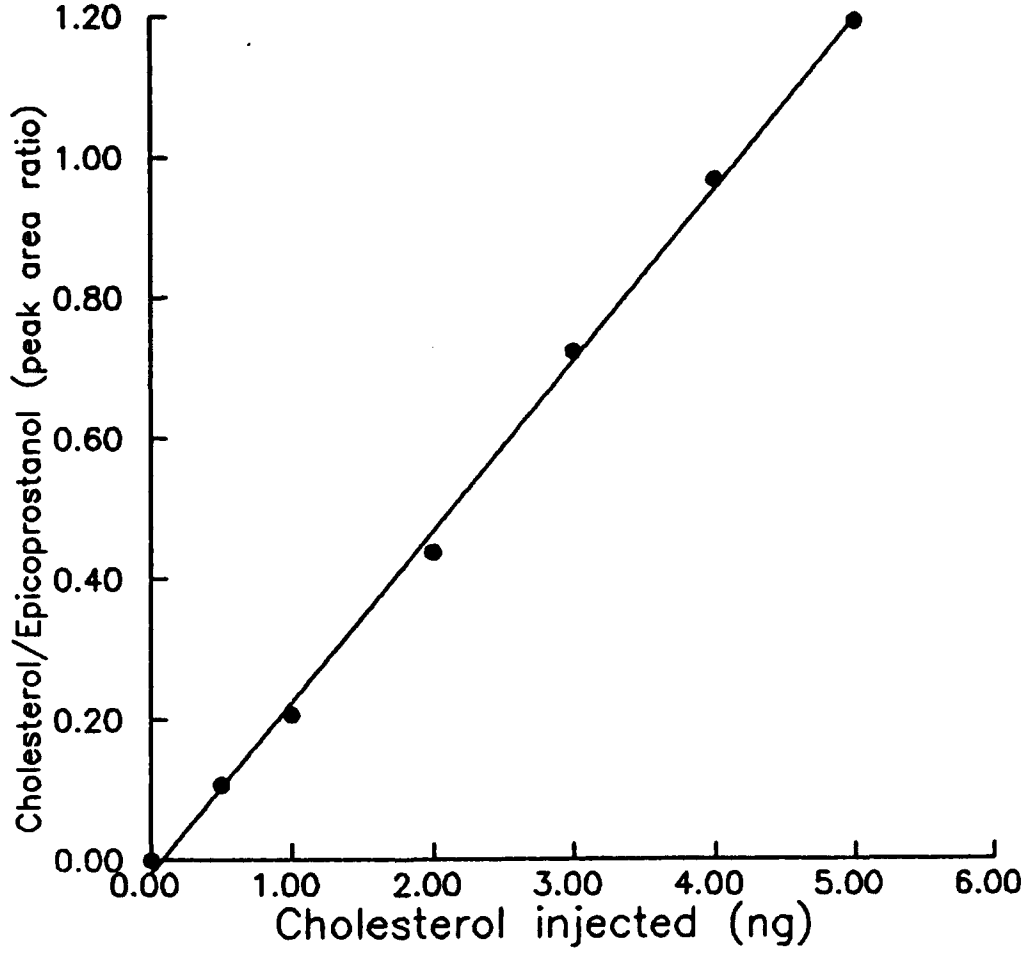
Oven temperature: 270 °C

Detector temperature: 280 °C

Carrier gas flow: 5% methane in argon, 6.0 mL/min

Sample injected: 1 µL (4 ng of each component)

Figure 2. Linear curve for cholesterol analysis using epicoprostanol as an internal standard.



5. For the determination of total cholesterol, add 2.0 mL 0.5 M KOH in methanol. Cap the tubes and heat for 20 min at 95 °C in a heating block. Vortex the sample for 5 sec at 0, 10, and 20 minutes during the heating period. For the determination of non-esterified cholesterol, add 2.0 mL methanol and proceed to step 6 without heating.

6. Cool the samples to room temperature and add 1.0 mL distilled water and 5.0 mL hexane. Shake for 10 min on an Eberbach shaker at 4 cycles per sec. Centrifuge at 600 x g for 15 seconds to separate the phases. Transfer approximately 4 mL of the hexane layer (top phase) to a clean 16 mm x 125 mm screw-capped culture tube. Evaporate the hexane with a gentle stream of nitrogen at 60 °C in a heating block.

7. Add 25 microliters of PFB-chloride to the dried extract, cap the tube, and heat for 60 min at 95 °C in a heating block. Evaporate the excess PFB-chloride with a gentle stream of nitrogen at 60 °C in a heating block. Dissolve the dried sample in 1.0 mL ethylacetate for injection into the gas chromatograph using the chromatographic conditions described in Figure 1.

The coefficient of variation for this assay on fourteen 2.0 mL aliquots of a single urine sample (mean value = 570 ng cholesterol/mL) was found to be 4.2%, which compares favorably with other less sensitive cholesterol assays reported in the literature. The utility of this method in the determination of total and non-esterified cholesterol in saliva and urine is shown in Figure 3. Figure 3A shows a urine sample with a non-esterified cholesterol concentration of 0.32 µg/mL. Hydrolysis of the same sample resulted in a total cholesterol (cholesterol plus cholesterol esters) concentration of 0.56 µg/mL (Fig. 3B), which indicates that the cholesterol concentration in the sample was 0.22 µg/mL. In a similar fashion, the non-esterified cholesterol concentration in a saliva sample was 1.32 µg/mL (Fig. 3C), while the total cholesterol concentration of the same saliva sample was determined to be 1.79 µg/mL (Fig. 3D).

In collaboration with Dr. Schwertner, we have prepared an abstract for presentation of these results at the 1989 meeting of the American Society of Biochemists and Molecular Biologists in San Francisco. We

Figure 3. Electron capture gas chromatographic analysis of cholesterol in urine and salivary fluid.

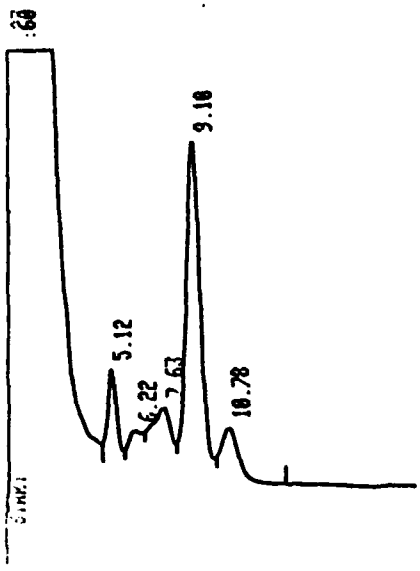



Figure 3A. Urine without hydrolysis
Cholesterol concentration = 0.32 µg/mL

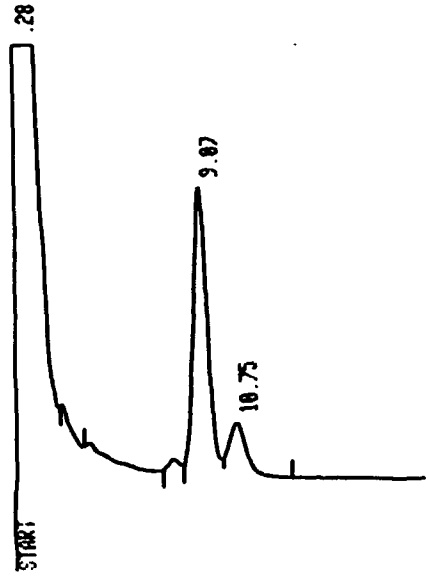



Figure 3B. Urine with hydrolysis
Cholesterol concentration = 0.56 µg/mL

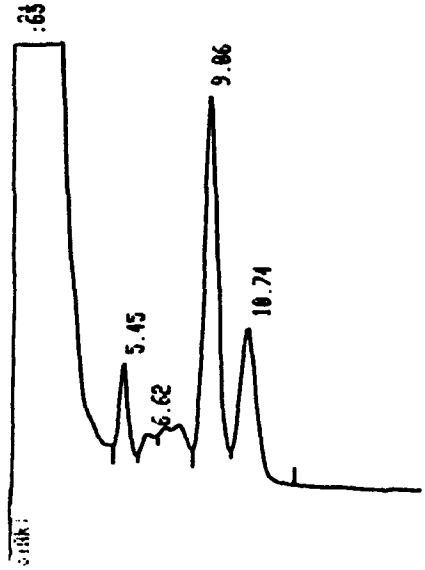



Figure 3C. Saliva without hydrolysis
Cholesterol concentration = 1.32 µg/mL

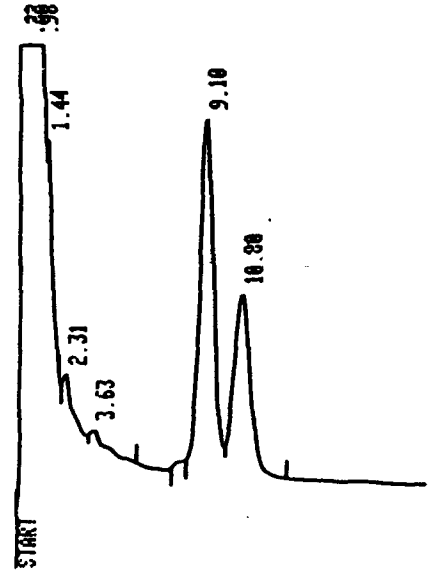



Figure 3D. Saliva with hydrolysis
Cholesterol concentration = 1.79 µg/mL

have also prepared a manuscript that will soon be submitted to an appropriate journal for publication.

II. Development of an improved HPLC system for analyzing HDL subfractions

Since the cholesterol assay development was progressing so well, little time was spent working on this objective, which was actually intended as a backup project to the cholesterol assay. However, we did optimize an HPLC system that provided good separation of low density lipoprotein (LDL), HDL, and albumin.

A. Evaluation of new molecular exclusion columns

Approach - Dr. Schwertner obtained several new molecular exclusion columns of higher resolving power than previously available which should enhance the separation of HDL subfractions. We intended to evaluate these columns for their ability to resolve $HDL_2$ and $HDL_3$ under several sets of chromatographic conditions using the same HDL sample.

Results - As a result of our focus on the cholesterol assay, we did not evaluate these columns.

B. Optimization of chromatographic parameters

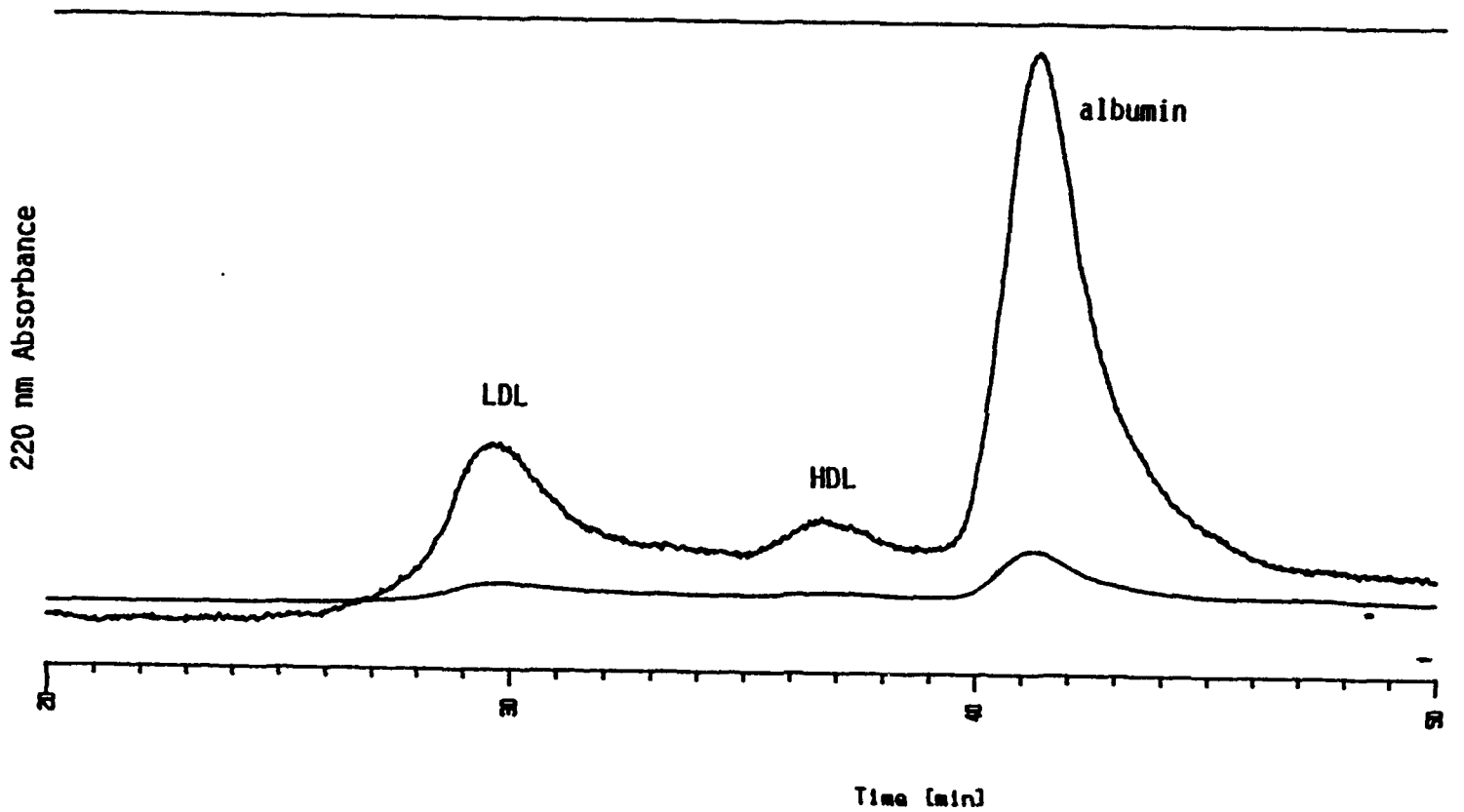
Approach - After selecting the best columns from the above evaluation, we intended to systematically vary the flow rate, ionic strength, buffer composition, pH, and temperature to optimize the separation of $HDL_2$ and $HDL_3$.

Results - To establish a foundation for accomplishing this objective, we optimized the separation of LDL, HDL, and albumin using a three-column system consisting of one TSK 5000PW (Toya Soda Manufacturing) column (7.5 mm x 60 cm) and two 3000SW columns (7.5 mm x 60 cm). The chromatogram of a mixture containing LDL and HDL, both purified from human serum by density gradient ultracentrifugation, and commercially available human serum albumin is shown in Figure 4. Optimal resolution was obtained at 22 $^{o}C$ with the three-column system using unbuffered 0.15 M NaCl at a flow rate of 0.9 mL/min. This resolution was sufficient to allow its use in the evaluation of serum fractions made by density gradient ultracentrifugation by Dr. Joe Ross, an SFRP colleague in Dr. Schwertner's lab.

III. Analysis of human samples

Approach - The serum of ten patients with proven coronary artery disease (CAD) and ten individuals without evidence of CAD were to be

Figure 4. Separation of a mixture of purified LDL, HDL, and albumin by molecular exclusion HPLC on a 3-column system (TSK 5000 PW + 3000 SW + 3000 SW) at a flow rate of 0.9 mL/min using 0.15 M NaCl.



assayed for unbound cholesterol, $HDL_2$ and $HDL_3$. The results would be statistically analyzed to detect possible correlations between the measured unbound cholesterol, $HDL_2$ and $HDL_3$ levels and the incidence of coronary artery disease.

Results - Since the membrane system for the separation of unbound cholesterol from serum remains to be developed, CAD patient samples were not utilized during the FSRP period. The applicability of the assay system to human saliva and urine was demonstrated on the samples of several volunteers from the laboratory. Examples of the results obtained from these samples are shown in Figure 3.

4. RECOMMENDATIONS

A. The success of the new sensitive cholesterol assay described in this report indicates that it may now be possible to measure the extremely small bit finite amount of cholesterol in serum that is likely to exist in a form which is not bound to proteins. The membrane technology for separating other non-protein-bound sterols such as testosterone already exists. This technology should be applied to the separation of non-protein-bound cholesterol from human serum. That fraction of cholesterol could be measured with the new assay and the potential of non-protein-bound cholesterol as a marker for CAD could be evaluated.

B. The newly developed high resolution molecular exclusion HPLC columns should be evaluated for their ability to resolve $HDL_2$ and $HDL_3$ in human serum samples. An HPLC system capable of resolving HDL subfractions would be very useful in the evaluation of $HDL_2$ and $HDL_3$ as potential markers for CAD. At present, one of the more promising methods to reliably assess $HDL_2$ and $HDL_3$ levels may be density gradient ultracentrifugation, which is time-consuming, labor-intensive, and much too costly for a routine clinical laboratory test (and really not very reliable by clinical chemistry standards). An HPLC-based system would result in much more rapid assays and could be used to monitor reliability of the double precipitation method (8) commonly used in the clinical laboratory. Promising results have been reported in the development of such an HPLC-based method (9).

C. Due to the presence of serum proteins of similar hydrodynamic volume to $HDL_2$ and $HDL_3$, the usual HPLC ultraviolet absorbance detectors

cannot be reliably used for the quantitation of lipoproteins directly from serum. These interfering proteins cause overestimates of the amounts of lipoproteins with which they co-elute. A potential solution to this problem lies in the continued development of a post-column cholesterol detector. The effect of interfering proteins that do not contain cholesterol is eliminated with the use of such a detector. The technology for such a system exists (10) and prototypes have been described (11).

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FINAL REPORT

# PLASMA CATECHOLAMINE ASSAYS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY


| | |
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| Contract No: | F49620-0013 |

# PLASMA CATECHOLAMINE ASSAYS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

by

Daisy White Kimble

## ABSTRACT

In a study of G-Loss of Consciousness (G-LOC) in pilots, there is a loss of blood flow to the brain. Catecholamines are central nervous system neurotransmitters which may be important in the maintenance of consciousness and in other neurophysiological functions such as heart rate control, etc. Additionally, these amines are released systemically and have direct influence on hemodynamics, heart function and thus G-Tolerance. In order to determine the concentration and physiological effects of catecholamines in blood plasma, an analytical assay was developed for norepinephrine (NE), epinephrine (E) and dopamine (DA). This assay consists of five steps: (1) separation of plasma from whole blood samples, (2) adsorption of the catecholamines from plasma onto alumina, (3) washing the alumina, (4) desorption of catecholamines from the alumina with acid and (5) quantitation by high performance liquid chromatography. The internal standard used was dihydroxybenzylamine (DHBA).

## ACKNOWLEDGEMENTS

I would like to thank the Air Force System Command and the Air Force Office of Scientific Research for their support and sponsorship of my summer research as a Faculty Fellow. I also feel that it would be most appropriate to thank Universal Energy Systems, Inc. for their timely manner in handling all of my administrative needs.

My experience was rewarding, exciting and enhancing. Dr. Paul M. Werchan was very supportive and aided me in all phases of my research project. I also have to acknowledge the help I received from Dr. Faust R. Parker, Jr., Mrs. Judy Barber and Ms. Star Forster and other persons from the Crew Technology Division, School of Aerospace Medicine. Ms. Dorothy Baskin and Mr. Frank Garcia were very much appreciated for their clerical support.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The main goals of my summer research was to develop an analytical assay for catecholamines from human and animal subjects exposed to high sustained $+G_z$ (head to feet) and rapid onset of $+G_z$. We hope that future research will (1) describe changes which occur before, during and following loss consciousness due to a single high G exposure and/or multiple high G exposure and (2) to develop and/or evaluate pharmacologic or physiologic interventions which could:

(a) alter brain physiology to extend the time period of G exposure before GLOC occurs, i.e., increase man's G tolerance and

(b) reduce the time period of recovery following GLOC.

Initially, to accomplish our goals, our assay method consisted of extracting plasma with boric acid gel, elution and then preconcentration on a cation-exchange column. However, during the course of our analyses, alumina was chosen as an adsorbant and reversed phased chromatography was used. The method used was adapted from the 1972 method of Anton and Sayre [1]. This method seemed to be more state-of-art.

During the course of my research and effort, we encountered many technical problems with the centrifuge and the HPLC system. Also, the funds needed for a segment of our research project was not appropriated. Therefore, the blood samples needed for the G-LOC study of catecholamines were not available. Thus, the bulk of my tenure was used to do an HPLC analysis of nucleotides found in the blood from another aspect of the G-LOC study.

## III. DISCUSSION:

Great interest is being shown at present by physiologists and pharmacologists in substances which are released locally and have local effects, such as vasodilation and vasconstriction in the surrounding tissue [2].

Catecholamines are synthesized from 1-tyrosine via a sequence of enzymatic steps in sympathetic nerves, the adrenal medulla and in chromaffin cells. The observation of chronic changes in plasma catecholamine levels has been shown to have utility in demonstrating the presence of various pathological states [3,4]. Generally, two areas of pathology may be defined by abnormal catecholamine levels. The first area involves tumors of the adrenal medulla (pheochromacytoma, neuroblastoma) which synthesize large quantities of catecholamines and give rise to elevated

circulating levels. The second involves the cardiovascular system. Elevated levels are indicative of hypertension and myocardial infarctions, whereas low levels are most notably indicative in hypotension.

The utility of plasma catecholamine determinations has been studied extensively over the past several years. High performance liquid chromatography with electrochemical detection (HPLC-EC) has become the method of choice for the analysis of catecholamines in biological fluids. For biological samples, such as plasma, a purification step is necessary to remove potential interfering compounds and increase the specificity. This purification step has been performed mainly on activated alumina [5]. However, this process introduces variability [6] and is relatively time-consuming.

Another widely used method of analysis for monoamines are related substances are variations of a fluorometric approach. The procedures involve the physical separation of each substance and conversion to the corresponding fluorescent derivative [3]. The procedures are, unfortunately, insufficiently sensitive for the concurrent determination of monamine transmitters and metabolites in samples.

In a method described by [7], gas chromatographic techniques were used to separate biologic amines, including catecholamines. This method requires the preparation of suitable derivatives. One major problem in the development of a gas-liquid chromatography (GLC) procedure for polyfunctional compounds such as epinephrine (E) and norepinephrine (NE) is that multiple products may be formed when converting to derivatives.

Ion-exchange [8] and reversed-phase [9] high performance liquid chromatographic (HPLC) techniques have been used successfully to resolve the catecholamines and their related metabolites in extracts of tissues and in body fluids. High performance liquid chromatography associated with electrochemical detection has allowed greatly increased sensitivity of noradrenaline and serotonin analysis [10, 11, 12, and 13]. Also, using reversed phase columns with ion pairing has given good separations at the chromatographic level [14]. We have modified the methods [15, 16, and 1] in our methodology. Although we met with technical problems, the clear separation and sharpness of peaks from our standard calibration show that the electrochemical detector with a reversed phase column gives a significant amount of selectivity.

## IV. PREPARATION OF REAGENTS AND INTERNAL STANDARD:

Norepinephrine (NE), epinephrine (E), dopamine (DA), and dihydroxybenzylamine (DHBA), the primary internal standard; were supplied by Sigma Chemical Co., St. Louis, Mo. The stock solutions were prepared in 100 ml of 80% methanol and 20% 0.1 N perchloric acid at concentrations of 1,000 ng/ml for NE, E, and DA and 500 ng/ml for DHBA. These solutions were stored at -0°C and freshly prepared each week. The working solutions were made on the day of analysis by serial dilutions in 0.1 N perchloric acid.

PREPARATION OF ALUMINA: Aluminum oxide (Neutral Activity Grade 1) was prepared under the hood. 10 grams of aluminum oxide were added to 100 ml 1.0 M sulfuric acid with stirring. The solution was allowed to boil for 20 min. Allowed the alumina to settle for 2 min. The supernatant was decanted and the alumina was added to 100 ml of distilled water and stirred with a magnetic stirrer for 5 min. The supernatant was once again decanted and the wash-stir cycle was repeated four to five times. The pH should be between 2.5 and 3.0. The supernatant was decanted and the alumina was allowed to dry overnight at 65.

HPLC INSTRUMENTATION: The catecholamine assay utilized a Waters Associates Model 711 System Controller, 510 Pump,

730 Data Module, 710 B Sample Processor, and ESA Model 5700 equipped with a 3 micron column and an ESA Model 5100 A Coulochem Electrochemical Detector with 5021 and 5011 cells in place. Integration was done by an SP 4270 integrator operated in the peak height mode.

CHROMATOGRAPHY: The optimal flow-rate was found to be 1 ml/min. The mobile phase consisted of 212 mg heptanesulfonic acid, 80 mg EDTA, 6.9 gm sodium phosphate and 3% filtered methanol (pJ 2.6). A 100 microliter volume of the eluate was injected onto a Regis Little Champ column (5 cm x 4.6 mm I. D.). The parameters for the control module were set at: conditioning cell +0.20 V; electrode 1: +0.10; electrode 2: +0.25; and gain 10 x 1 on electrode 1, 60 x 100 on electrode 2: and responses for both electrodes were 4 seconds. The pressure was approximately 2800 psi.

SAMPLE COLLECTION AND STORAGE: Blood samples were collected from subjects directly into ethylenediamine tetracetic acid (EDTA) treated silicone coated vacutainer tubes and immediately centrifuged. The plasma was separated by use of a CMS serum filter. The plasma samples were frozen until needed.

EXTRACTION OF CATECHOLAMINES: In this assay 1-2 ml of plasma and 50 microliters of internal standard (DHBA) were placed in a polyethylene centrifuge tube. One ml of 0.1 N

perchloric acid was added, stoppered and vigorously shaken for 5 minutes and then spun at 4,500 x g at 25°C for 20 minutes. The clear supernate was adjusted to 3 ml with 0.1 N perchloric acid and 50 microliters of sodium metabisulfite was added. The solution was centrifuged at 3,000 x g for approximately 10 min. The supernate was transferred to 50 ml beaker containing 25 mg of acid washed alumina and 10 mg EDTA. Under constant and rapid stirring, the mixture was brought to pH 8.6 with 1.5 M Tris-hydrochloride buffer. The alumina was allowed to settle. The alumina and supernatant were transferred into an ESA 2-ml extraction column (Bedford, MA). The supernate was washed four times with distilled water. The catecholamines were eluted with 2 microliters of 0.1 N perchloric acid. The tubes were shaken vigorously and vortexed for 10 min at a shaker speed of 4. The catecholamines were eluted with a vac-elute (Analytichem International) placed in a sample vial and 100 microliters was injected onto the HPLC.

RESULTS (ANALYTICAL VARIABLES):

In order to establish the optimum mobile phase conditions required for the baseline resolution of the catecholamine, we used an acetate-citrate buffer. Needless to say, we did not have any success with monitoring a baseline. The next attempt to effect a separation of NE, E, DA and DHBA were successful employing the stock solutions of heptane-sulfonic

acid and monobasic sodium phosphate buffer containing 0.274 mM EDTA served as the basis for the mobile phase solutions. The pH was varied between 2.6-3.6 using concentrated phosphoric acid. It was noted that the amine retention times decreased with the decrease in pH. Figure [1] shows the chromatogram of the standards NE, E, DHBA internal standard, and DA at retention times of 2.90, 4.42, 5.85 and 9.90 respectively. After an adjustment of the pH to the mobile phase to approximately 3.6, the retention times for the standards were 3.69, 6.00, 8.29 and 14.90.

V. CHROMATOGRAPHIC CONDITIONS:

The parameters for the chromatographic instrumentation had unpredictable consequences. The potentials had to be optimized by experimentation. We were fortunate to find a starting point, even with a detector which had not been utilized in over a year.

By optimizing the components of the mobile phase and instrument conditions, the separation shown in Fig. [1] was obtained. As stated earlier in my paper, because of technical problems with the HPLC pump, the electrochemical detector and the centrifuge, there were no samples available for our study.

We were able to obtain a few blood plasma samples from human subjects and a baboon. Fig. [2] shows a typical chromatogram of a plasma sample from a human subject. The retention time of 2.57 was reported for an unknown peak. However, this may be a peak shift of the NE in a plasma sample. Time did not permit for an analysis of peak shifting. Fig. [3] is also a typical chromatogram of another plasma sample from a human subject, showing the same isolated peak at a retention of 2.64. Fig. [4] shows a typical chromatograph of a baboon plasma sample. There was no detection of any catecholamines.

An ESA extraction kit was initially used to isolate the catecholamines. This column was prepared with acid washed alumina in a buffered solution. Several samples were worked up with the column. Some recovery was made with the internal standard and possibly an isolation of NE. It was later discovered that the extraction kit materials had expired.

## VI. RECOMMENDATIONS:

1. After collecting blood from subjects, it should be centrifuged immediately and placed in the presence of sodium metabisulfite for preservation and longer stability of the catecholamines.

2. The mobile phase should be allowed to degas for a period of 24-36 hours. The pH should be checked occassionally throughout the analysis. This will eliminate the increase or decrease in retention times of the catecholamines and make for better quantitation.
3. The use of extraction columns gave an isolation of the internal standard in the plasma sample, whereas without the use of the extraction columns, the internal standard was not recovered.
4. The HPLC should be calibrated for each new batch of mobile phase used.
5. The catecholamines should be eluted with at least 2-ml of perchloric acid and evaporated to dryness and brought up to 1-ml.
6. Never use a flow rate over 1 ml/min., because this will cause a pressure shut down on the system.
7. Back-up pressure is frequent on the HPLC system, therefore, make sure that the pump is primed for each new batch of mobile phase.
8. The electrode potentials should be checked for each new batch of mobile phase and also for column changing.
9. Sample preparation should be done with an ice bath. Losses of catecholamines will be lessened.

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## Chromatographs for Standards And Plasma

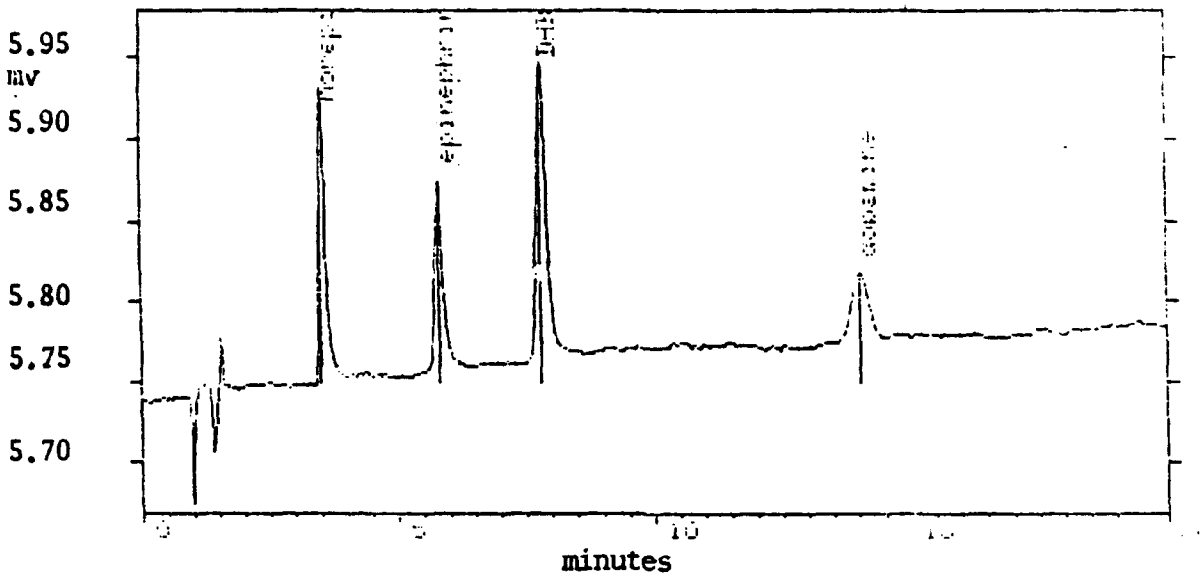



**Fig. 1**
**Catecholamine Standards**

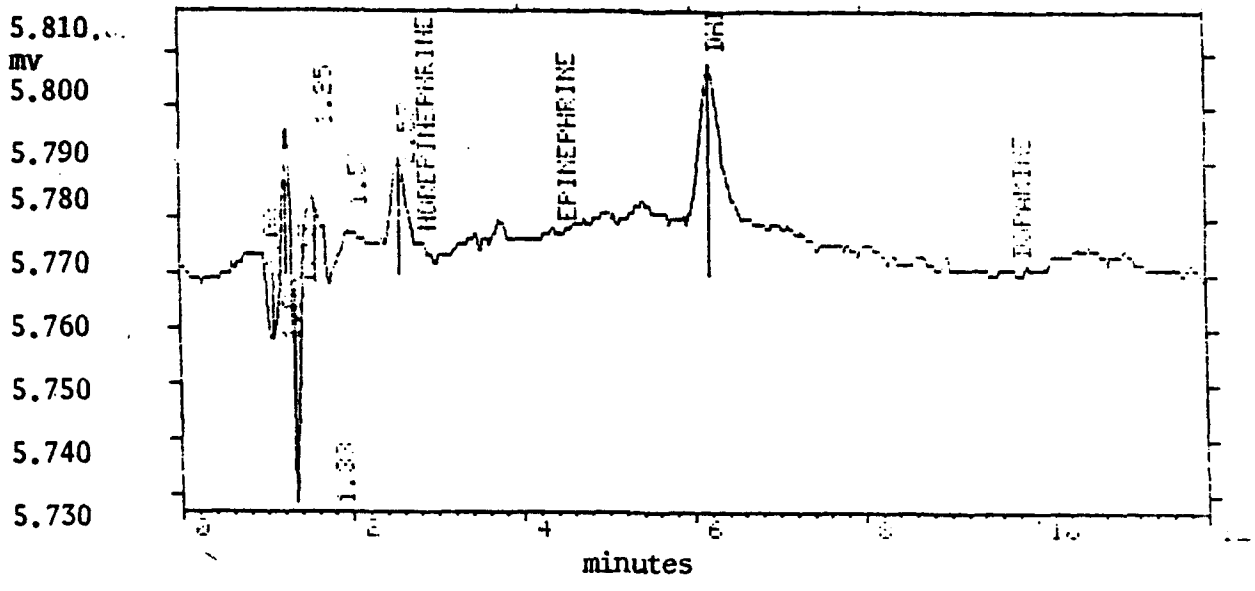



**Fig. 2**
**Human Plasma**

Typical Chromatographs of Plasma Sample

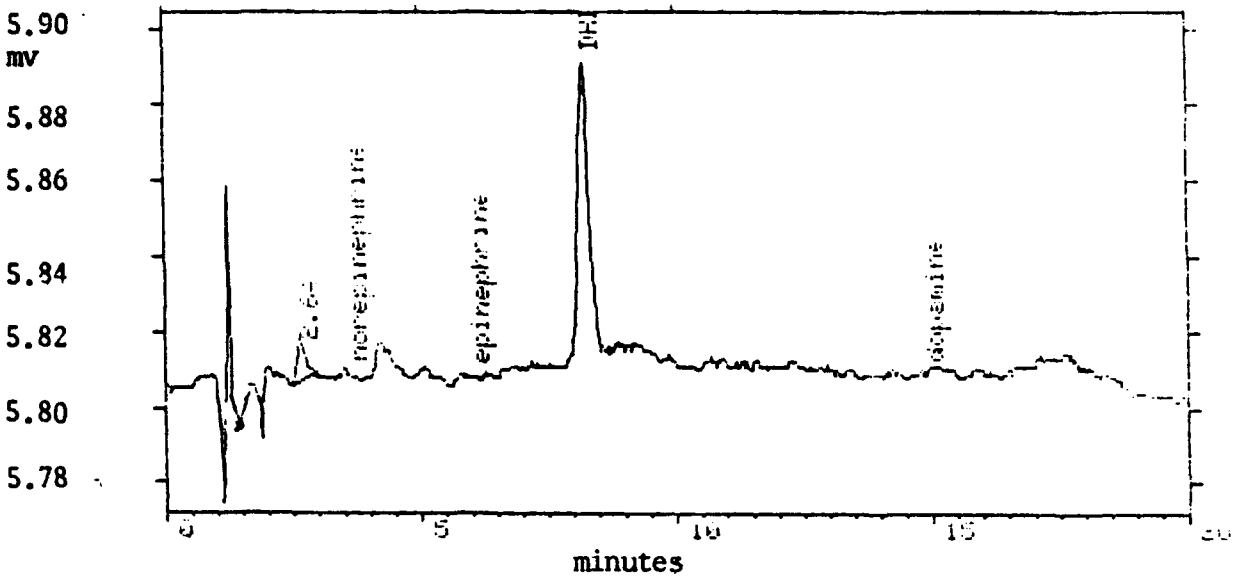



**Fig. 3**
**Human Plasma**

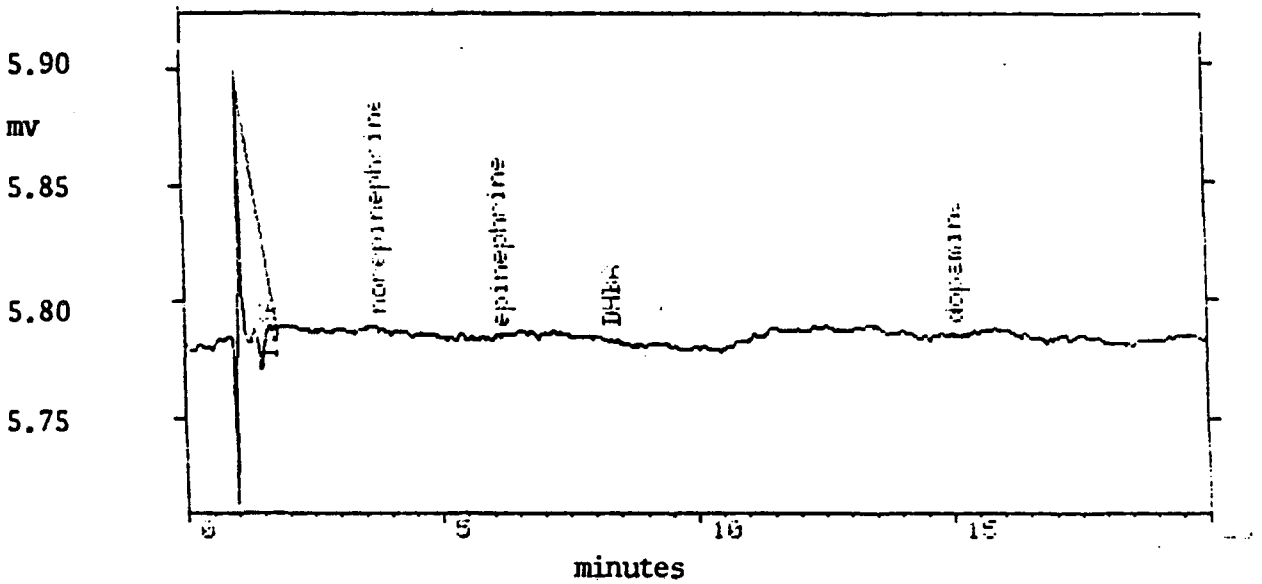



Fig. 4
Boon Plasma
140-17

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FINAL REPORT


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# Application of Nonlinear Filters to VEP Data

by

Harold Longbotham, Ph.D.
Jim Roberts, Graduate Student

## Abstract

To date, data reduction of steady state VEP data has relied on properties of linear filters. While linear filters are useful in the frequency domain, the technique of noise reduction in analysis of VEP data relies on estimating an unknown constant signal imbedded in an unknown noise. The averager is used currently to estimate this constant signal.

It has been shown that if one assumes the noise is zero mean, white, and considers the nonlinear class of OS (order statistic) filters, the averager is not the optimal filter unless the noise is normally distributed. In this work we demonstrate that for the VEP data considered and the six OS filters used, there is one OS filter that is consistently better than the averager, one that is equivalent, and the averager is better than the other four. This indicates not only that new filtering techniques should be used, but also gives an indication as to the noise distribution.

## ACKNOWLEDGEMENTS

Gratitude must be expressed to the Air Force Systems Command and Air Force Office of Scientific Research for sponsorship of this research. Universal Energy Systems is to be commended for the excellent administrative aspects of the program.

I wish to personally thank Captain Norman Barsalou and Dr. Randy Glickman for support above and beyond what would be normally expected in the initiation of this research effort.

# APPLICATION OF NONLINEAR FILTERS TO VEP DATA

HAROLD LONGBOTHAM

## I. INTRODUCTION

### Historical Perspective

D. Regan [1] has defined a steady state evoked potential (SSEP) to be "scalp potentials that are locked in time (or in phase) to the sensory stimulus which evokes them". We also know the system is nonlinear due to the following observation by J.E. Desmedt [2], "The fact that the VEP (visual evoked potential) to sinusoidally modulated light contains higher harmonics indicates directly that the human VEP system is nonlinear". The above statements lead us to consider steady state VEP data collection as consisting of inputting a periodic signal into a noisy nonlinear system whose parameters are unknown, but with a response one could assume in the noiseless case to be of the same period as the stimulus. This yields the classical approach (outlined below) to raw data noise elimination that has been used to date, and the modifications we consider in this paper.

The signal to noise ratio in VEP data varies from fair (in anesthetized monkeys with implanted probes) to poor (in alert humans with scalp electrodes) necessitating filtering of the data to improve the signal to noise ratio (a graph of raw SSVEP data from an anesthetized monkey is in Appendix 1). The previous definition of SSEP

allows the current processing of raw data to be done by two methods which we will now describe, for the improvement of these two methods is the focus of our application.

Let us assume the stimulus and response are sampled simultaneously, at N sample points in each period of the stimulus, yielding a sequence of points $\{x_i\}$, $1 \leq i \leq L$, where the number of complete periods of sample data is the largest integer in L/N. We will assume the jitter is constant, i.e. the stimulus is sampled at exactly the same points in each period. The first method is to reduce all information to the time frame encompassed by one period of the stimulus indicated by the N different averages of the data sets $\{x_{J + jN}\}$, where J is a fixed integer such that $1 \leq J \leq N$ and j is a nonnegative integer such that $J + jN \leq L$. We note the result is a set of N means of responses, where each mean is assumed to be the mean of a constant value perturbed by noise. This technique is justified by noting the mean is the MLE (maximum likelihood estimate) of an i.i.d. (independent and identically distributed) sample from a Normal distribution[3]. The second method is to use an FIR filter of length M that windows each of M consecutive values in the data set $\{x_i\}$ and replaces the central value with the average of the M values in the window. This method is justified by noting that the FIR averager filter is the BLUE among all linear filters for a constant signal that is corrupted by additive Gaussian noise[4]. We note the first method makes the assumption of the noise being independent from point to point and identically (normally) distributed at each point and the

second method is only optimal over linear combinations of the sample values. Since we are assuming the system is nonlinear, it seems reasonable to believe a new nonlinear approach may improve on the linear (averager) technique presently used in each of the above methods.

## The Median Filter

The median of a data set with an odd number of values is the data value in the set for which at least half of the values in the set are greater than or equal to it and at least half of the values in the data set are less than or equal to it. The median filter of length 2N+1 is defined as the filter which considers each consecutive set of 2N+1 values and replaces the central value with the median of the 2N+1 values. For an even number of values there are other definitions, however from now on we will restrict ourselves to data sets/windows with an odd number of values since the theoretical results on which we will base our discussion are only derived for odd length filters [6, 8].

## Properties of the Median Filter

Tukey [5] was the first to suggest the use of the median in the place of the averager because of its ability to totally eliminate impulses and maintain monotone regions. The reason for the recent surge of interest in the median filter lies not only these facts, but also from recent theoretical work that has accurately described the

pass band of the median. Gallagher and Wise [6] have shown that a median filter of length 2N+1 will pass a finite length signal unaltered (such a signal is called a root) if and only if each set of N+2 consecutive values is monotone. This assumes the signal is initially padded with N values equal to the first value. They have also shown that any signal is reduced to a root by a finite number of passes (depending on the signal length) of the median filter. Lately the median filter has proven successful in a variety of applications involving impulsive noise (including estimation of sonar signals [10] and real-time TV signal processing with impulsive noise [11]).

The median filter is applicable in our situation since a constant signal is definitely monotone and filter length may be adjusted to compensate for the varying lengths of artifacts. As an example assume the stimulus is at 10 cycles per second, and the response is of the same period. Assume our subjects are alert humans with muscle artifacts on the order of one second. This would produce a burst of impulsive noise our "assumed" constant samples of duration 10. Therefore we would require a median filter of length 21 to completely remove the artifact.

The OS (Order Statistic) Filter

An OS filter [7] is very similar to an FIR filter except that when a set of consecutive values is windowed from the data sequence, the values in the window are ordered before weighting. The median is a simple example of an OS filter where there is an odd number of weights

and the weights are all zero except for the central weight, which is one. It is also noted that the averager is both an OS filter and an FIR filter, since if all data values are equally weighted ordering has no effect on the outcome of the filtering.

### Applications of OS Filters

Optimal OS filters (using the MSE, mean square error, criterion) have been found for the estimation of a constant signal with additive i.i.d. noise, where the noise is from a variety of distributions [7]. It has been shown that in order for the OS filter to provide an unbiased estimate (of a constant signal with additive noise) the coefficients must sum to one, and if the noise distribution is symmetric, so will be the weights. One of the more popular of the OS filters is the $\alpha$-trimmed inner mean, where the coefficients are symmetric, zeros on the outside and M values of 1/M in the center. This allows one to use editing of outliers (due to outer zeros) and smoothing (due to the averaging of the inner ordered values) simultaneously.

## II. OBJECTIVES OF THE PRESENT RESEARCH

### Elimination and Identification of Adaptive Noise

To determine the form of an additive noise component we will determine the order statistic (OS) filter that yields the most periodic response. It has been shown that if the additive noise

component is Gaussian, the optimal OS filter for the additive noise component will turn out to be the averager already in use [7], but if the signal is more heavy tailed (or shallow tailed) than the Gaussian, other OS filters are more useful. One might note the determination of the optimal MSE coefficient set is equivalent to determination of the additive noise distribution. Therefore we will be able to make an informative guess as to the noise distribution.

## III. RESULTS

### Measure to be Used in the Comparison of Various Filters

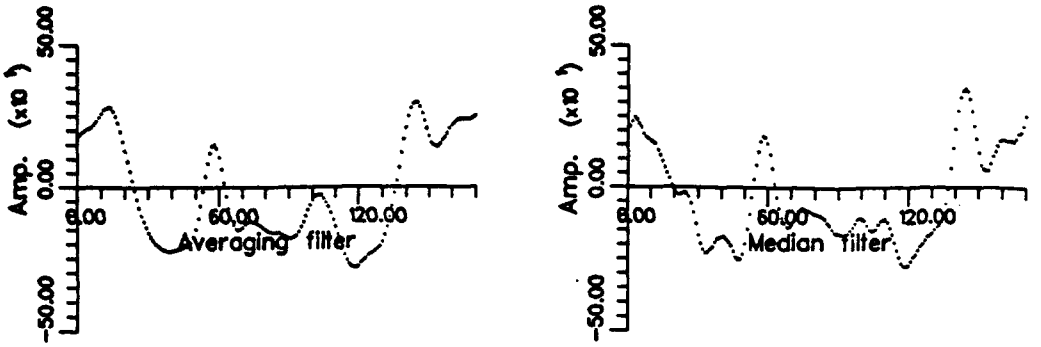
The basic assumption in the analysis of SSVEP data is that the response is periodic, in the same period as the stimulus. This assumption, in the absence of noise and jitter will result in constant sequences. A common measure of the how much sequence deviates from an assumed signal is the MSE or mean square error. Therefore if the assumed signal is constant and estimated by the average of the sequence values, we need only calculate the sum of squares of the deviations of the sequence values from the average. We note this is just the variance of the signal. If the raw data has N points in each period, there will be N of these variances. In the results reported later the measure of periodicity is the average of the N square roots of the variances (or the standard deviations).

## Software Package and User's Manual

A software package has been developed that will enable the investigator to use any OS filter (i.e. one may specify the filter length and coefficient set) to filter SSVEP data. A laser print out is then generated by the graphics package that will graph the raw data, the reconstructed data (the data with assumed constant sequences filtered with the entered OS filter and then reconstructed), and a graph of the filtered data (the reconstructed data passed through an user selected OS filter). Care must be taken that the coefficients sum to one, so as not to bias the measure of repeatability. One must also be sure the data segment to be evaluated is padded with enough data points at the front and end for there to be an output for the first and last values in the segment. This is described in more detail in the user's manual.

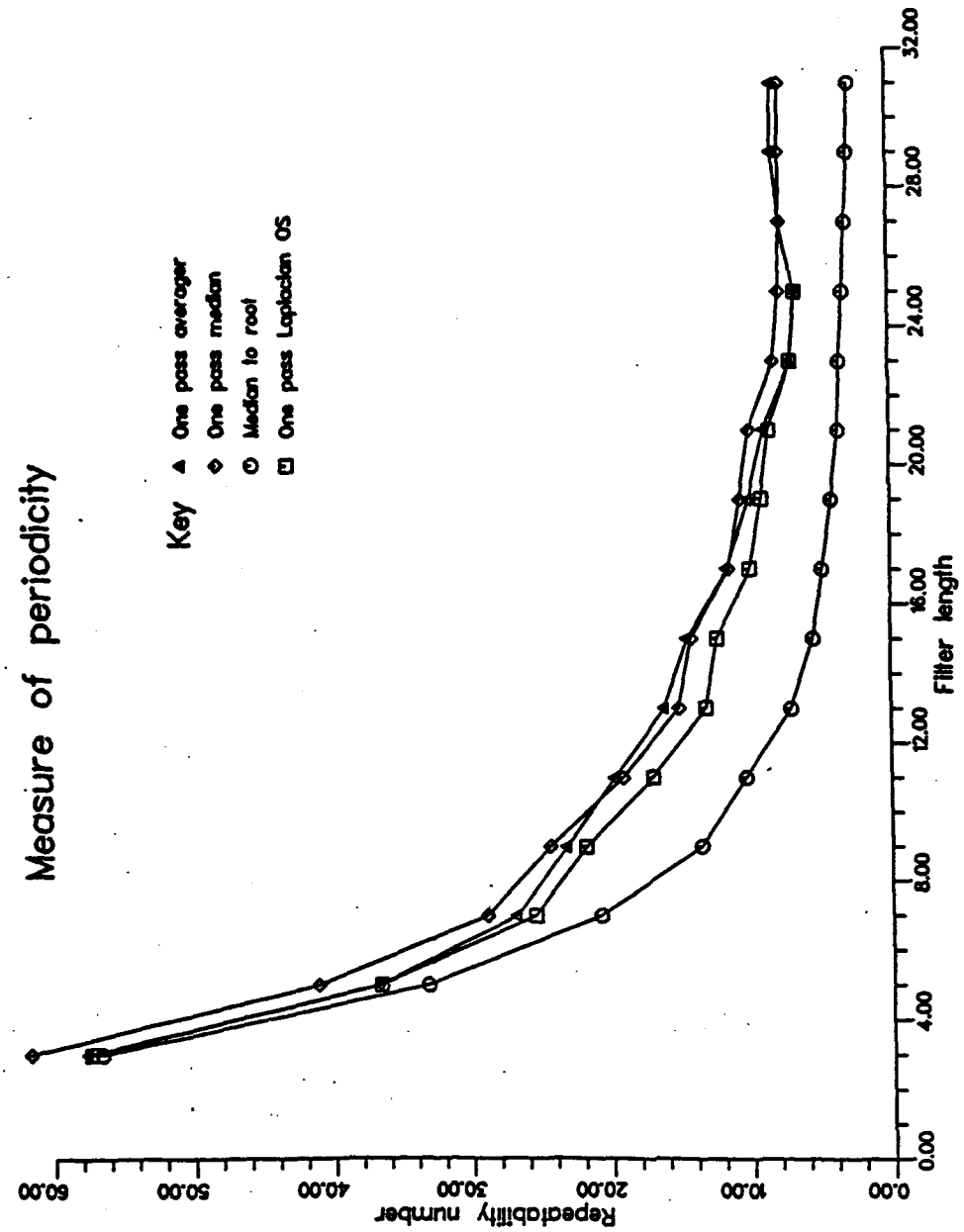
## First Method of Data Reduction

In the following figure we show the reduction of a data segment of eleven periods to one period by using the average and median of the corresponding constant signal. The result of each was then smoothed by a moving average filter of length five. It is to be noted that the method of data reduction is the same as that described above (and used in method II below) with the window width so large that only one output is generated from the data sample.




**Second Method of Data Reduction**

Using the previously described data package runs were made for the averager, median, and "Laplacian" coefficients of odd filter lengths from 3 to 31. The Laplacian coefficients are defined as the OS filter coefficients that are optimal for detecting a constant signal in the presents of additive Laplacian noise. It is obvious there is not enough room for inclusion of all the resulting graphs (they are available upon request from the authors). The figure on the following page summarizes the results, and a table of the values plotted is given in the appendix. The averager is slightly better than the median in most cases with the "Laplacian" being the best in all cases. If one recalls the averager is optimal for Gaussian noise (an exponential with the variable squared), the median is asymptotically optimal for heavy tailed noise, and the "Laplacian" is optimal for Laplacian noise (an exponential with the absolute value of the variable raised to the first power, it is obvious the additive noise component should be modeled as an exponential distribution with exponent positive and less than 2. One shortcoming of the above is that it considers only


Measure of periodicity
Key
One pass averager
One pass median
Median to rool
One pass Laplacian 05
Repeatability number
0.00
10.00
20.00
30.00
40.00
50.00
60.00
Filter length
0.00
4.00
8.00
12.00
16.00
20.00
24.00
28.00
32.00

symmetric exponential distributions. Runs have also been made with OS coefficients that are optimal for triangular, uniform, parabolic, and U-shaped distributions with filter lengths of 5,7,9 and 11. Results in each case were much worse than the averager or median and are tabulated in the appendix. Therefore exponential distributions (distributions with a tail of positive area) seem the most promising. The case of nonsymmetric distributions has yet to be studied and will be discussed as a future area of research.

## IV. FUTURE STEPS TO BE TAKEN

### Variability of Noise From Level to Level

It is to be noted that in our measure of repeatability, it is assumed that the noise distribution is the same for each of the N assumed constant sequences. This assumption must be justified.

### Nonsymmetric Filters

We have only considered symmetric filters. Assume one wishes to find the optimal OS filter of length five for a given data segment. In general this might prove impossible but it would be possible to write a program that approximated it. One would first find the best rank order filter, then find the best OS filter with only 2 coefficients, etc. We note one would have to restrict the study to increments of say one tenth in the process, because of the length of time involved in each iteration. If one is then interested in inferring the

corresponding distribution there may be problems. There is no general theory to the author's knowledge that allows one to deduce the noise distribution from a given set of optimal filter coefficients.

**Impulsive Noise**

Once the additive noise component has been identified it will be possible to estimate the impulsive noise component using the median filter. Then one can examine whether it is best to follow the optimal OS filter for the additive noise component with a median filter or to simultaneously filter the additive component and the impulsive component by appending zero's on either end of the optimal OS filter.

**Extend Results to Other Data Sets**

It is to be noted that only one segment of one channel of one SSVEP data collection has been examined. What is optimal for this data set may not be optimal for another. Therefore we must look at other extended data sets.

**Adaptive Filters**

Once the optimal filter coefficients (there will be more than one depending on the noise distribution) have been determined one may design an adaptive filter that will select the appropriate filter coefficients for a given data segment, that minimizes the measure of repeatability over that data segment.

### Comparison to Statistical Methods

Our main objective is the determination of the optimal set of filter coefficients (with optimality with respect to the repeatability measure). As an aside we point out that one may infer what the noise distribution might be. We may check ourselves at this time by a determination of the additive noise component by statistical means.

## V. SIMILAR STUDIES USING OS FILTERS

### Periodicity of the Data and Alternate Filters

What should the period of the response be? Are we forcing periodicity upon the data? Is there an alternate method to that of Method 1 or 2, would a 2-dimensional filter be better?

### Non-Steady State Raw Data

For data in which the subject is flashed the periodicity of the stimulus is disturbed, but one may proceed as above with the period of the stimulus taken to be the period of the flash. For this experiment it is noted the original stimulus will need to be synchronized with the flash.

### Processing of the Filtered Data

OS filters may be written for peak detection, phase detection, and latency detection. Detection of other points or regions of interest is also possible using OS filters.

**Detection of Nonlinearities**

Assume that an increase in the input stimulus causes a corresponding increase (or decrease) in response of the system, and that following an abrupt change in the stimulus (i.e. it changes instantaneously from increasing to decreasing), the system has a nonlinearity that causes a delay before it can respond to the stimulus change. It might be possible to detect this type of nonlinearity by use of higher sample rates and median filtering.

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**APPENDIX**

**Example of graphics printed out by the developed software**

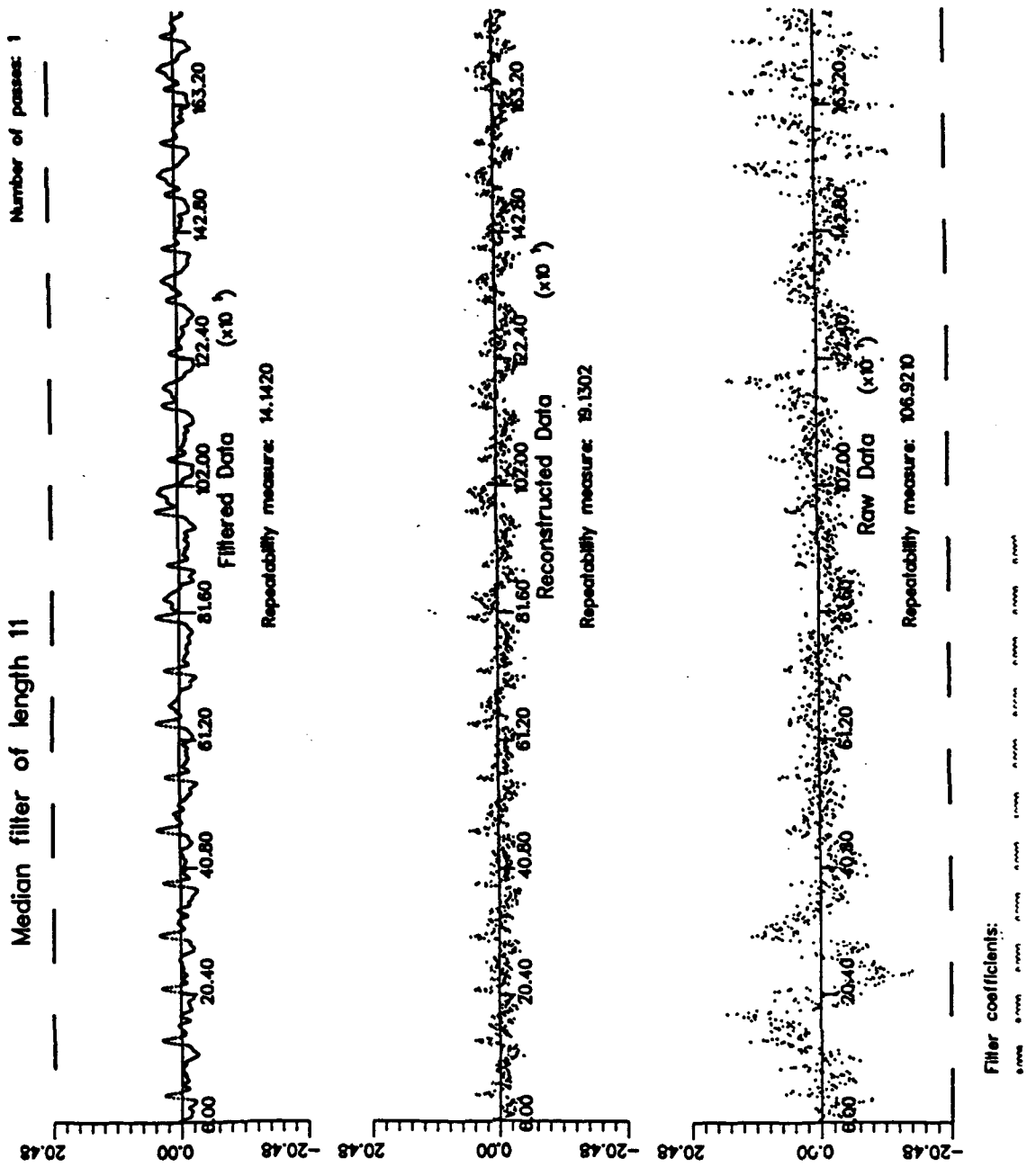


**Table of Plotted Values in Figure 2**

| Filter Length | Averager 1-pass | Median 1-pass | Median to root | "Laplacian" 1-pass |
|---|---|---|---|---|
| 3 | 57.63 | 61.71 | 56.61 | 57.38 |
| 5 | 36.78 | 41.16 | 33.23 | 36.66 |
| 7 | 26.74 | 28.87 | 20.74 | 25.41 |
| 9 | 23.17 | 24.37 | 13.61 | 21.78 |
| 11 | 19.71 | 19.13 | 10.37 | 17.00 |
| 13 | 16.12 | 15.11 | 7.14 | 13.19 |
| 15 | 14.48 | 14.20 | 5.52 | 12.36 |
| 17 | 11.62 | 11.51 | 4.84 | 9.97 |
| 19 | 10.03 | 10.66 | 4.18 | 9.12 |
| 21 | 8.92 | 10.01 | 3.63 | 8.56 |
| 23 | 7.01 | 8.19 | 3.47 | 6.94 |
| 25 | 6.65 | 7.77 | 3.21 | 6.62 |
| 27 | 7.66 | 7.63 | 2.96 | —— |
| 29 | 8.22 | 7.68 | 2.77 | —— |
| 31 | 8.14 | 7.62 | 2.62 | —— |

## Table of Non-exponential Noise Distributions

## and

## Corresponding Measures of Repeatability

Repeatability results for different noise distributions

| Filter Length | "Uniform" | "Parabolic" | "Triangular" | "U-shaped" |
|---|---|---|---|---|
| 5 | 48.76 | 42.98 | 39.64 | 52.13 |
| 7 | 44.74 | 36.18 | 31.44 | 48.67 |
| 9 | 43.41 | 33.76 | 28.99 | 46.94 |
| 11 | 41.32 | 31.01 | 26.19 | 44.07 |

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FINAL REPORT

# Extensions of Several Difference Score Approaches for the Analysis of Time Ordered Repeated Measures


| | |
|---|---|
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| Date: | 12 Aug 88 |
| Contract No. | F49620-87-R-0004 |

# Extensions of Several Difference Score Approaches for the Analysis of Time Ordered Repeated Measures

by

David A. Ludwig

## ABSTRACT

Extensions of several difference score approaches for the analysis of repeated measures experiments involving more than two time periods are presented. They are easily understood and interpreted by research workers while being mathematically equivalent, and in some cases, an improvement over more complex forms of the split-plot analysis of variance and covariance.

## 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.

I also wish to thank all of the researchers in acceleration effects especially Larry Krock and Russell Burton.

## I. INTRODUCTION:

A commentary appearing in the American Statistician by Laird (1983), on an alternative approach to the analysis of pretest-posttest designs, has proven to be not only elegant, but easily understandable and interpretable by researchers with limited statistical knowledge. The approach uses the difference score calculated between the two time periods in which the experimental unit was repetitively measured. The difference score is used as the dependent variable with the pretest measure acting as a covariate allowing partial correction for regression toward the mean prior to the evaluation of treatment group or classification effects. Since a difference score is used as the dependent measure, adjusted group means can be tested that each is equal to zero by placing the estimates over their respective standard errors. The former test addresses the question of interaction, while the latter can be used to evaluate within group changes.

Traditionally, a split-plot format would be used to analyze such an experiment (Brogan and Kutner, 1980). Although both the split-plot approach and the covariance difference score approach are technically correct, the split-plot approach fails to correct for regression toward the mean and it also requires the interpretation of a two-factor interaction (Huck and McLean 1975). The two-factor interaction can be eliminated by differencing the crossed factor and using it as the dependent variable without correction for the pretest. This basic difference score approach reduces the complexity of the statistical model while retaining information as to within group changes. However, the problem of regression toward the mean still remains. The covariance difference score approach is also superior to the covariance model in which the posttest is used as the dependent variable. This approach, which corrects for regression toward the mean, looses information

about the degree of change within each group and is only concerned with posttest results (Bock 1975). It is more of a classical analysis of covariance approach than a repeated measures analysis.

II. METHODS AND PROCEDURES:

Since the basic and covariance difference score approaches are so intuitively appealing and easily explained to research workers, an extension of these techniques to situations in which the experimental units are measured across more than two time periods would be useful. Repetitive differencing would not provide an extension to these methods, since the crossed effect would only be reduced by one and the split-plot format would still need to be utilized. The problems associated with the assumption of a constant variance-covariance matrix would also need to be addressed. This in turn may lead to a multivariate approach which is difficult to interpret for most researchers and in many cases not even defined. Brogan and Kutner (1980) state that there is no logical extension of the basic difference score approach when there are more than two levels of the repeated measures. Fortunately, this is not exactly true. When the repeated measures are time ordered, a linear function could be fit across the time periods separately for each subject, and the resulting slope estimates used as the measures of gain. Since the slope estimate is not affected by the variance-covariance structure of the repeated measures, the assumption of a constant covariance matrix is not an issue. All that is required, along with the usual parametric assumptions of the analysis of variance, is that each experimental unit be observed at the same points in time. In the event a linear function is not a true representation of the gain, variable reexpression or polynomial regression estimates can be used. The same ends are pursued when polynomial decomposition is used in split-plot repeated measures analysis (Winer 1971).

In fact, a one-way analysis of variance on the slopes using treatment group as the independent variable is mathematically equivalent to partitioning a linear polynomial on the group by time effect in the split-plot analysis and testing this with the linear part of the within subjects error term. This is a natural approach when time is a factor in the design, since some form of a linear or higher order polynomial function would be expected if true variation across time is being observed (Dawkins 1983). As with the two period difference model, in the event of no changes overtime, mean slope estimates within a group would be zero and follow-up statistical tests would be meaningful.

In most time ordered repeated measures experiments, an initial baseline measure is taken prior to the administration of the treatment. This baseline measure can be used as a covariate to correct for regression toward the mean. Although this type of covariate (constant across all levels of the repeated measures) can be included in the traditional split-plot analysis, the sub-plot (within factors) sources of variation remain unchanged. In the case of regression toward the mean, meaningful covariate adjustment needs to be made on the repeated measures part of the analysis. This can be accomplished by placing the baseline measure into the analysis of variance which used the individual slope estimates as the dependent variable. As with the covariance difference score approach, adjusted means can be tested against each other and against zero. Thus, all of the advantages of the covariance pretest-posttest difference score approach are preserved for the situation when the number of repeated measures exceeds two.

## III. SUMMARY AND RECOMMENDATIONS:

More often than not, the worth and utility of a statistical procedure are evaluated on a purely mathematical bases with little or no concern given to issues such as interpretability, intuitive appeal, or flexibility. It is

felt that the statistical methods advocated here are easily understood. Provide an improvement over more traditional approaches and by the majority of research workers. Much more so than split-plot approaches involving multiple error terms and interactions or multivariate approaches which are usually not fully understood by the typical researcher.

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FINAL REPORT


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# Spin Label Studies of Oxygen in Biological Systems

by

William Z. Plachy, Ph.D.

## ABSTRACT

Experimental studies were initiated to examine the effect of high oxygen tensions on the membrane of the red blood cell, RBC. Electron Spin Resonance, ESR, spectroscopy was employed using custom designed spin probes. The product of the oxygen concentration in the membrane times the oxygen diffusion rate in the membrane was shown to be significantly less than that same prodect in a simple phospholipid bilayer model membrane. The experimental evidence suggests that high oxygen tensions employed (2.6 atm) do not cause a significant change in the membrane viscosity after 16 hour exposure times. However, evidence was also obtained that suggests that the spin probes themselves may protect the RBC membrane from oxy free radical damage by acting as radical scavengers.

Experiments were designed to test the idea that significant quantities of hyperbaric oxygen could be delivered to the body via a transcutaneous pathway. This pathway would likely require that the natural barrier to transcutaneous oxygen absorption be disrupted temporarily by the use of a permeation enhancer. It is suggested that perfluorinated chemicals may serve as good permeation enhancers for oxygen. The proposed experiments use spin labels in excised porcine skin samples. It is suggested that this transcutaneous mode may prove to be a viable alternative to the conventional mode of delivery of hyperbaric oxygen to some patients.

## Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this project.

I thank the staff of the Hyperbaric Medicine division at the School of Aerospace Medicine at Brooks AFB for their hospitality.

Thanks are also due to Universal Energy Systems for smoothly handling the many administrative details of this project.

Spin Label Experiments on Oxygen in RBC Ghost Cells:

Introduction:

Molecular oxygen in the RBC membrane is of interest since the oxygen must cross this membrane before it is bound to the hemoglobin inside the cell. This membrane is also a prime candidate for lipid peroxidation by oxy free radicals since, A, it contains many unsaturated lipids, and, B, it is exposed to some of the highest oxygen tensions found in the body.

These experiments were performed with the assistance of Dr. Daniel Chiu, Ph.D. Dr. Chiu is a Research Biochemist at Childrens Medical Center in Oakland. Dr. Chiu is an expert in this field. He very kindly agreed to supply me with a freshly prepared batch of RBC ghost cells. Thus I was able to spend extra time in the lab doing ESR experiments.

I have wanted to spin label RBC membranes with my monofunctional and approximately spherical spin probes for about ten years. My early attempts with pd-di-t-butyl nitroxide, called 44NO, met with failure because the probe is not sufficiently hydrophobic to give a useful signal from the RBC membrane (the signal from the probe in the surrounding water dominates despite packing of the cells). The 44NO probe gives excellent signals from simple aqueous dispersions of phospholipid vesicles at about 10%w/w. It is thus evident that the RBC membrane does not partition solutes as favorably as does a simple phospholipid vesicle dispersion by at least one order of magnitude. It wasn't until Jason Vierick (last year in my lab in San Francisco) synthesized the closely related, but more hydrophobic, probe called 47NO (pd-t-butyl-t-heptyl nitroxide) that this experiment was possible.

RBC membrane ghoasts were labeled with the 47NO probe at 37°C. The sample was alternately equilibrated with both nitrogen and with oxygen at a total pressure of one atm. The oxygen dependent line width of the membrane line is proportional to the product of the oxygen diffusion coefficient in the membrane times the oxygen Henry law coefficient in the membrane. These results were contrasted with the same data for vesicles of the phospholipid, DMPC, dispersed in water and labeled with the 44NO probe. It is clear that the slope is significantly less for the probe in the RBC,

suggesting that either oxygen is less soluble in the RBC membrane or it has a smaller diffusion coefficient in the RBC membrane relative to the DMPC bilayer membrane.

A second result of these experiments is that the probe line width is much greater in the membrane than in the DMPC dispersion. This demonstrates that the RBC membrane is much more viscous than is the DMPC vesicle. It is interesting that line widths similar to these RBC values are found for the probes in vesicles that are 50 mol % DMPC and 50 mol % cholesterol. Thus this lipid mixture may be a good physical model system for the lipid part of the RBC membrane (cholesterol makes up about 50 mol % of the lipid fraction in the RBC membrane).

A second point of interest to me was that there is no evidence of a strongly immobilized spin probe in the RBC membrane despite the large protein content of this membrane. When we use these probes to spin label stratum corneum samples we see two signals, one fluid signal that we assign to the lipid regions of the stratum corneum, and a second strongly immobilized signal that we assign to the keratin rich regions of the stratum corneum. Evidently the proteins of the RBC are very unlike keratin!

Of more interest to hyperbaric oxygen, HBO, therapy was our final experiment where we took two identical RBC samples spin labeled with the 47NO probe. Both samples were kept at 37°C overnight (16 hours), but one was maintained under 2.6 atm of pure oxygen while the second was kept under nitrogen. The next day ESR spectra were taken on both samples with all remaining molecular oxygen removed. In this experiment we were attempting to see if lipid peroxidation had occurred to the extent that it would change the membrane viscosity and thus modify the probe line width under nitrogen. There was no significant difference in probe line width between the two samples. However, the sample that was under oxygen had lost about 80% of the spin label initially present. This could be a result of free radical chemical reactions going on in the oxygen rich membrane (recall that our spin probes are stable free radicals and are sometimes used as free radical chain reaction terminators). A control experiment was performed in which a dilute aqueous solution of the 44NO probe was sealed in two identical gas permeable Teflon tubes. One tube was held at 3 ATA of oxygen while the second tube was kept under pure nitrogen. After 16 hours at

37°C there was no difference in the ESR spectrum of the probe in the two tubes. Since the probe is thus stable to these oxygen concentrations this means that the probe loss in the RBC experiment was likely due to the spin probe scavenging oxy free radicals.

The sample under oxygen also showed a significant increase in the probe partition coefficient, suggesting a disruption of some of the RBC membrane structure.

## Transcutaneous Oxygen Delivery-Proposed Experiments

### Introduction:

"Oxygen is toxic" thus read a banner hung over a 1984 symposium on free radicals in micro-circulation held in Oxford, England. "Oxygen is a drug" is a common theme of those who practice hyperbaric oxygen, HBO, therapy. There is no conflict between these two statements, as several medical treatments (chemotherapy or radiation therapy for example) are toxic, but they are administered in measured doses and, insofar as is possible, they are targeted for delivery to the proposed site of action. The benefits of HBO therapy have been evaluated and documented by Davis and Hunt specifically for problem wounds[1] and more generally[2]. HBO is the primary treatment of choice for decompression sickness, gas embolism, and carbon monoxide poisoning. It also may be used as adjunctive therapy for gas gangrene, non-healing wounds, infected wounds, osteomyelitis, radiation necrosis, crush injory, and skin flaps.

In normal HBO therapy oxygen is administered in carefully measured doses, but it is administered to the whole body in a systemic mode at about 2.5 ATA using the very efficient cardio-vascular-pulmonary, CVP, system. In this mode the oxygen concentration rises at the proposed site of action, but it increases more at sites where it may cause damage due to the formation of reactive oxy free radicals. Sites of special concern in this regard include the lumen of the lung, the central nervous system, the cells of the blood, and the retina of the eye. A recent lead article in Science[3] cites "oxygen stress", including hyperbaric oxygen, as a potential source of modification of DNA molecules via oxy radicals produced in vivo.

In this proposal we investigate the prospects of using hyperbaric oxygen as a drug that is targeted toward the site of disease or injury. In particular we wish to consider and test the possibility that some wounds and disease states may benefit from hyperbaric oxygen administered to selected regions of the body by a transcutaneous route we call TC-$O_2$. Because ir skin has a significant barrier to TC-$O_2$ we will investigate use of

permeation enhancers to increase the oxygen permeability of skin.

Life Before the Lung:

In the Metazoic era multicellular organisms evolved with differentiated cells forming organs to facilitate oxygenation of tissue. Prior to that time aerobic organisms obtained oxygen from the air by a passive diffusion pathway not unlike that which we are proposing for human skin. Theoretical estimates of maximum organism size based on estimated oxygen metabolic requirement, oxygen solubility, and oxygen diffusion coefficients show that organisms existing at 150 mmHg oxygen partial pressure could have developed to a size of about 2 mm (2,000 μm) before being limited by the diffusion rate of the oxygen[4]. We are proposing that passive diffusion through the skin, driven by hyperbaric oxygen tensions and aided by permeation enhancers, may be able to get doses of the drug oxygen, over and above that delivered by the CVP pathway, into tissues to a depth of a few thousand micrometers.

Respiration, Lung vs. Skin:

The human skin is no match for the lung as an organ to dissolve oxygen. The surface area of the lung is about 100 square meters while the surface area of the adult human skin is only 1.5 to 2.0 square meters (however only use a small fraction of our lung capacity at rest). Although some amphibious organisms, eg. the salamander, receive most or all of their oxygen via a transcutaneous pathway, we are not suggesting that the human skin can ever replace the human lung as the vehicle to supply oxygen to the major oxygen consuming organs of the body. However, under favorable conditions the TC-$O_2$ route, coupled with HBO, may be able to provide significant additional oxygen to sites at or near the skin that may benefit from oxygen therapy. At ambient temperature and 60% humidity the partial pressure of water vapor is about 12 mmHg so that the ratio of the partial pressure of oxygen tending to dissolve the TC-$O_2$ for HBO relative to air is (2.5x760-12)/(159-12) or a factor of 12.8.

The Stratum Corneum, Other Skin Strata, and Drug Delivery :

A recent development in pharmacology is the use of permeation enhancers, PE's, to facilitate transcutaneous drug delivery[5,6]. These PE's are chemicals that are applied topically to the skin. Selected PE's have been shown to reversibly permeabilize the barrier of the stratum corneum, SC, the outermost 15-20 μm of normal epidermis. In favorable cases these PE's have demonstrated significantly enhanced transcutaneous delivery rates of some drugs[7]. We expect that similar enhancements may be possible with transcutaneous oxygen using a suitable PE.

The stratum corneum, not unlike the lung surface, is rich in lipids. The current "brick wall" model for the structure of the stratum corneum has keratin rich "bricks" of dead cells embedded in a "mortar" consisting of a polar and non-polar lipid mixture[8]. The anucleate cells of the stratum corneum require about 2 weeks to reach the surface, where they are gradually desquamated. Oxygen is a lipophilic molecule (oxygen is an order of magnitude more soluble in organic liquids than it is in water)[9]. Thus, under HBO conditions, the SC has the potential to dissolve large quantities of oxygen without the risks involved with similar concentrations in living cells.

In considering the barrier function of the skin, work in my laboratory in San Francisco using ESR, electron spin resonance, spin probes (in collaboration with Dr. Peter Elias at the San Francisco V. A. Medical Center), is consistent with the work of others in establishing that the lipids of the SC are relatively fluid. In spite of this lipid fluidity the SC has been identified in the literature as the primary barrier to TC-$O_2$ transport[10]. For a stratum to be an effective barrier to a solute it should have a low concentration of the solute, and/or a significant thickness, and/or a low diffusion coefficient for the solute. Our knowledge of oxygen and of the SC suggests that oxygen should penetrate the SC by passive diffusion. Transport across the stratum corneum is favored for small and largely lipophilic molecules like oxygen.

Oxygen, unlike some drugs, is consumed by all viable cells such as those in the inner epidermis. Thus, a steady state would eventually be reached where the oxygen supplied from both the TC-$O_2$ route plus the CVP route is balanced by the oxygen consumed at

each point in the skin. Fortunately the skin (dermis plus epidermis) has a relatively low overall oxygen metabolic requirement (about 5 mL/min[11], although about half of this metabolic activity takes place in the inner epidermis), and that demand can be reduced further by lowering the skin temperature. The thickness of the epidermis is often little more than 0.1 mm or 100 μm (it is considerably thicker on the palms of the hands and the soles of the feet).

Horikoshi et al.[12] have measured the effect of oxygen on the growth of human epidermal keratinocytes and found the optimun oxygen pressure to be about 130 mmHg. These cells in vivo are found in the lower epidermis where the oxygen tension is normally about 20 mmHg. Thus the use of hyperbaric oxygenation should be beneficial to these cells. Epidermal regeneration over a wound begins with increased mitosis, with a corresponding increased oxygen demand, in the viable cells of the wound border epidermis[13a,b].

Beneith the epidermis is found the dermis, a skin layer that is fed nutrients (including oxygen) by the micro-capillaries of the CVP system. The dermis is typically about 2 mm thick and makes up about 95% of the skin (not including subcutaneous fat). The dermis has a very low total oxygen consumption rate, about equal to the much thinner epidermis[13c]. As the site of collagen production the dermis is important in wound healing. The need for adequate oxygen at the site of collagen production has been well documented.[14]

History of TC-$O_2$:

Goldschmidt et al[15] observed that an arm bathed in pure oxygen retains a normal skin tone after all circulation is cut off (in air or nitrogen the same arm would rapidly appear cyanotic). This same group also observed that when naked human subjects were sealed from the neck down in a gas tight can the subject's metabolic rate was found to be a function of the composition of the gas in the can. The metabolic rate was monitored by a metabolism machine from which they breathed. A significant drop of a few percent in the metabolic rate was observed when the skin was bathed in pure oxygen at 1 ATA compared to that found with nitrogen[16]. In this bimodal oxygen delivery scheme the demands of the major oxygen consuming organs (kidney, heart, brain, liver, gut, muscle, etc.[17]) are

being satisfied by the more efficient CVP mode. Thus, even in the absence of PE's, it is clear that a very significant amount of oxygen could be delivered to the outermost few millimeters of tissue by the HBO TC-$O_2$ mode.

For example the resting oxygen consumption rate, $V(O_2)$, for an adult human is about 250 mL/min. Thus the 5 mL/min for the skin represents about 2% of the total. It has been estimated from the work of several groups that $V(O_2)$ for TC-$O_2$ in air is somewhere between a trace and 2 mL/min[18]. If we assume a value of 0.5 mL/min then the TC-$O_2$ route accounts for only 10% of the oxygen delivery to skin under normal conditions. However, under HBO conditions of 2.5 ATA of oxygen, the TC-$O_2$ rate to skin should rise by a factor of 12.8 to a value of 6.4 mL/min which will more than double the normal oxygen delivery rate to the skin. If a suitable PE could enhance the oxygen delivery rate by a factor of three then the HBO TC-$O_2$ will result in a <u>five fold</u> increase in the normal rate of oxygen delivery to the skin (of course the patient will also receive additional oxygen due to the systemic effect of breathing 2.5 ATA of air, see the proposed patient protocol section below).

Rabkin and Hunt[19] point out that "topical oxygen" may be of some value in "promoting both epithelization and clearing of superficial infections." However these authors doubt that hyperbaric oxygen can penetrate much past the epidermis. They cite theoretical work that suggests that even under HBO conditions oxygen can diffuse only about 70 micrometers from a capillary in tissue with active respiration. However the capillary model is <u>not</u> appropriate for diffusion into tissue from a <u>planar</u> resevoir such as the air outside the skin. The calculations are also flawed by using a specific metabolic rate much too high for even the epidermis. Calculations with the correct geometry and a more reasonable metabolic rate show that penetration of a few millimeters should be expected[4].

Gruber et al.[20], working with early oxygen micro-electrodes, were able to show a significantly enhanced current due to HBO transcutaneous oxygen in excised human skin at a depth of 300 μm into the dermis, but no enhancement at a depth of 3 mm into the deep dermis. This work should not, however, suggest a barrier function for the dermis. in vivo the microcirculatory system of the dermis will aid in transporting the oxygen once it penetrates the epidermis.

None of the literature work with TC-$O_2$ cited above used a PE.

Choice of the Permeation Enhancer:

Some PE's are better for lipophilic solutes and others are better for lipophobic solutes[5]. We will test several PE's since some may not be stable in the presence of hyperbaric oxygen (for example cis-oleic or vaccenic acids are frequently used as permeation enhancers, but these unsaturated molecules may be too reactive under HBO conditions). Most applications of PE's require that they remain active and tolerated by the skin for a period of days. We want the stratum corneum permeability to be enhanced for only the duration of the HBO treatment (a few hours).

The best PE's for oxygen may be those that take advantage of the small size and hydrophobic nature of this solute. It is known that the diffusion of oxygen, and other very small solutes, in a medium of macro-molecules does not follow Stokes's law[21]. Oxygen appears to diffuse in these media by jumping between "holes" or defects about the size of oxygen. Thus the viscosity of the medium is relatively unimportant. In this way oxygen is able to diffuse relatively rapidly through some viscous plastics, e.g. Teflon. Several pieces of evidence itemized below suggest that some perfluorinated chemicals, PFC's, may be excellent PE's for oxygen in the sratum corneum:

1. Oxygen is at least twice as soluble in PFC's as it is in typical organic solvents[9]. The oxygen concentration in air saturated liquid PFC's is about 4 mM. Recall that PFC emulsions such as Fluosol-DA (Green Cross Corp., Osaka, Japan) are under investigation as blood substitutes. We will include this emulsion as one of our trial PE's.
2. PFC's are very chemically stable due to the strong C-F bond. Thus there is no fire hazard or other chemical oxidation possible under HBO conditions.
3. Most PFC's have been evaluated for use in the body and several have been shown to be safe (for example the $LD_{50}$ for Fluosol-DA in adult rats is 150 mL/kg).[22a] The exceptions are largely those PFC's with vapor pressures at body temperature in excess of 5 mmHg. This work was stimulated by the interest in PFC's as blood substitutes.
4. Although PFC's are excellent oxygen solvents they are relatively

poor solvents for most other species[22b]. Thus they will not extensively extract lipids from the skin so that skin irritation following their application should be minimal (delipidization of the SC when many organic solvents are applied to the skin[22c]). We expect to use PFC's with an oxygen heteroatom in order to achieve sufficient solubility of the PFC in the SC.

5. PFC's are relatively small molecules with a finite vapor pressure. Thus they will rapidly diffuse into the skin, disrupting the ordered lipid structure[23] and creating the "holes" needed for rapid oxygen diffusion. The PFC's will slowly evaporate from the skin and thus restore the barrier function of the skin in a reasonable length of time.

## Experimental:

### A. Questions to be tested:

Many experimental tests must be performed before trials on humans can be considered. At what rate can oxygen diffuse into and within the stratum corneum and what is the oxygen solubility of the SC? What permeation enhancer(s) would be best? What is the optimum dose and best method of application for the PE? How long does the PE remain effective? How long and at what pressure can the skin be safely treated with the high pressure oxygen? What temperature and relative humidity of the SC is required to optimize oxygen transport?

### B. Procedures:

Sample preparation: We propose to test these questions using stratum corneum patches obtained from pig skin, as skin from this animal is a good model for human skin. Several patches of skin each with an area of a few square centimeters are obtained from the animal after sacrifice. The SC from these patchs is obtained free from the inner layers of the epidermis with the aid of a trypsin treatment[24] (the skin sample is spread dermis-side-down on the filter paper impregnated with the trypsin to avoid sample damage from the enzyme). I have asked Dr. Elias to assist us by training my

personnel in these techniques that are standard in his laboratory.

Spin label procedure: The SC patches will be uniformly doped with one of our "high resolution" ESR lipophilic spin probes[25] at about 30 ppm. These probe molecules are monofunctional and approximately spherical. For best ESR peak resolution they are totally perdeuterated. An example is pd-t-butyl-t-heptyl nitroxide spin probe. Our recent work on SC demonstrates that the spin probes report independently from both a fluid lipid phase as well as an immobile protein rich phase[26]. It is the narrow lipid phase lines that will be of primary interest in these oxygen experiments. The SC sample (about 6 mg with an area of about 5 $cm^2$) will be mounted on both sides of a thin quartz plate. The mounted sample will be placed in the variable temperature gas flow apparatus inside the ESR sample cavity.

Rate of oxygen absorption by the stratum corneum: The oxygen dependent part of the ESR line width of the probe in the stratum corneum is proportional to the product of the oxygen concentration times the oxygen diffusion coefficient in the SC[27]. We will first obtain an ESR spectrum of the SC patch equilibrated in a nitrogen atmosphere. The ESR spectrometer will be placed in the second harmonic detection mode and the magnetic field positioned on the best resolved probe line. In this mode the ESR peak height is inversely proportional the the cube of the line width. At this point the gas will be rapidly switched under computer control from nitrogen to oxygen and the time evolution of the probe ESR peak height will be monitored by the computer. As the oxygen permeates the sample the ESR line will broaden, reducing the peak height, until the oxygen tension is uniform over the sample. We estimate that the characteristic time for this experiment will be of the order of seconds. The use of the time evolution of the second harmonic ESR spectrum of spin probes to monitor changing oxygen concentrations has been recently published by Belkin et al.[28]

Sample stability to oxygen: Our "gas-switch" experiment will be cycled to check for sample decay (our newer ESR spin probes have a sufficiently low vapor pressure that little probe should be lost to the gas phase). The high sensitivity of the probe ESR line width to dissolved oxygen will allow us to use nitrogen and air as the two gases. At the partial pressure of oxygen in air at 1 atm little or no

lipid peroxidation of the SC should be seen in the lifetime of the experiment. We will then repeat the experiment using higher partial pressures of oxygen (1, 2, or 3 ATA). At these higher oxygen pressures lipid peroxidation may be seen.

Effect of temperature and humidity: The dependence of the oxygen diffusion rate on temperature and relative humidity will also be established. Blank et al[29] have established that the permeability of the SC to water is dependent on the degree of hydration of the sample. The temperature of the sample and of of the flowing gases will be controlled with the Varian Variable Temperature Accesssory. The relative humidity of the flowing gases will be maintained using the design of Schear[30] and measured with a commercial humidity monitor.

Test of the ability of several compounds to act as PE's for oxygen: The final phase of the initial round of experiments will be to test several permeation enhancers using the porcine SC system. The selected oxygen stable PE's will be applied to spin labeled SC samples and allowed to penetrate the sample. In order to administer a controlled dose we may use either an inert vehicle or a calibrated aerosol spray depending on the nature of the PE. Those enhancers which have the greatest positive effect on the rate at which oxygen diffuses throughout the SC in the "gas-switch" experiment will be considered for tests on excised human tissue.

The oxygen diffusion-solubility product in the SC vs PE: The magnitude of the infinite time line width increase due to dissolved oxygen is a measure of the diffusion-solubility product of the oxygen in the permeabilized SC. At a given temperature, relative humidity, gas composition, and PE dose this product is a mesure of the efficiency of the different PE's. The diffusion-solubility product will be measured as a function of the dose of the PE, and these measurements will allow us to establish the optimum dose for each PE of interest.

Equipment needs: The major equipment items necessary to perform these experiments, including a Varian E-12 ESR spectrometer and associated computerized data system, are in place in my laboratory at San Francisco State University.

Proposed Patient Protocol vs. Conventional HBO Therapy:

Consider a patient requiring HBO for a problem wound on a limb.

With the new protocol a permeation enhancer would be applied to the skin in the area of the wound edge. The patient would then enter the hyperbaric chamber and become acclimatized to breathing compressed air at 2.5 ATA (the value consistent with conventional HBO therapy, other pressures may be found to be optimal). At this point a cuff or a bag would be placed about the limb over the wound area and sealed. Partially humidified oxygen gas at an optimal temperature would be delivered to the interior of the bag and thus to the skin of the limb. Some high pressure oxygen will be taken up by the healthy skin in the vicinity of the wound edge while additional oxygen will be absorbed by the wound tissue directly. The target site for this oxygen is the hypoxic injured or diseased tissue where the oxygen tension is probably 20 mmHg or less[1]. The newly oxygenated tissue should be stimulated to increase vascurization and to produce collagen and glycosaminoglycans as key steps in the dermal regeneration process[13a]. Increased oxygenation due to TC-$O_2$ with the PE will fuel increased mitosis in the wound border epidermal cells with subsequent cell migration into the wound in order to regenerate the epidermis[13b].

This protocol differs from conventional HBO therapy in only a few significant ways. In conventional HBO therapy the patient is exposed to 2.5 to 3 ATA of compressed air and breathes 100% oxygen from a cylindrical plastic tent placed over the head and sealed around the neck. During the approximately two hour HBO session the patient takes periodic "air-breaks" from breathing pure oxygen for his/her safety. These breaks would not be required in the proposed new procedure. Finally, in conventional HBO therapy no permeation enhancer is employed as the only significant oxygen delivery to the tissues is assumed to be via the CVP pathway.

Summary:

If hyperbaric oxygen could be administered transcutaneously at the site of the wounded or diseased tissue the patient could obtain the benefits of HBO without most of the potential risks. This mode of HBO delivery appears especially suitable for those conditions where the target for the oxygen is within a few millimeters of the skin surface (an example is the dermis layer of the skin where

collagen is synthesized for wound healing). If suitable PE's are found the method can be applied to many patients with relatively minor changes in conventional HBO protocol. Possible oxygen toxicity in the lung, in the central nervous system, in the eye, in the blood, and other critical organs would be reduced. By thus minimizing concern over possible unnecessary patient risk the popularity of HBO treatment may increase.

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FINAL REPORT

# The stinging wasps (Hymenoptera:Vespidae) of south Texas


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| Date: | 29 July 88 |
| Contract No: | F49620-87-R-0004 |

# The stinging wasps (Hymenoptera: Vespidae) of south Texas

by

Hal C. Reed

## ABSTRACT

A survey of stinging wasps and their importance to the U. S. Air Force was conducted at Brooks AFB, TX. Collections of individuals and colonies and examinations of regional insect museums revealed the presence of 19 species in south Texas, but only 12 of these occur in the San Antonio area. The most abundant paper wasp in this area is Polistes exclamans as 75% of all nests collected or encountered belonged to this species. Three yellowjackets occur in this area and Vespula squamosa is the most common of the three species. A revised fact sheet on wasps, a preliminary field key to vespid wasps, and a reference collection was provided the Medical Entomology Section. Examining the pest control records of three local USAF bases reveal that 4% of all animal-related control jobs involved destruction of stinging wasp colonies. Wasp sting allergy among patients at Wilford Hall (Lackland AFB) was the second most important arthropod-induced allergy next to fire ant allergy cases. A preliminary experiment on alarming chemicals in paper wasps demonstrated that methylene chloride extracts of the venom sac elicited defensive behavior.

ACKNOWLEDGEMENTS

I am very grateful for this research opportunity awarded by the USAF Systems Command, Air Force Office of Scientific Research and the Medical Entomology Section (EKEN), Epidemiology Division, School of Aerospace Medicine, Brooks AFB. I greatly appreciated the support, encouragement, space and assistance provided by Lt.Col. Pinkovsky,(Chief, EKEN) and his staff (Dr. Chad McHugh, Amn VanderBerg, Pat Miller and Capt. Jerome Goddard). I extend my thanks to the following pest control operators (PCO) who made their records accessible to me: Mr. Mustifel (Kelly AFB), Mr. Popham (Randolph AFB), and Mr. Gafford (Lackland AFB). Mr. Popham kindly donated the large yellowjacket nest to the EKEN lab. Mr. Higdon (PCO, Brooks AFB) helped greatly by directing me to nests on base. The following institutions and individuals allowed me to examine their vespid collections: Trinity U., Witte Museum (Sarah Kerr), San Antonio College (Mr. John Fardell), Ft. Sam Houston-Medical Zoology section (Lt.Col. Bosworth), U. Texas-Austin (Dr. Allan Hook), and Texas A&I U. Interaction with the following vespid researchers helped me complete this project: Dr. Roger D.Akre (Washington State U.), Mr. Robert Jacobson (East Carolina U.), Dr. John MacDonald (Purdue U.) and Dr. James Gillaspy (retired, Texas A&I U.). William Freeman, M.D. (USAF allergist, Wilford Hall, Lackland AFB) provided allergy patient data and showed great interest in the project.

## I. INTRODUCTION:

Stinging wasps are primarily social wasps that belong to the family Vespidae in the insect order Hymenoptera which also includes ants, bees, and other wasps. Vespid wasps are known for their propensity to sting and inflict pain in defense of their colony. Due to their aggressive nature, large colonies, and habit of nesting or foraging around man's dwellings they can pose a serious threat to human health. Approximately 40-50 deaths occur each year in the U. S. due to the stings of Hymenoptera; probably over half due to social wasps, and possibly over 2.5 million Americans suffer from hypersensitivity to Hymenoptera venoms (Levine and Lockey 1986). This hypersensitivity may range from a large local reaction to a more serious, systemic reaction (i.e. anaphylactic shock) that may result in death. At this time allergists are unable to predict who among this hypersensitive group may develop a potentially fatal reaction upon their next sting.

The Medical Entomology Section of the Epidemiology Division of the USAF School of Aerospace Medicine at Brooks Air Force Base (AFB) is concerned with insects affecting the health and performance of USAF personnel and strategies for their control. The Section serves as a center for mosquito and tick identification for North American USAF bases but also serves as a consulting group for a wide array of other insect-related problems. They must be prepared to identify and answer questions about the biology and control of venomous arthropods such as stinging wasps. Their need for such expertise in this area is evidenced by their production of a pamphlet on venomous arthropods (AFP-161-43 1977). My research background in identification, behavior and ecology of the two major groups of stinging wasps in North America

(paper wasps and yellowjackets) made me a suitable candidate for assignment to the Medical Entomology Section.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

My assignment as a participant in the Summer Faculty Research Program (SFRP) was to investigate aspects of vespid biology and the problems associated with stinging wasps, and thus provide an information base for dealing with future wasp-related problems. Our preliminary goals for the project were: (1) conduct a survey of stinging wasps in the local area: (2) develop a representative collection of the local species; (3) revise current USAF literature on venomous arthropods, especially stinging Hymenoptera: and (4) collate current information on incidence and subsequent treatment for envenomization among USAF personnel. During the course of the research period objective 4 was modified to focus on the pest status of vespid wasps based on the incidence of wasp control at the local USAF bases and frequency of wasp sting allergy reported at the local USAF clinics. Also, a fifth objective was added: to test for the presence of a pheromone(s) that elicits alarm and defensive behavior in paper wasps. Since the approaches and results of the first three objectives are interrelated, they are discussed together in section III. Objectives 4 and 5 are discussed separately in sections IV. and V., respectively.

## III. Survey of stinging wasps in the San Antonio area

a. This aspect of the project involved intensive collecting of active colonies and individuals in the area. Collecting

was primarily done on Brooks AFB, but other local sites were also searched. The base contains a suburban area (i.e. base housing) and a more natural area (i.e. mesquite savannah), both of which are good potential sites for nesting and foraging activities of these wasps. In addition to my personal collecting efforts, active wasps nests were located with the help of base homeowners, maintenance workers, and the base pest control operator (PCO). Their assistance was solicited through an article in the base paper (Discovery), leaflets, and personal contact. These efforts provided a list of species and estimation of their occurrence on Brooks AFB. Also, colony collections provided information on various aspects of vespid biology such as colony size and nesting habits. Insect museums at various colleges and institutions in south Texas were visited and examined to determine the species composition and distribution in this area. Also, the assistance of other vespid researchers was sought through letters and telephone calls, and one visit was made to a retired, experienced researcher of vespid wasps in south Texas. These insect museums plus my personal collecting efforts assisted me in developing a species list for the San Antonio area, an identification scheme and distribution maps for vespids in south Texas, and a representative collection of the principal species in south Texas. USAF literature on stinging wasps was revised based on this research effort, previous experience, review of the scientific literature, and personal interaction with vespid researchers and medical allergists.

b. Nineteen species of stinging wasps inhabit south Texas. The following is a list of species from south Texas with an * indicating the ones that were reported in San Antonio:

Vespidae (social wasps)

Polistes (paper wasps)

* P. annularis (L.)
* P. apachus
  P. carnifex (Fab.)
* P. carolina (L.)
  P. comanchus Saussure
* P. dorsalis (Fab.)
* P. exclamans Viereck
* P. fuscatus fuscatus (Fab.)
* P. fuscatus bellicosus Cresson
  P. instabilis Saussure
  P. major Beauvois
* P. metricus Say
  P. pacificus Fab.
* P. perplexus Cresson

Polybiinae

Mischocyttarus mexicanus Saussure
Brachygastra mellifica (Say)

Vespinae (yellowjackets)

* Dolichovespula maculata (L.)
* Paravespula maculifrons (Buysson)
* Vespula squamosa (Drury)

The more common species encountered at Brooks AFB during this period (May 23-July 29, 1988) were: P. exclamans. P. dorsalis, P. apachus, P. carolina, P. fuscatus bellicosus, and P. metricus. Over one hundred colonies were located and 53 of these were collected and the adult and brood population analyzed. The most ubiquitous stinging wasp in this area, and indeed probably all of Texas is the paper wasp, P. exclamans. Over 75% of all nests collected or located on base belonged to this species. This species often nests around man-made structures and usually in well-exposed, visible sites (e.g. under eaves and porches). Nests are also built in a wide variety of shrubs and trees in the semi-natural area of Brooks AFB. One to several smaller nests, called satellites, may be present in close proximity to a larger, parental nest. The average colony size of P. exclamans varied widely during this period. For example, nest size in June ranged from 35 to 209 cells, but averaged 85 total cells (N=25). Larger parental colonies in July usually had 25-50 adults and 150-300 total cells.

The next most common species collected was P. apachus (11 colonies). This large, brightly-colored yellow and brown wasp occurred primarily in the semi-natural area of Brooks AFB but was found nesting in man-made structures as well as vegetation. Six nests were located in dark, less-exposed sites (e.g. inside enclosed sheds) while the other five were in open, well-exposed sites (e.g. under eaves). Colony size was usually 9-12 adults and 90-190 total cells in June and early July. Nests undoubtedly get larger as the season progresses. One mature, abandoned nest from the previous season had 301 cells.

The smallest-sized paper wasp, P. dorsalis, was probably as abundant at Brooks AFB as P. apachus although fewer nests (9) were located. Their colonies usually contained 14 adults and 100-200 cells. Nests were found in shrubs and under roof eaves but often in sites less exposed than the nests of P. exclamans. Only one nest of the red paper wasp, P. carolina, was collected on base although numerous individuals were collected in the field. No nests were found in association with man-made structures on base but one colony collected from under an eave in Comal Co. One nest was collected in vegetation in the semi-natural area. This species will nest near human habitations in other areas of Texas, usually in well concealed sites (e.g. attics, wall voids) (Reed and Vinson 1979). No nests of P. metricus were found on base but a few individuals were collected foraging in the field. One nest was found under an eave in central San Antonio. This species can be very common around houses in Texas (Reed and Vinson 1979). Many individuals of P. fuscatus bellicosus were collected in the field but no nests were located near structures or in the vegetation in the semi-natural area. This species apparently shuns man-made structures and may be as abundant as P. apachus in some localities of Texas (Gillaspy 1986).

The relative abundance of the local vespids likely varies in other ecological zones in San Antonio. The Ft. Sam Houston-Medical Zoology Section has an insect collection from their students over the past five years. Nearly all specimens were collected on base and most from a semi-forested area (e.g. live oak) in Salado Creek Park area. Based on the abundance of specimens in this collection the relative frequency of vespid species in this area is: P. exclamans > P. annularis > P. carolina = P. apachus = P. metricus > V. squamosa > P. dorsalis > P. f. bellicosus = P. perplexus > P. maculifrons > P. f. fuscatus. This more wooded habitat will be preferred by P. annularis, P. metricus, P. perplexus and the yellowjackets (V. squamosa and P. maculifrons); species that were absent or rarely encountered at Brooks AFB.

This study during late May through July cannot accurately determine the importance of yellowjackets in this area as they become more evident in the late summer and fall. In the fall colonies reach peak size and are more troublesome as scavengers at man's food and drink (Akre et. al. 1981). However, only a few yellowjacket adults were in the collection at Ft. Sam Houston (stated above) and most PCOs on the local AFB had only rarely controlled a yellowjacket nest (see section IVb). Two colonies of the southern yellowjacket, V. squamosa, were examined during this study. One colony (1 comb and 17 adults) was collected in mid-July by an allergy patient and another large mature colony was destroyed at Randolph AFB the previous winter (Dec. 10, 1987). The nest sites of the two colonies were very similar as each was located in a box in a garage. The latter nest contained 7,263 cells in 8 combs and 572 males, 112 queens, and 244 workers. The adult population of such a colony may contain 1000-1500 wasps (Akre et. al. 1981). Obviously, such a colony would be a tremendous stinging

hazard to man. This species is probably the most important yellowjacket in this area. No nest and only four specimens of P. maculifrons, the eastern yellowjacket, were located in the regional collections. However, nests have been found in Austin, Texas only 70 mi. north of San Antonio. Although no specimens of the baldfaced hornet, Dolichovespula maculata, were found from Bexar Co., a local PCO has stated that he has destroyed their large grey aerial nests in the San Antonio area. They are probably more abundant in the hill country north of San Antonio.

A 7 page fact sheet on wasps, a 17 page document titled "A preliminary field key to the stinging wasps of south Texas" and a list of recommended revisions to the Venomous Arthropod Handbook (AFP-161-43) were completed and are on file with Lt.Col. Pinkovsky, EKEN. A representative collection of North American yellowjackets (51 specimens representing 9 species) and paper wasps (59 specimens representing 10 species) from south Texas was also provided EKEN. Nests of the major species of stinging wasps were also placed in the collection. Other representative collections of south Texas species were provided for William Freeman, M.D. (Allergy Clinic, Wilford Hall, Lackland AFB) and Lt.Col. Bosworth (Ft. Sam Houston-Medical Zoology). Two seminars on stinging wasps were given : one to the Epidemiology Division, Brooks AFB and the other at a meeting of the local military medical allergists in San Antonio.

## IV. Pest status of social wasps in San Antonio

a. The PCOs on Kelly, Lackland, and Randolph AFB maintain records of all jobs done on base reporting insect pest, building, date, and pesticide usage. Although the records did not give the species of stinging wasp, nearly all cases

were paper wasps. These records were examined to determine the frequency of wasp control relative to other insect pests. The records on incidence of sting allergy were provided by the Allergy Clinic at Wilford Hall.

b. Stinging wasps were not a major pest on the three local bases relative to the frequency of control of cockroaches and fire ants. Overall, about 4% of all animal-related control jobs each year were wasp control jobs; however, the frequency varied depending on the year and the base. During the years (1983-1987) the Randolph AFB PCO had 11,471 pest control jobs (average=2,294/year) and only 218 (1.9%) of these involved destroying paper wasp colonies and two additional jobs involved yellowjacket colonies. More vespid colonies were eliminated in 1983 and 1985 (each 2.8%) than in 1984 (1.3%) and 1986 (1.4%). Most colonies were controlled during August (23.2% of 220 colonies) and July (17.3%). Kelly AFB had even fewer wasp control jobs than Randolph AFB. The Kelly PCO had an average of 1,524 pest control jobs/year during the 6 year period 1981-1987. Only 1.5% (158) of these cases involved destruction of wasp colonies. All jobs were paper wasps, except for two cases of yellowjacket control. The frequency of wasp control varied from a high of 47 jobs in 1985 (3.1%) to only 9 jobs in 1984 (0.6%). Wasp control was at a high frequency during 1985 at both Kelly and Randolph AFB; possibly reflecting a high wasp population. As at Randolph AFB, most wasp control was done at Kelly AFB during July (20.9% of 158 cases) and August (18.6%) . Considerably more wasp problems were encountered at Lackland AFB. Approximately 1,980 pest control jobs were performed each year on this base during 1976-1981 and 8.9% of these (a total of 1,063 cases) involved elimination of paper wasp nests. The frequency of control ranged from 12% (237) in 1977 to a low of 5.5% (108)

in 1980. Again, most nests were controlled during July (27.7% of 1,063 cases) and August (23%). However, as seen in Fig. 1 wasp nests or aggregations were controlled during every month of the year. The temporal occurrence of wasp control reflects the growth of the nesting population of paper wasps in the San Antonio area. Colonies are initiated in April and May and thus less likely to be of concern to residents and maintenance workers. However, during June, July and August colony size increases and thus nests become more evident and more likely to be destroyed. Some colonies may remain active in September and October but they begin to decline in the fall. Most wasp cases during mid October through December are mating and hibernation aggregations. Significantly, such aggregations often occur in the same place year after year and show a special attraction to the control towers at Kelly and Randolph AFB where they disturb traffic controllers.

The frequency of wasp sting allergy among the U. S. population is not well documented. One study at Ft. Stewart, Georgia revealed that only 4% of all persons reporting to the clinic for treatment of arthropod stings or bites were due to wasps and nearly half (49%) were due to the imported fire ant (Adams and Lofgren 1982). Data from other regions or on a national scale are lacking. However, in the past two years, 84 patients were referred to the Allergy Clinic at Wilford Hall, Lackland AFB because of reactions to bites or stings of arthropods. Nearly 30% of these patients showed sensitivity to paper wasp and/or yellowjacket stings while another 42% were sensitive to the imported fire ant. Persons sensitized to honey bees comprised only 6% of this patient population. Sixty-four of these 84 patients showed systemic reactions and the rest had nonsystemic reactions. Twenty-one of the 64 patients had sensitivity to paper wasp and yellowjacket stings (cross

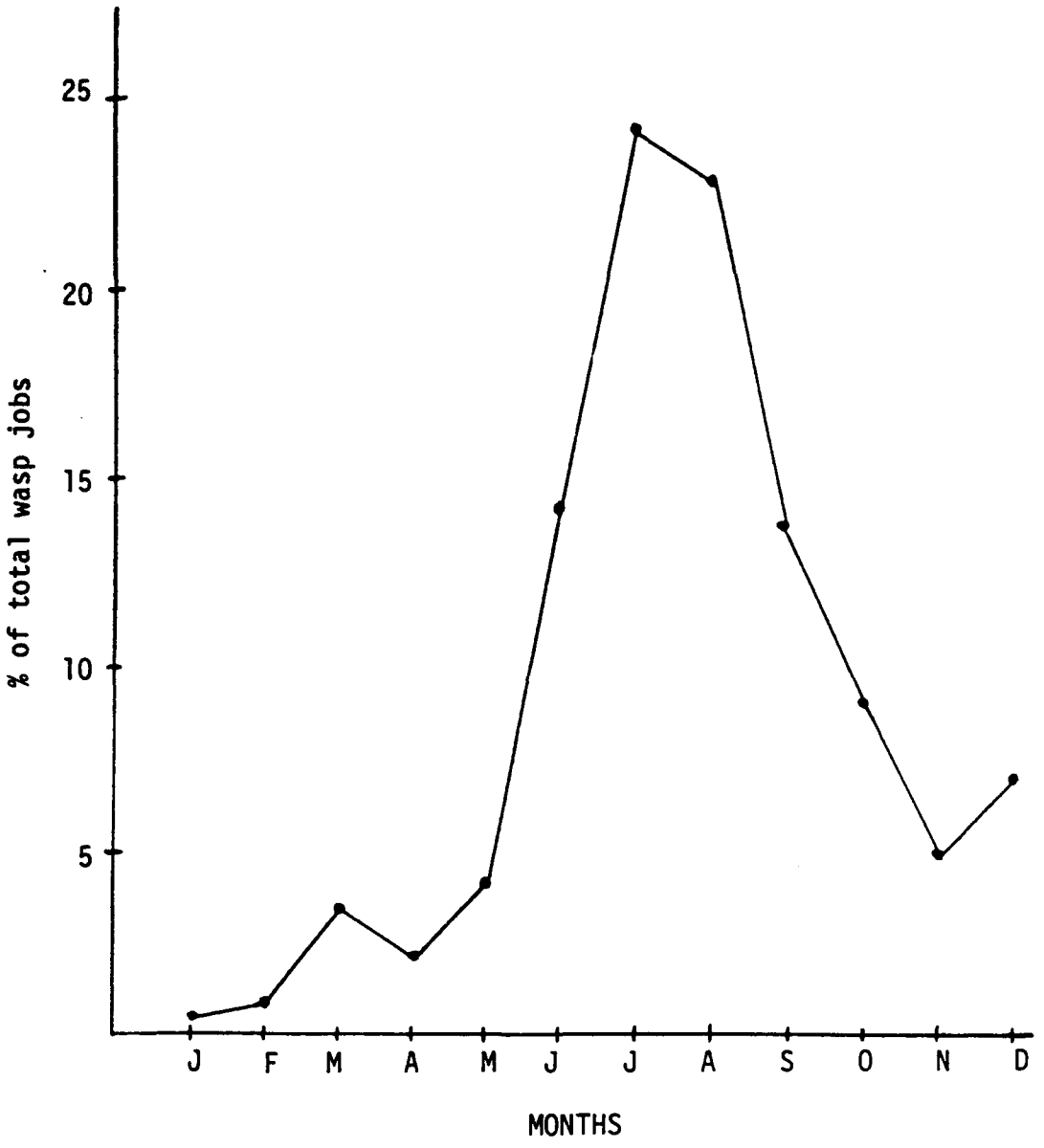



Figure 1. Monthly occurrence of wasp control at three USAF bases: Kelly (1981-1987), Lackland (1976-1981); Randolph (1983-1987). Percentages are based on a total of 1,440 wasp control jobs.

reactivity between these two groups is common), and 4 other patients reacted only to paper wasp stings. Four of the 20 patients with nonsystemic reactions reacted to vespid stings. Although these patients are referrals only and may not necessarily represent the frequency of wasp stings or allergy in the population of San Antonio, it does suggest that the incidence of sensitivity to stinging wasps is higher than that indicated in the Georgia study. Further study is needed in this area to substantiate this claim.

V. Tests for an alarm pheromone in paper wasps

a. Little research has been done on the factors eliciting defensive and alarm behavior in social wasps. Recently Heath and Landolt (in press) identified a compound in the venom that stimulates attack behavior (i.e. alarm pheromone) in the southern yellowjacket, Vespula squamosa. Many researchers considered that vision and substrate vibration, rather than pheromones, were the primary stimuli to evoke attack in paper wasps. However, Post et. al. (1984) demonstrated that fresh venom sacs in conjunction with black models elicited attack in P. exclamans. The objectives of the present experimentation were to repeat the results of Post et. al. (1984); simplify the experimental design, and test extracts of the venom sac for effectiveness in eliciting alarm as the initial step in chemical identification of the active substance. A successful bioassay was developed using a styrofoam sphere (10 cm diam) painted black and mounted on a wooden dowel. The test substances or solvents were placed on filter paper pinned to the top of the sphere. This "target" was placed onto the handle of a telescoping net handle to present to colonies 15-20 feet high. The target was held very still for 1 min

14-15 inches upwind from the colony. Distances closer to the nest or movement alone would evoke attacks. The number of visible or audible hits were presumed stings and were recorded. The solvent control (hexane or methylene chloride) was done prior to the extract test. The extract was prepared by removing the sting apparatus and venom sacs of 30 frozen P. exclamans adults and placing them in 1 ml of solvent. A glass rod was used to crush the venom sacs. The extract was used at a concentration of 0.2 ml (=6 venom sac equivalents) per test. Ten trials were done on one colony (20-25 adults) over a 2 week period. Consecutive tests were done on different days or morning and afternoon of the same day. Whole body extract (minus the sting apparatus and venom sac) of 50 adults in 11 ml of methylene chloride was also tested.

b. Methylene chloride extracts of venom sacs proved successful in evoking attack behavior in 8 of the 10 trials whereas only one wasp hit the sphere in the 10 control trials. In the two unsuccessful extract trials there was a wind shift that may have affected the results. In the 8 successful trials an average of 4.8 hits in 1 min were recorded. Other behavior such as anemotaxis and tandem flying were observed. However, a stinging defender did not seem to alert the rest of the colony upon return to the nest. The control test did not elicit defensive behavior as observed during the extract trials but in three control trials a wasp flew off the nest in the direction of the target but returned without contacting the sphere. Three trials with whole body extract (6, 8 and 14 wasp equivalents) did not elicit attacks. Tests using venom sac extract on another colony also resulted in hits to the sphere. One test using a higher concentration (10 venom sac

equivalents) evoked a stronger response of 20 hits in 1 min. Interspecific tests with P. apachus were inconclusive.

VI. RECOMMENDATIONS:

a. The Medical Entomology Section now has the information, identification scheme, and reference collection to address many of the stinging wasp problems encountered throughout North America. The study has established the principal stinging wasp in San Antonio during the summer and the incidence and seasonality of wasp control on the local AFB. My recommendation is that EKEN establish a long-term relationship with the USAF Allergy clinics to develop a program for identifying and recording the specific arthropod inducing allergic reactions. The development of reference collections at EKEN and Wilford Hall (Lackland AFB) is an initial step in this direction.

b. This preliminary research project has established several definite avenues of future research to be addressed in the application for a mini-grant from AFOSR. Research during the fall of the year in San Antonio needs to be done to establish the incidence and importance of yellowjackets. Further work is needed on the role of pheromones in Polistes defensive behavior. Chemical identification of the active substance of the methylene chloride extract would require gas chromatographic separation and analysis and subsequent testing of the principal fractions for alarm pheromone activity. Another research project of potential interest to the USAF is to investigate the "tower phenomenon" among fall aggregations of paper wasps. Large groups of males and females often occur around the control towers and other tall buildings on the bases. One possible

approach is to investigate the role that pheromones (e.g. mating, aggregation) may have in forming these aggregations. Another important area to pursue is to develop a feasible program for the USAF clinics to identify and record the specific arthropod inducing allergic reactions (see section VI.a.). A relationship with the clinicians would be established including communication, training, a reference collection or photographs, and a common system for entering the causative agent into the computer system. Such a system would better equip allergists to treat their patients and would also provide more accurate data on the incidence of stings due to paper wasps, yellowjackets, fire ants, etc. (see section IV.b.). These data would then enable allergists to better prepare for their potential patients by keeping adequate supplies of venom for testing and desensitization procedures.

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FINAL REPORT

# MODELING OF BLOOD FLOW IN THE SYSTEMIC HUMAN ARTERIAL TREE


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| Contract No: | F49620-87-R-0004 |

# MODELING OF BLOOD FLOW IN THE SYSTEMIC HUMAN ARTERIAL TREE

by

Thomas R. Rogge

## ABSTRACT

The development of a mathematical/computer model of blood flow in the systemic human arterial tree was the project undertaken for the summer. An existing program, based on the finite element method for certain arterial segments, was used as a starting point. The model uses the one-dimensional field equations to simulate pressure and flow waveforms in the arterial segments. The model takes into account taper, nonlinear material behavior, constriction of the arteries (stenosis), and allows a set of different boundary conditions at the proximal and distal ends of the tree. A set of parameters, such as arterial segment geometry and compliances, was compiled and estimates of the resistances and compliance in the distal Windkessel model were obtained. The effect of G-forces was included in the model and simulations comparing a zero G-force to a 1G-force in the arm and leg arteries were made.

## ACKNOWLEDGMENTS

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.

Many people contributed to making this experience a rewarding and enjoyable one. My thanks go first to Dr. Sherwood Samn for his interest and consultation on the research project. I am indebted immensely to Tom White, Billy Jackson, and the machine room operators for their help with all the computing problems. I also wish to thank all the people in Building 110, in particular, Dr. Michael Henderson, Mary Gonzalez and Maxine Newsom, for all the support which these people afforded me.

## I. INTRODUCTION:

Cardiovascular diseases account for almost one-third of all deaths in Western countries and in the United States there are more deaths from cardiovascular-system-related diseases than from any other disorder. Current methods for diagnosis of these diseases are mainly invasive in nature and under certain conditions there are risks involved with these methods themselves. Hence there is a need for better and more accurate noninvasive diagnostic techniques. At present, ultrasonic devices are being used to obtain flow and pressure variations noninvasively, but reliable techniques to calibrate such instruments under in vivo conditions are yet to be found. With the development of these instruments, a need arises to satisfactorily interpret the data collected. The need is to be able to distinguish a healthy system compared to a diseased system. Mathematical modeling can be used to simulate various types of cardiovascular disorders and thus reduce the cost and number of lengthy experiments using live subjects. The present high-speed computers and work stations make it possible to use mathematical modeling which can yield fast, inexpensive and very useful methods to detect and/or better understand abnormalities in the cardiovascular system.

The Air Force is very concerned about the effect of high +G environments on pilots of high performance aircraft. In particular, the effect that +G loadings may have on the cardiovascular system is of primary concern. In an attempt to understand the effect of +G loading on the cardiovascular system, experiments with animals and humans are being conducted. One way to complement these tests is with a mathematical model of the cardiovascular system. The development of such a model is a formidable task, but with knowledge gained in cardiovascular research and the advances in computer science, it is feasible to begin to develop a high fidelity, comprehensive model of the cardiovascular system.

I have been working on models of segments of arteries for approximately the last nine years. This work has been a combination of experimental work carried on by my colleagues at Iowa State University and computer modeling done by myself and graduate students. The existence of a program which would simulate the blood flow in the arm or leg arteries contributed to my assignment in the Decision Support Division at the School of Aerospace Medicine.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

The objectives of the research project were to take the existing program and modify it so that +G loadings could be simulated and to begin to model the complete systemic human arterial tree. Once this first objective was realized, a study of the pressure and flow waveforms due to different +G loadings and with abnormal heart pressure pulses or flow pulses was to have been undertaken. Time did not permit the accomplishment of all of these objectives and funding from a minigrant will be sought to continue this project.

## III. PREVIOUS WORK:

A literature review of previous work in this area is beyond the scope of this report; however, references (1,2,3,4) give an indication of the work which has been done in this area. The beginning point in the mathematical modeling of the cardiovascular system is with the conservation of mass and the conservation of momentum, which yield the following equations (5):

$$\frac{\partial Q}{\partial x} + \frac{\partial A}{\partial t} = 0 \qquad (1)$$

$$\frac{\partial Q}{\partial t} + \frac{\partial}{\partial x}(BQ^2/A) = \frac{A}{\rho}\frac{\partial P}{\partial x} + \tau_o \frac{\pi D}{\rho} + f \qquad (2)$$

where $Q(x,t)$ = flow at time t and averaged over axial location x; $P(x,t)$ = pressure at time t and axial location x; A = cross-sectional area; B =

one-dimensional momentum transfer coefficient; D = diameter; $\tau_o$ = wall shearing stress; f = body force; and $\rho$ = blood density.

In addition to the above two equations, a constitutive equation which relates the area to the pressure is taken as

$$A(P,x) = A_o(x) + C_o(x)\,(P-P_o) + C_1(P-P_o)^2 \qquad (3)$$

where $C_o(x)$ and $C_1(x)$ are coefficients calculated by fitting experimental data of the variation of local cross-sectional area with pressure. Other models have used equations of states based on pulse wave velocities.

The boundary conditions specified at the proximal end of the artery are that of a pressure or flow pulse, while at the distal end of the arterial segment either a pressure or flow pulse may be specified or some form of a distal resistance may be used. Two types of distal resistance are used; one is a pure resistance and the other is a modified Windkessel model which has a resistance $R_1$ in series with a resistance $R_2$ and a capacitance C in parallel. The equation governing the flow in this lumped system is

$$C\frac{dP}{dt} - R_1C\frac{dB}{dt} + (P/R_2) - (1 + R_1/R_2)B = 0$$

where B is the flow in the branch and the venous pressure has been taken as zero (6).

The simulation of a stenosis is accomplished from the equation developed by (7)

$$\Delta P = \frac{4K_v\mu}{\pi D^3}Q + \frac{8K_t\rho}{\pi D^4}(A_o/A_1 - 1)^2|Q|Q + \frac{4K_u\rho L}{\pi D^2}\frac{dQ}{dt}$$

where D is the diameter of the unobstructed lumen, $A_o$ the corresponding area, and $D_1$ and $A_1$ the lumen diameter and area of the stenosis. The

coefficients $K_v$, $K_t$, and $K_u$ are empirical quantities which are determined experimentally.

There are three mathematical methods which can be used to solve this system of equation. The first is finite difference (3), the second is the method of characteristics (4), and the third is the finite element method (2) and (5). The work described here uses the finite element method and this method is so chosen for the ease of 1) handling changing material properties, 2) inserting of stenosis, and 3) handling different boundary conditions.

IV. WORK ACCOMPLISHED:

A program was brought from Iowa State University to the Brooks Air Force Base and installed on the VAX 8650 computer system. This program was then used as the starting block for the development of the human systemic arterial tree. Certain modifications of the program were first needed, such as using a different plotting routine, allowing a flow input boundary condition to be used, including the effects of +G acceleration, and testing for accuracy. Once these above modifications were made, it was decided to model the aorta-abdominal-leg arteries, shown schematically in Fig. 1, as an initial building of the arterial tree. This allows the input condition of either a heart pressure pulse or a left ventricle flow pulse to be used. In order to model this segment of the tree, a set of data needed to be obtained and/or calculated. A preliminary set of this data has been obtained and a preliminary simulation of flow in this system has been attempted with a pressure input.

The inclusion of the +G term in the program was finished where the reference of +G acceleration is normal G environment to which the arterial tree is subjected.

A simulation on the brachial-radial-ulnar arterial segment and the leg artery was conducted. Figure 2 shows the schematic for modeling the arm and leg arteries.

V. RESULT:

Figures 3 and 4 show the pressure and flow for the brachial/radial arm simulation with a G acceleration force of zero and a G acceleration force of 1G (980cm/sec$^2$). This force was applied in the direction of flow and should give the maximum effect of a G-force on the flow and pressure. As can be seen, the inclusion of a nonzero G term in the direction of flow increases maximum peaks of both the pressure and the flow. The effect of a nonzero G loading also changes the mean flow and pressure. Table 1 shows the effect of the mean flow and the mean pressure of three different G loadings; the effect of all these accelerations is along the length of the artery.

TABLE 1. MEAN FLOW AND PRESSURE IN THE ARM ARTERY

| G Value | Mean Flow | | Mean Pressure |
|---|---|---|---|
| cm/sec$^2$ | cc/sec | | dynes/cm$^2$ |
| | Proximal | Distal | Distal |
| 980 | 5.48 | 0.93 | $1.43 \times 10^5$ |
| 0 | 1.16 | 0.62 | $0.99 \times 10^5$ |
| -980 | -3.16 | 0.30 | $0.55 \times 10^5$ |

The effect of G loading on the leg artery was also studied. Again, the total artery was considered to be affected by the G loading. Similar to the arm artery, there were changes in the flow and pressure and Table 2 gives the mean flows and pressures for the leg subjected to G loadings.

TABLE 2. MEAN FLOW AND PRESSURE IN THE LEG ARTERY

| G Value | Mean Flow | | Mean Pressure |
|---|---|---|---|
| $cm/sec^2$ | cc/sec | | $dynes/cm^2$ |
| | Proximal | Distal | Distal |
| 98 | 11.67 | 2.04 | $1.60 \times 10^5$ |
| 0 | 9.47 | 2.27 | $0.999 \times 10^5$ |
| -98 | 6.56 | 1.93 | $1.74 \times 10^5$ |

A number of different simulations with varying values of the G acceleration were made and Fig. 5 shows how the mean flow at the distal end of the arm artery varies as a function of the acceleration. The behavior is seen to be linear, i.e. increased mean flow is proportional to increased value of G acceleration.

In the simulations presented above, no compensation by the arterial system was taken into account. As a crude approximation to the behavior of the arterial system when subjected to a G acceleration force, the resistances in the modified Windkessel model were increased by a factor of two when the arm artery was subjected to a 1G acceleration. Figure 6 shows a plot of the flow at the distal end for the case of no change in resistance and when the resistance is increased by a factor of two.

The increase in the resistance decreases the mean flow at the distal end from $0.93 cm^3/S$ to $0.47 cm^3/S$ (a factor of two), while at the proximal end of the segment the mean flow increases from $5.5 cm^3/S$ to $8.3 cm^3/S$.

The simulations of flow in the aorta-abdominal-leg model were attempted and results are encouraging even though the input data still

need to be refined so that the proper response can be obtained. This particular aspect of the project will continue upon return to the university.

## VI. RECOMMENDATIONS:

The study described above is only the beginning of an attempt to create a model for the arterial system which will reproduce as close as possible the actual physical system. The material behavior of the systemic system is viscoelastic in nature and thus an element needs to be developed which will reproduce this response. The development of this element and the inclusion of this element into the developed computer code will take a significant effort. It is important, though, that in order to model the arterial tree properly that this modification be made.

In addition to the above, future effort will need to be directed toward the following model extensions and applications:

a) A study of the peripheral "Windkessel" model and modification of this boundary condition to more accurately reflect how the distal beds affect the flow and pressure waveforms.

b) A study of compliant stenosis and inclusion of these types of stenosis into the computer code. A great portion of this study will require experimentation on both flexible tube models and arteries in animals. The purpose here is to obtain equations which would describe the relationship between pressure and flow through stenosis of this type.

c) A study of pressure and flow waveforms and appropriate indices, such as the pulsatility index, to determine what are the appropriate measurements which need to be taken so that disorders in the cardiovascular system can be detected early in their development.

d) A study of pressure and flow waveforms to better understand GLOC and its relationships with various cardiovascular parameters.

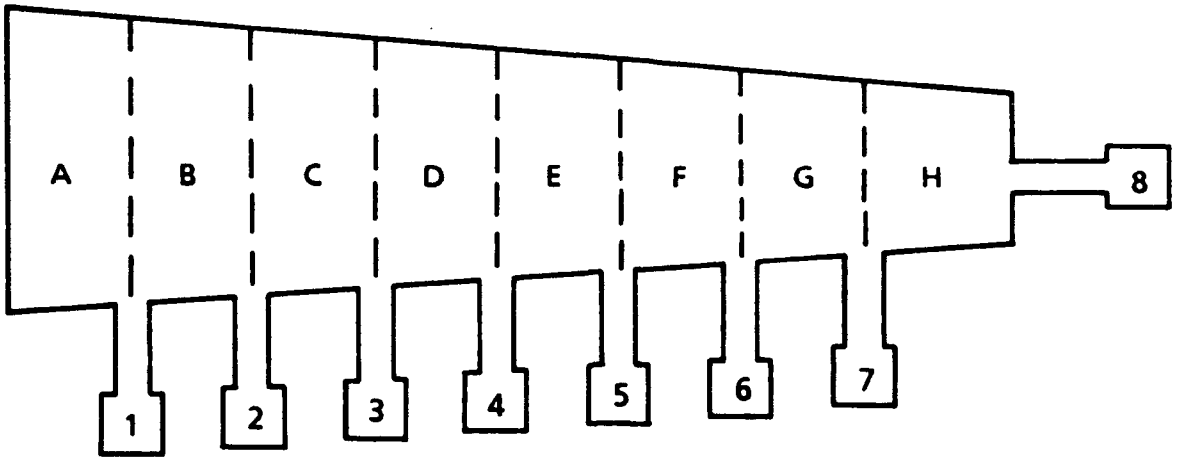
e) The use of parameter estimation which can use noninvasive measurements on the systemic system and then estimate some of the more pertinent parameters, such as the existence of a stenosis, the value of the arterial compliance (which gives information about arteriosclerosis), or the waveform of the heart pressure pulse or the ejection pattern of the left ventricle. This area holds great hope for being able to use the model in a diagnostic mode in conjunction with measured pressure/flow waveforms.

f) The inclusion in the model of feedback and control systems which will reproduce the body's ability to change such parameters as the distal resistance, increase the heart rate, develop collateral circulation, etc. This particular aspect of the study is very complex and can only be accomplished under a long-term commitment of time.

The above give some idea of the magnitude of the problem involved in developing a model of the human arterial system and give some indication of the magnitude of time and effort which must be spent on this development. The result of this endeavor, though, is to be able to aid the medical profession in the detection of many cardiovascular disorders with minor risk to the patient. It is also thought that in many situations early detection of cardiovascular disorders will be possible and thus preventive measures be possible.

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| SEGMENT | | SIDE BRANCH | |
|---|---|---|---|
| A | A. Ascendens | 1 | Coronar A., Truncus Brachiocepahlicus |
| B | Arcus Aortae | 2 | A. Carotis Sinistra<br>A. Subclavia Sinistra |
| C | A. Thoracalis | 3 | Truncus Coeliacus |
| D | A. Thoracalis | 4 | A. Renalis |
| E | A. Abdominalis | 5 | Bifurcation |
| F | A. Iliaca Com. | 6 | A. Iliaca Interna |
| G | A. Femoralis | 7 | A. Profunda Femoralis |
| H | A. Femoralis<br>A. Poplitea | 8 | A. Tibialis Ant.<br>A. Dorsalis Pedis<br>A. Tibialis Post.<br>A. Peronaea |

Fig. 1. Schematic of aorta-abdominal-leg arteries.

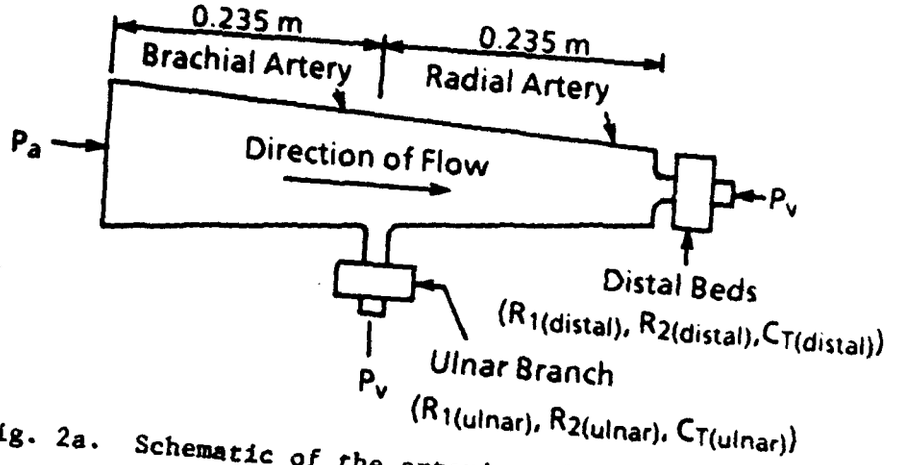



Fig. 2a. Schematic of the arteries of the human arm model.

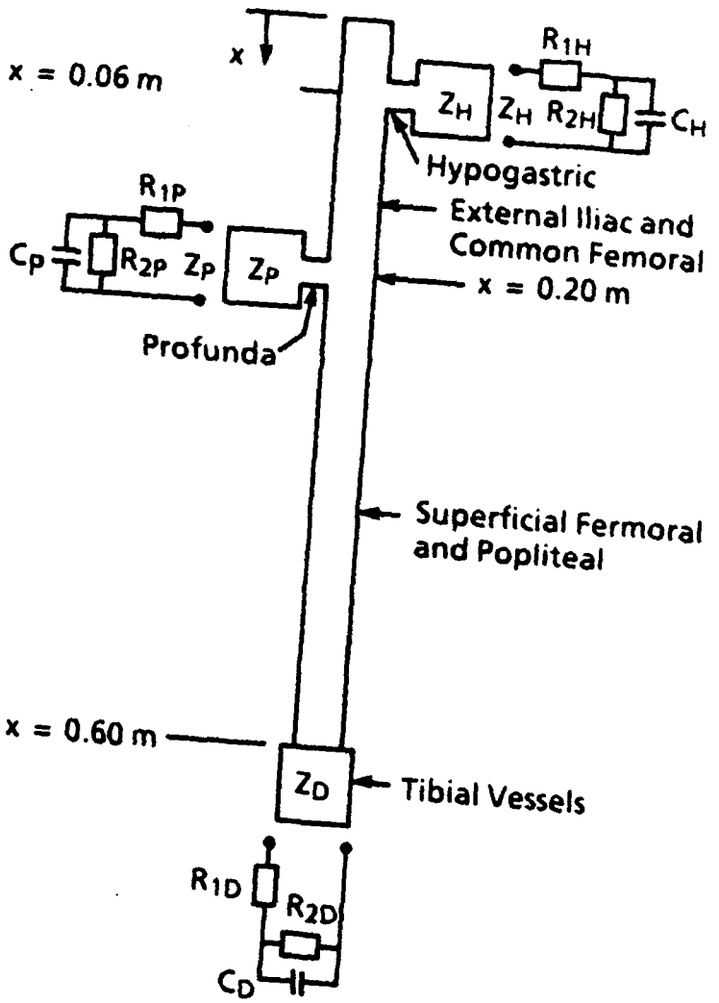



Fig. 2b. Schematic of the arteries of the human leg model.

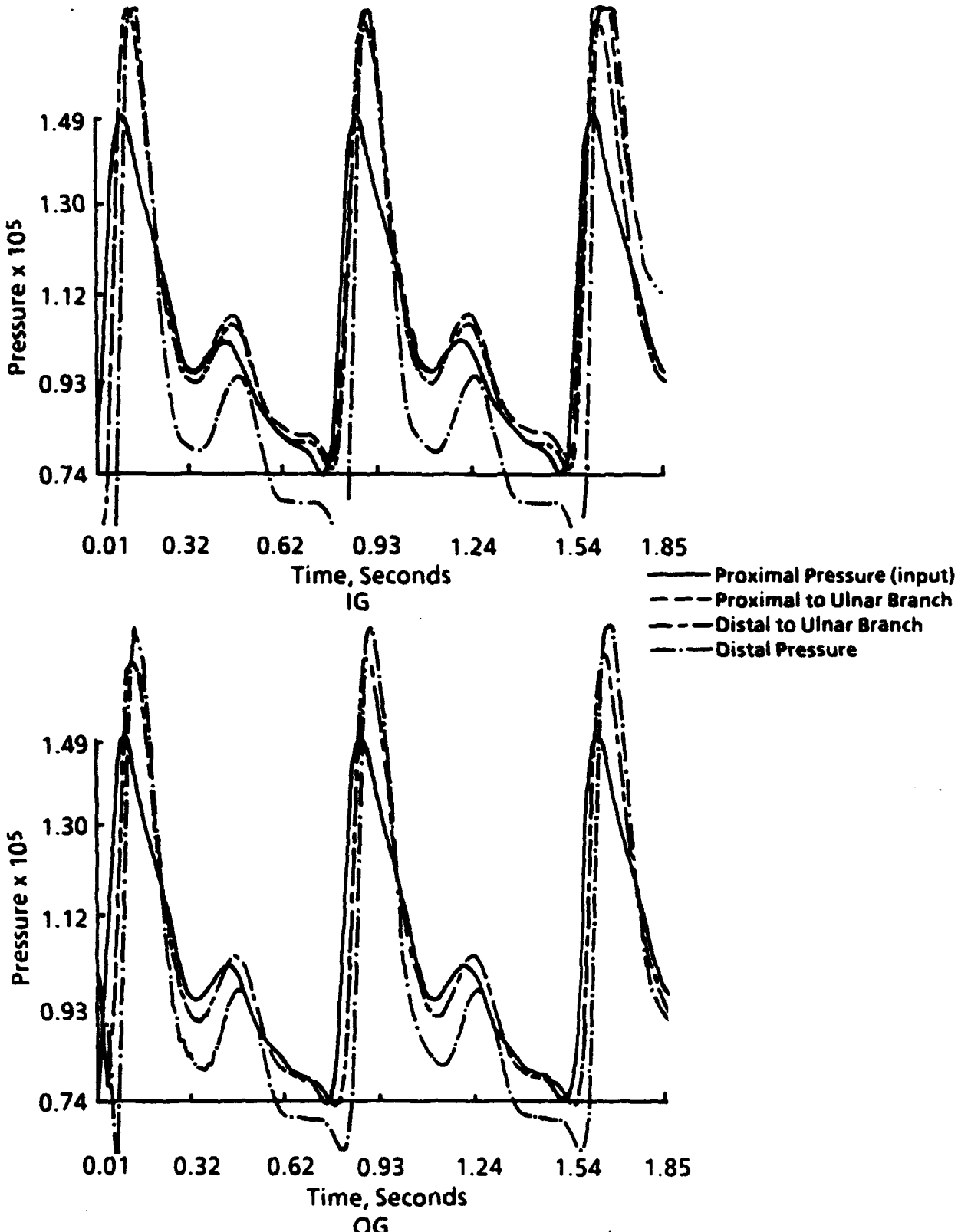



Fig. 3. Pressure waveforms along the Brachial/Radial model with 1G and 0G acceleration.

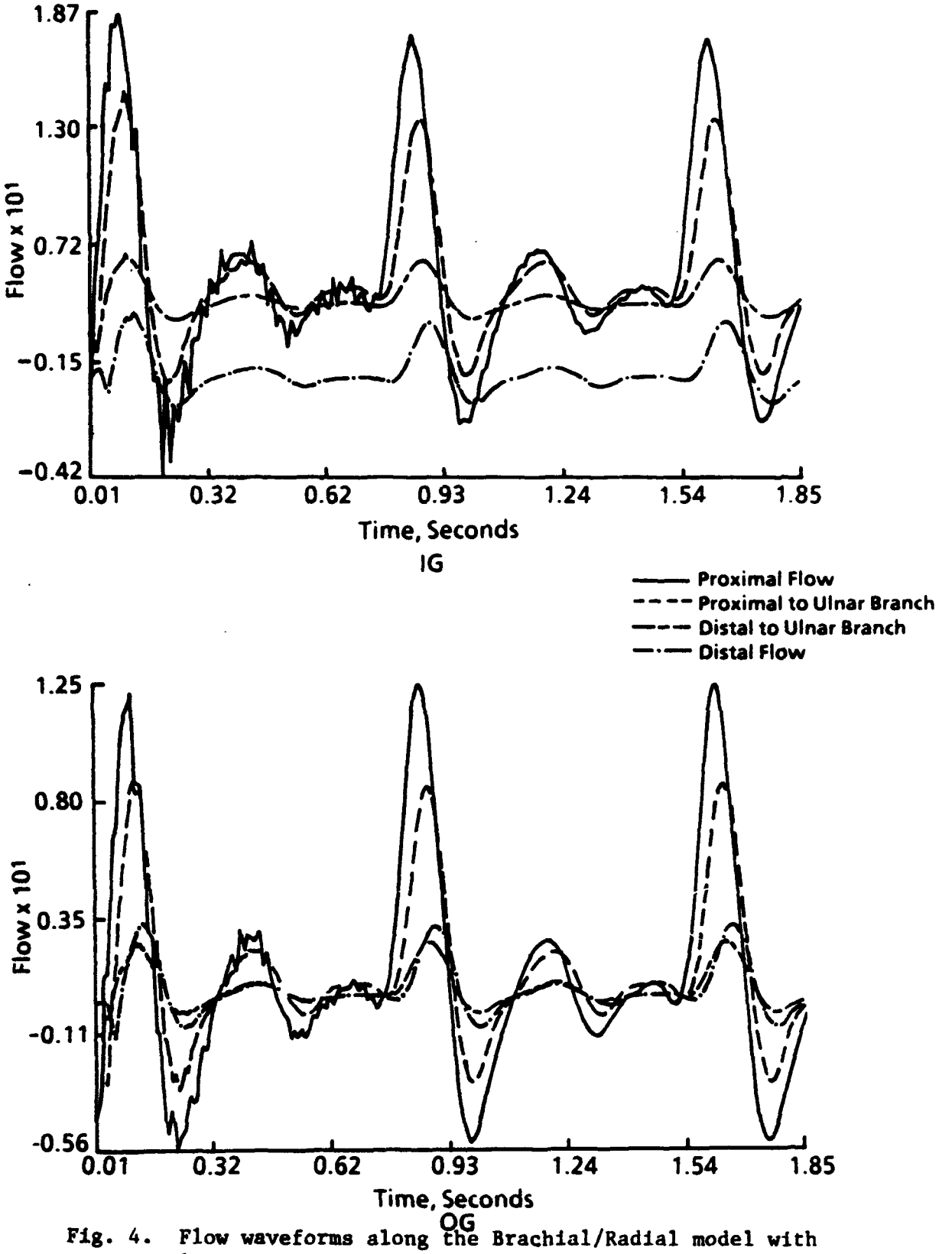



Fig. 4. Flow waveforms along the Brachial/Radial model with 1G and OG acceleartion.

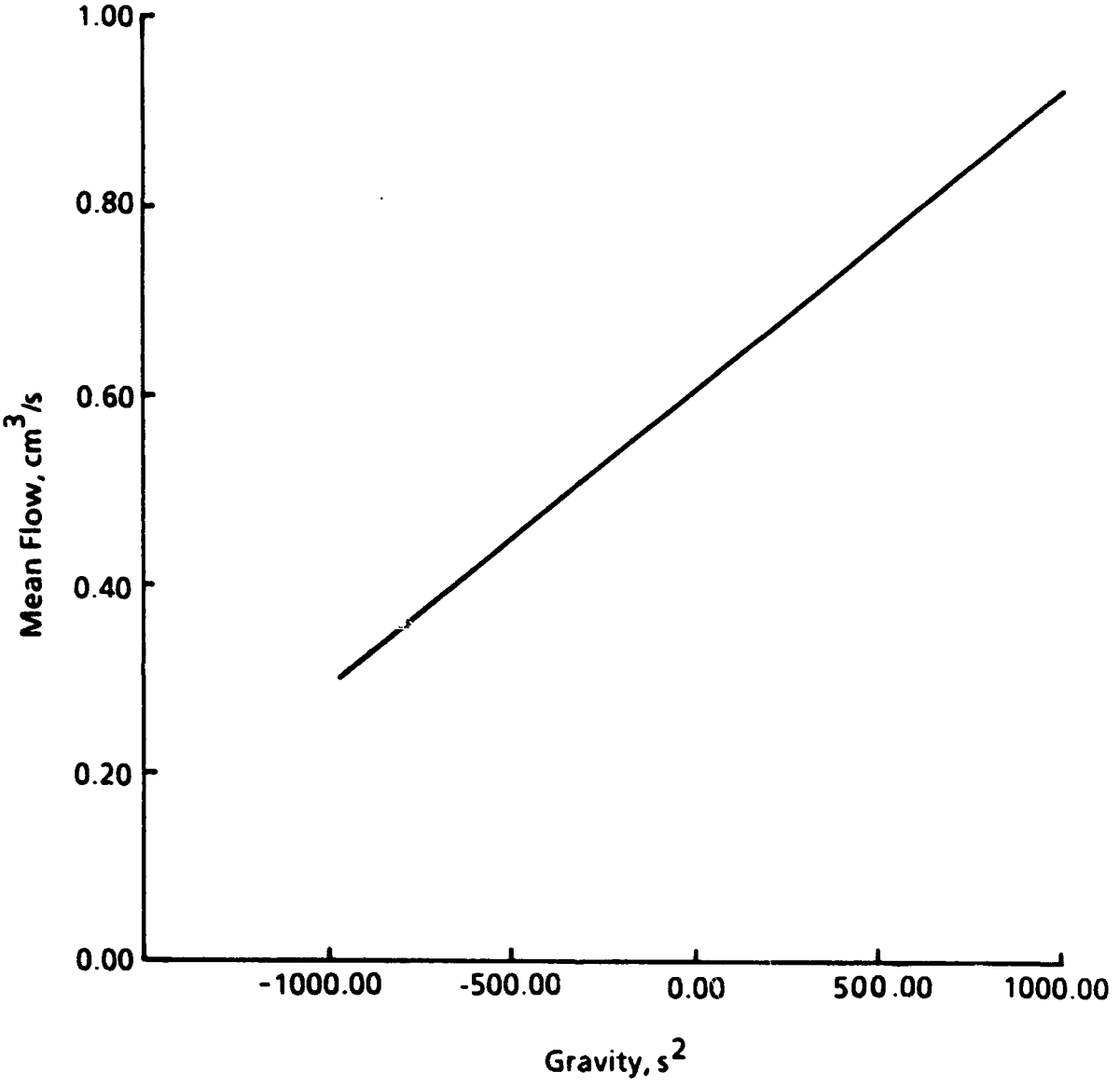



Fig. 5. Mean flow at distal end of the Brachial/Radial model versus G acceleartion.

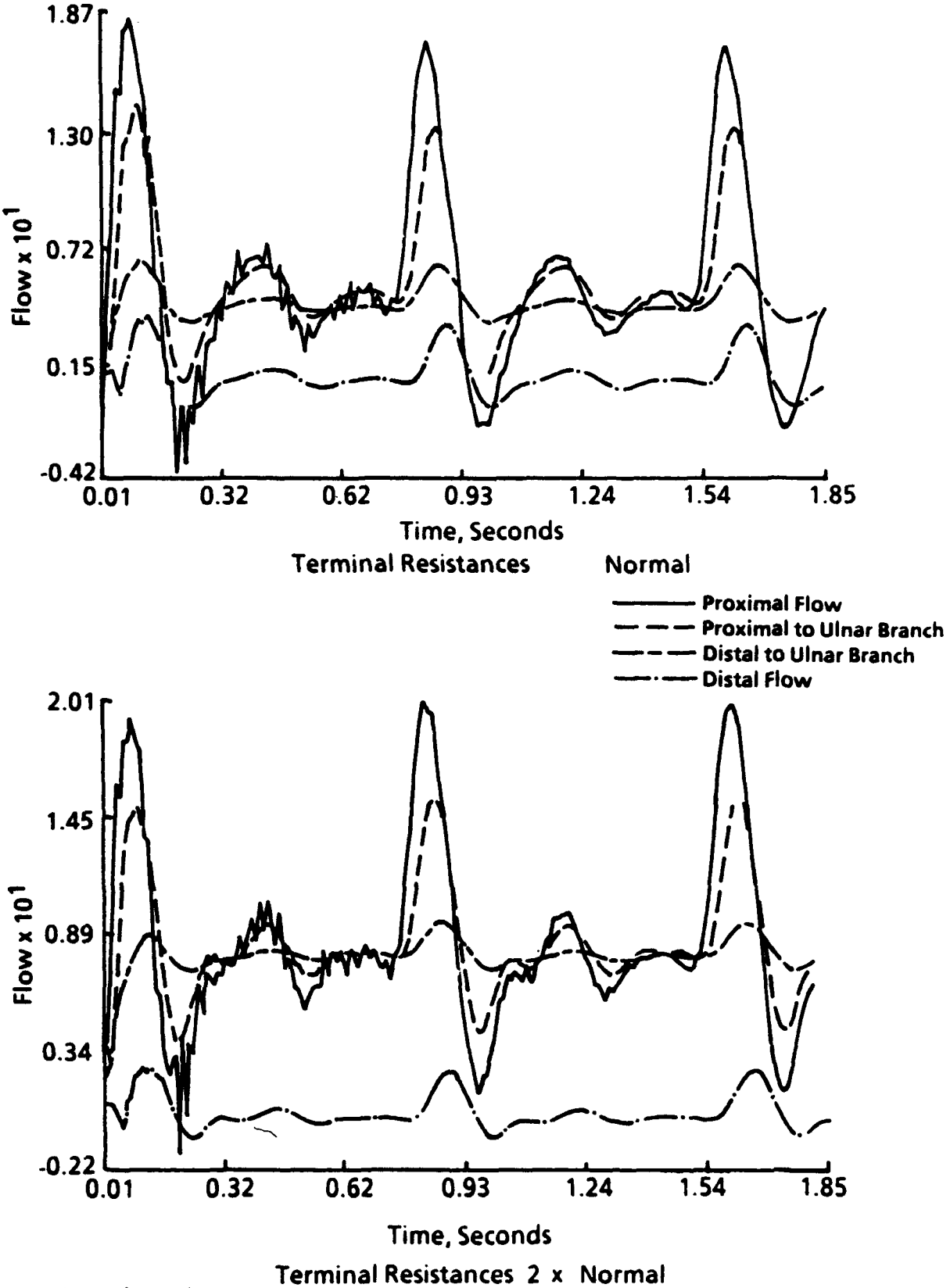



Fig. 6. Flow waveforms in the Brachial/Radial model with 1G acceleration with different values of terminal resistances.

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FINAL REPORT

# THE SEPARATION OF HDL2 AND HDL3 USING THE TECHNIQUE OF ULTRACENTRIFUGATION


| | |
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| Contract No: | F49620-87-R-0004 |

# THE SEPARATION OF HDL2 AND HDL3

# USING THE TECHNIQUE OF ULTRACENTRIFUGATION

by

Joe M. Ross

## ABSTRACT

A Beckman TL100 ultracentrifuge fitted with a TL55 swinging bucket rotor was used to:

(1) separate while serum into two fractions - one containing both LDL and HDL and one containing the remaining serum proteins;

(2) separate whole serum into four fractions - one containing only LDL, one containing only HDL2, one containing only HDL3, and one containing the remaining serum proteins.

After centrifugation, fractions were collected using a Beckman fraction recovery system. The purity of each fraction is checked using a Hewlett Packard HP1090 liquid chromatograph.

## Acknowledgments

I thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. I appreciate Universal Energy Systems help with the administrative aspects of the program.

A number of individuals were instrumental in making the program an enriching one. Dr. Harvey Schwertner provided a number of useful hints with respect to data collection. He also provided encouragement when the experiments were not going smoothly. Without the help of Sgt Clifford Sage, the project would have been impossible. He did a brilliant job in procurement of equipment and supplies. He also provided much needed instructional help with the liquid chromatograph. Dr. Eric Johnson of Ball State University is to be thanked for his preparation of the liquid chromatograph for easy use by a novice. Finally, I thank Leo Mosser for helpful ideas and techniques for the recovery of fractions following ultracentrifugation.

## I. INTRODUCTION

An important aspect of research involving coronary heart disease (CHD) is the identification of biochemical markers. These biochemical markers should predict predisposition towards the disease so that early measures can be implemented to prevent or reduce the effects of the disorder.

Naito (1988) has provided an excellent review of the use of diagnostic markers for assessing coronary heart disease risk. One of the markers that appears to have much promise for the future is a subcomponent of high density lipoprotein (HDL) called HDL2. Studies of persons with CHD and patients with various types of hyperlipoproteinemia seem to indicate that HDL2 is lower in these individuals than in individuals without CHD (Demacker, et al, 1983; 1986). The HDL3 component of HDL, however, does not appear to be different in individuals with and without CHD (Farish, 1983; Gidez, et al, 1982). In order to improve our ability to predict CHD, a rapid and quantitative method for analyzing both HDL2 and HDL3 is needed.

The method of ultracentrifugation is an excellent method and is often referred to as the reference method for lipoprotein analysis. It is however a difficult and slow procedure. Ultracentrifugation does produce pure fractions that can be used for the development of other methods for analyzing lipoprotein subfractions such as high performance liquid chromatography (HPLC). With fractions containing only LDL AND HDL, conditions amenable to the separation of HDL into HDL2 and HDL3 may be investigated without the added complications of other serum proteins. Using the

ultracentrifuge to produce separated fractions of HDL2 and HDL3, allows the investigation of conditions that preferentially affect one versus the other. With clean fractions of HDL2 and HDL3 available, a broad spectrum of separation techniques and conditions for separation and quantitation may be looked at. Thus, the long term objective is to automate the process for quantitative clinical applications. Our objective here is to develope an ultracentrifugation method for reproducibly preparing a total lipoprotein fraction (HDL, LDL, VLDL) as well as fractions of HDL2 and HDL3.

My research interests have always centered around the structural properties of macromolecules. The major classes of molecules of interest have been lipoproteins and dehydrogenases. My earlier work involved cholesterol analyses of both serum and atherosclerotic lesions in the arteries of rabbits fed high cholesterol diets. During the first years of post-doctoral research I worked exclusively with the yolk lipoproteins of the frog Xenopus laevis. This work involved separation, lipid analysis, and low-angle x-ray diffraction. My work with the dehydrogenases also involved many techniques of separations, analysis, and quantitation. Over the years I have been exposed to instruments as diverse as the Model E Ultracentrifuge and computer controlled Enraf-Nonius CAD4 X-ray Diffractometer. This experience may have contributed to my assignment to the Clinical Sciences Division at the School of Aerospace Medicine.

## II. OBJECTIVES OF THE RESEARCH REPORT

My assignment as a SFRP participant was to adapt existing ultracentrifugation techniques to the Beckman TL100 table top ultracentrifuge. The

specific objectives were as follows:

(1) Develop an ultracentrifugation method for isolating total lipoproteins, i.e., HDL, LDL, and VLDL free of albumin and other serum proteins.

(2) Develop a gradient system suitable for the separation of HDL2 and HDL3 free of albumin and other serum proteins.

(3) Monitor the homogeneity of the lipoprotein fractions using molecular exclusion HPLC.

## III. APPROACHES AND RESULTS

### a. Approaches

(i) To prepare the serum fraction containing only low density lipoprotein (LDL) and high density lipoprotein (HDL), fresh fasted serum is adjusted to a density in the range of 1.200 g/ml to 1.220 g/ml. This is accomplished by using a calibrated solution of potassium bromide (KBr) of density equal to 1.362 g/ml. The desired density is obtained by mixing 3.95 ml of serum with 4.85 ml of the KBr solution. These values are not to be rounded. Choose appropriate integer - and fractional-valued pipetters. A total of 8.80 ml of adjusted serum is distributed equally between four Beckman ultraclear centrifuge tubes whose maximum capacity is 2.20 ml each. The unsealed tubes are placed in the centrifuge buckets, capped, checked for balance, and seated in the model TL55 swinging bucket rotor. Centrifugation is for 18.5 hours at 55000 rpm (g average of 201,247) and a temperature of 20 degrees Celsius. The centrifuge is programmed to accelerate to 5000 rpm in one minute, after which it accelerates to

55000 rpm at full acceleration. The centrifuge is set to deaccelerate without braking.

After centrifugation, each tube is fractionated using a Beckman fraction recovery system. Tubes waiting to be fractionated are placed in a refrigerator to help reduce diffusion. Each tube is mounted in the recovery apparatus and punctured. The needle is inserted just far enough into the tube to initiate drainage. (The depth of needle insertion should be determined prior to actual fractionation by using tubes containing water.) The first 1.6 ml of solution is collected and discarded. The remaining 0.6 ml is collected in three 0.2 ml aliquots. A 40 microliter sample of each aliquot is injected into a Hewlett Packard HP1090 liquid chromatograph to check for albumin. Commercially obtained non-denatured human serum albumin is used as a marker.

(ii) The procedure for preparing HDL2 and HDL3 subfractions is essentially that of Demacker, et al (1983). Due to the small size of the centrifuge tubes used in the present study, fiduciary marks were placed on the tubes with a fine point water proff marker. Eleven dots were placed such that the space between dots represented 0.2 ml. This is accomplished by pipetting 0.2 ml aliquots of water with an Eppendorf pipetter and placing a dot on the surface of the tube after each addition. The tube is then emptied and dried by blotting the inside with an appropriate tissue. On the opposite side of the vertical column containing the 11 dots, a column of 4 dots was placed on the surface of the tube by pipetting in sequence the following volumes of water; 0.370 ml, 0.366 ml,

0.732 ml, and 0.732 ml. The set of 11 dots is used as markers when collecting separated lipoprotein bands. The set of 4 dots is used for layering the discontinuous gradient. Due to the small tube size, each must be individually marked using the above scheme.

Two 2 ml aliquots of serum is adjusted to a density of 1.250 g/ml as described by Demacker (1983). To one of the 2 ml aliquots 20 microliters of freshly prepared and filtered Coomassie Brilliant Blue (15 g/L) is added. At least one tube per centrifuge run should contain stained serum so that the separated bands can be easily visualized. This aids considerably in collecting the fraction from tubes containing unstained serum.

Tubes are filled according to the following scheme. Using an appropriate pipette, add 0.370 ml of stained or unstained serum (density=1.250 g/ml). This brings the volume to the first layering fiduciary mark. Then using a 1 ml tuberculin syringe fitted with a 22 gauge needle, carefully layer a potassium bromide (KBr) solution (density=1.225 g/ml) to the second layering fiduciary mark. Follow this by layering a KBr solution (density=1.100 g/ml) to the third layering fiduciary mark. Finally, layer distilled water to the fourth layering fiduciary mark. The total volume of layered liquids is 2.20 ml. Since the TL55 swinging bucket rotor holds a maximum of four tubes, either 2 or 4 tubes (one of which contains stained serum) should be prepared. However, with tubes of the size used in the present study, mixing of the layers due to diffusion is a big problem. It is suggested that initially only two tubes be prepared and that centrifugation is started

as quickly as possible. Tubes that have been layered should be placed in the tube buckets, capped, and stored in a refrigerator until the others are prepared. After all tubes have been prepared, capped, and checked for balance; they are centrifuged for 18.5 hours at 55000 rpm and 20 degrees Celsius. These conditions are identical to those described earlier. The collection of fractions from these tubes will be described at the appropriate point in the next section.

b. Results

(i) To check the purity of the total lipoprotein fraction (HDL, LDL, VLDL), an HP1090 liquid chromatograph fitted with a TSK5000PW and two TSK3000SW molecular exclusion columns was used. The mobile phase was 0.15 M sodium chloride. The flow rate of the mobile phase was 0.9 ml/min. With this experimental setup, LDL, HDL, and albumin have retention times of approximately 37.8, 50.1, and 52.8 minutes, respectively. When 40 microliters of the 0.2 ml fractions (collected as described earlier) are injected, the top most fraction produces only two peaks with retention times equal to those of LDL and HDL. The second (from the top of the tube) 0.2 ml fraction contains only traces of HDL. The third 0.2 ml fraction contains a small amount of albumin and no HDL. Thus the ultracentrifugation procedure developed here, using serum which has been adjusted to a density between 1.200 and 1.220 g/ml, results in the complete separation of serum lipoproteins from other non-lipid containing proteins. The lipoproteins are also concentrated in the top 0.2 ml of the centrifuge tubes.

(ii) The collection of HDL3, HDL2, and LDL fractions resulting from the layering technique are based on observations made with stained serum. Tubes are mounted in the Hewlett Packard Fraction collecting system and punctured as described earlier. Starting from the bottom, the first 0.6 ml is collected and discarded. This fraction contains albumin and the other heavy serum proteins. The next 0.6 ml, 0.4 ml, and 0.6 ml contain HDL3, HDL2, and LDL, respectively. These fractions are collected individually.

## IV. RECOMMENDATIONS

a. As stated in the objectives, the purpose of this project was to develop an "in house" procedure for fractionating serum lipoproteins. In this study we did not address the problem of quantitation. Follow-up research should include investigations of methods to assay quantitation. Accuracies and reproducibilities should also be considered. The pure lipoproteins can also be used to establish electrophoretic and/or chromatographic techniques for the separation of HDL into its subcomponents, HDL2 and HDL3, that are suitable for hospital laboratories. Such research would be aided by the fact that individual fractions obtained by ultracentrifugation could be used as reference standards or markers.

b. Two very important aspects of this work are the layering of gradients and the collection of individual lipoprotein fractions. A rapid procedure for layering the density gradients into the small tubes would be useful. Such methods can be developed and should be developed in order to speed

up and simplify the procedure. One should also consider using a tube slicer for the collection of the individual lipoprotein fractions. A tube slicer would minimize mixing during the collection process, thereby resulting in more accurate and reproducible results.

c. All lipoprotein fractions contain large quantities of potassium bromide. Thus, each fraction should be dialyzed against buffered 0.15 M NaCl. Also, the volume of each fraction should be carefully determined so that the concentrations of protein and/or cholesterol can be related to those of whole serum.

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# FINAL REPORT

# "An Experimental Design To Demonstrate The Dispersion Effects Of Salt Water On OPTICAL PULSES"


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| Date: | July 22, 1988 |
| Contract No.: | F49620-87-R-0004 |

# An Experimental Design To Demonstrate The Dispersion Effects Of Salt Water On Optical Pulses

By

Dhiraj K. Sardar

## ABSTRACT

First, the dispersion properties of electromagnetic pulses are elucidated in this report, especially, the spreading of electromagnetic pulses traveling through a dispersive medium is discussed in detail. Second, the qualitative differences between the classic and the modern descriptions of the so-called precursors are illustrated. Finally, described is a detailed experimental design which can be employed to observe the spreading of optical pulses propagating through salt water and thus demonstrate their dispersion effects.

## ACKNOWLEDGEMENTS

I wish to thank both the Air Force Systems Command and the Air Force Office of Scientific Research for their sponsorship of this Research. I also wish to gratefully acknowledge the Universal Energy Systems for their sincere assistance to me in all administrative and directional aspects of this program.

My experience in the Summer of 1988, has been professionally rewarding and enriching. I would like to express my deepest gratitude to Dr. Richard A. Albanese of USAFSAM/RZM for his invaluable help and encouragement throughout this research. The help from 2Lt. Erik Nielsen in the excellent drawing of schematic of the experimental design is greatly appreciated. I wish to acknowledge the important conversation I had with Dr. K.E. Oughstun of University of Vermont. Finally, I would like to thank all the people at USAFSAM for providing me a truly enjoyable working atmosphere. The discussions with Dr. Ralph Allen regarding the possibility of performing the proposed experiment using the facility of the laser laboratory here at USAFSAM/RZV have been very helpful and I sincerely appreciate his help.

Finally, I wish to extend my thanks to Dr. Phelps Crump, Dr. David Erwin, Mr. John Penn, Mr. Richard Medina, Ms. Julia Rennenkampff, Ms. Edith Wood, and Ms. Elizabeth Toth for their help which made my work here as enjoyable and memorable as could be.

# AN EXPERIMENTAL DESIGN TO DEMONSTRATE THE DISPERSION EFFECTS OF SALT WATER ON OPTICAL PULSES

## I. INTRODUCTION:

Although the consequences of dispersion effect of electromagnetic fields propagating through dispersive media have recently become of critical importance in numerous practical applications such as optical communication, integrated optics systems, etc., rigorous theoretical investigations of the propagation of electromagnetic field was undertaken by a number of eminent scientists in the early 1900's. The problem of wave propagation through an absorptive medium with a strong absorption band, a region of anomalous dispersion, was first considered by Laue [1], and then by Sommerfeld [2]. If the medium is nondispersive, the pulse would propagate with its initial phase velocity through it without being distorted. But the pulse shape becomes greatly modified as it propagates through a dispersive medium because both the phase- and amplitude-relationships among the spectral components of initial pulse change with propagation distance. These phase and amplitude changes result in a complicated structural change in the propagating field. Due to such intricate distortion of the signal, it is very difficult to define propagation velocity for signals whose frequencies fall within an absorption band where the group velocity can exceed the vacuum speed of light c, without contradicting the special theory of relativity. Sommerfeld [2], however, showed that no signal can travel at a velocity greater than c. Brillouin [3,4] then extended the theoretical work and successfully defined the signal velocity which is a function of frequency in the absorption band. The important result

of their classic work dictates that for initial pulses with appropriate rise- time, the dispersive nature of the medium can be manifested through the formation of precursor fields followed by the signal. The formation of these precursors exclusively depends on the excitation field and the dispersive properties of the medium.

The acceptable level for electromagnetic radiation is currently set for only continuous wave (CW) fields. Nonetheless, the exposure level due to transient fields generated by electromagnetic pulses in dispersive media e.g., body tissues, can be significantly different from that due to CW field of the same frequency and energy density. Since the radiation level received by the human body from various electromagnetic sources is of great concern from the health point of view, it is critically important that a complete understanding must be gained of the dynamical evolution of pulsed fields in dispersive media as well as the materials conditions of media which are responsible for the formation of such fields.

## II. OBJECTIVE OF THE RESEARCH EFFORT:

Even though the dynamical evolution of the transient precursors and the steady-state signal fields in dispersive medium has been extensively studied theoretically for quite sometime, the experimental observation of these precursors only in the microwave regime was first reported in 1969 by Pleshko and Palocz [5]. Due to the lack of adequate technology however, no experiment demonstrating the precursors in the optical domain has hitherto been reported. The type of excitation field [6] necessary for the formation of these precursors is not yet

available. Since the body tissue contains a high percentage of water and is highly dispersive, it will be of importance to determine the effect of dispersion of water in optical domain. As a participant of the 1988 Summer Faculty Research Program (SFRP), my major objectives are to have an in-depth understanding of the physics of such a critically important field as this and to design an experiment that will enable us to demonstrate the effect of dispersion of water on optical pulses. This experiment will hopefully be performed here at the Brooks AFB, San Antonio, with funding from the Mini Grant Program. The proposed experiment will serve as a pilot research project and we are optimistic that we shall be able to demonstrate the existence of Brillouin precursor in the optical domain in the near future.

## III. BRIEF REVIEW OF THE THEORY

The propagation of an optical pulse through a dispersive medium has an enormous influence on the frequency dependence of index of refraction of the medium. In such a medium, the pulse shape is modified during its propagation. As a consequence, the main body of the pulse travels with a velocity quite different from either the phase velocity or vacuum speed of light c, and is preceded by the so-called precursors or forerunners. In order for an experimentalist to observe these precursors, the conditions for the medium as well as the excitation field must be properly satisfied. The foundation of the theoretical study was laid by Sommerfeld and Brillouin in their classic work. Brillouin first introduced the method of steepest descent path to analyze the propagating electromagnetic pulses through the classical

Lorentz medium. Their theory provides analytic expressions for pulse dynamics under proper dispersive conditions in a Lorentz medium which serves as a model system. After a good length of time, this work is however, reexamined and extended by several researchers [7-9] to include more complicated systems using asymptotic technique. Recently, Oughstun and Sherman [10] have shown quantitative improvement of Brillouin's results using numerical technique and rigorous asymptotic analysis. Their work provides more complete description of the entire precursor field evolution and signal arrival for optical pulse propagation in a Lorentz medium. A brief account of the dispersion effects of optical pulses propagating through dispersive media is presented here.

The wave equation obtained from the Maxwell's equations can be expressed as:

$$\nabla^2 u - (1/v^2)(\partial^2 u/\partial t^2) = 0 \qquad (1)$$

where u represents the electric (E) or magnetic (B) components, and

$$v = c/\sqrt{\mu\epsilon}$$

The plane wave solution of Eq.(1) can be expressed in the following form:

$$u = A \exp[ikx-wt] \qquad (2)$$

where $k = w/v = (w/c)\sqrt{\mu\epsilon}$

$= (w/c)\sqrt{\epsilon}$ (for nonmagnetic medium, $\mu=1$)

The phase velocity of the propagating wave is defined by

$$v_p = w/k$$

If a medium is dispersive, the dielectric constant is a function of frequency w, and is given by [11] :

$$\epsilon(w) = 1 + w_p^2 /(w_o^2 - w^2 - 2i\delta w)$$

where $w_p = 4\pi Ne^2/m$ is known as plasma frequency of the medium, N is the number density of electrons of charge e and mass m bound with resonant frequency $w_o$ , and $\delta$ is the damping constant of these bound electrons.

The wave number can now easily be written as

$$k = (w/c)[1 + w_p^2 /(w_o^2 - w^2 - 2i\delta w)]^{1/2}$$

Thus k is a complex wave number which can be conveniently expressed in the following form :

$$k = \beta + i\alpha/2$$

where the imaginary part $\alpha$ represents the attenuation constant or the absorption coefficient.

In the foregoing discussions of plane-wave solutions and their properties, only monochromatic waves having definite frequency and wave number were considered. But in reality, even in the best available laser, we find a finite spread of wavelength or frequencies governed by

the Heisenberg's uncertainty principle:

$$\Delta w.\ \Delta t \gtrsim 1 \qquad (3)$$

The peak power of a pulsed laser is normally very much higher than the output power of a CW laser. The more the light energy is concentrated in time, the more it spreads spectrally. However, the frequency spectrum of a light pulse whose envelope is of Gaussian shape has a Gaussian spectral distribution about the central frequency [12]. Here $\Delta t$ can be considered as full width at half maximum of Gaussian pulse and $\Delta w$ as that of the spectrum. This spreading may arise from many sources such as finite duration of pulses, inherent broadening in the source, etc. The pulse can therefore be expressed as a linear superposition of the monochromatic components with different frequencies, each of them propagating through the medium with its own phase velocity and absorption condition. Since the dielectric constant of the medium is a function of frequency of the field, the phase velocity is different for each component. As a consequence, the relative phases and amplitudes (assuming the medium is also dissipative) of the components change as the initial pulse propagates through the medium, resulting in a distortion of the entire optical field. The important ingredients of the dispersive effects are implied in the idea of Fourier integrals [13].

In order to include the dispersive effect in the plane wave solutions (2), one might consider the frequency w as a function of wave number k, or vice versa. In general, the frequency is a smoothly varying function of wave number, except for the regions of anomalous dispersion where w varies quite abruptly over a small band of

wavelength. Using Fourier representation [13,14], the general plane-wave solution (2) can be written as:

$$u(x,t) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} A(k) \exp[i(kx-w(k)t)]dk \qquad (4)$$

The amplitude A(k) describes the properties of the linear superposition of waves with different frequencies. At t=0, the amplitude is given by the following :

$$A(k) = (1/\sqrt{2\pi}) \int_{-\infty}^{\infty} u(x,0) \exp[-ikx] \, dx \qquad (5)$$

If u(x,t) represents a finite wave train with a finite pulse-width, then A(k) is not a delta function [13], and it has rather definite spread of frequency $\Delta w$, obeying the condition given by Eq.(3), which means that shorter the pulse, wider is the distribution of frequency. As the pulse propagates, the different components having different w and k in the pulse envelope move with different phase velocities. As a consequence, the original pulse shape loses its initial coherence and becomes distorted. Also the velocity of wave propagation is then expected to be conspicuously different from its initial value.

a. Spreading of Pulse in Dispersive Medium:

It is now important to state the initial-value problem for the wave equation which demands initial values of both u(x,0) and $\partial u(x,0)/\partial t$. If we consider the real part only, Eq.(4) can be written as:

$$u(x,t) = (1/2)(1/\sqrt{2\pi}) \int_{-\infty}^{\infty} A(k) \exp[i(kx-w(k)t)] \, dk + c.c. \qquad (6)$$

then A(k) can easily be expressed in terms of initial values as:

$$A(k) = (1/\sqrt{2\pi})\int_{-\infty}^{\infty}(u(x,0) + [i/w(k)][\partial u(x,0)/\partial t]\ \exp[-ikx])\ dx \qquad (7)$$

If A(k) is sharply peaked around $k = k_0$, then w(k) can be expanded as:

$$w(k) = w_0 + (dw/dk)_o (k-k_o) + \cdots \qquad (8)$$

Substituting (8) in (4), and after integrating and rearranging them we arrive at

$$u(x,t) \simeq u[x-(dw/dk)_o t,\ 0]\ \exp[i(k_o(dw/dk)_o - w_o)]t \qquad (9)$$

This shows that besides an additional phase factor , the pulse travels along undistorted in shape with a velocity, called the "group velocity":

$$v_g = (dw/dk)_o \qquad (10)$$

For an optical wave, w(k)= ck/n(k), hence the phase velocity can be expressed as:

$$v_p = w(k)/k = c/n(k) \qquad (11)$$

Considering now frequency as a function of wave number, Eq.(10) can be conveniently expressed in the following form:

$$v_g = c/[n(w) + w(dn/dw)] \qquad (12)$$

In order to elucidate the spreading property of pulses in a dispersive medium, a specific model needs to be considered in which k depends on w. It is then possible to calculate without approximation the pulse propagation in that model medium. Following Jackson [13], if we take a Gaussian modulated oscillation as the initial shape of the pulse:

$$u(x,o) = \exp\left[-x^2/2L^2\right] \cos k_o x \tag{13}$$

The time derivative of this pulse is assumed to be zero. This means that at times $t < 0$, the wave consisted of two pulses, both moving towards the origin so that at $t = 0$, they coalesce into the shape given by Eq.(13). At times $t > 0$, the pulse breaks into two, one moving to the left and the other to the right. The amplitude A(k) in Eq.(7) can now be written by substituting and performing straightforward integration in the following form:

$$A(k) = L/2 \left\{\exp\left[-(L^2/2)(k-k_o)^2 + \exp\left[-L^2/2\right)(k+k_o)^2\right]\right\} \tag{14}$$

For a dispersive medium, the dispersion relation of plasma can be approximated [13] as follows:

$$w(k) = \omega_c\left(1 + a^2 k^2/2\right) \tag{15}$$

where $\omega_c$ is a constant frequency, and a represents a typical wavelength at which dispersive effects become prominent. Since the initial pulse is a modulated wave at $k=k_o$, the two pulses will travel with the

following group velocity:

$$v_g = (dw/dk)_o = \omega_c a^2 k_o \qquad (16)$$

and will remain almost unaltered in shape provided the pulse is not narrow enough in space.

Substituting (14) in (6), and performing a little mathematical jargon, the exact nature of the pulses can be given by the following expression:

$$u(x,t) = 1/2\ \mathrm{Re}\Big\{ \frac{\mathrm{Exp}[-(x-\omega_c a^2 k_o t)^2 / 2L^2(1+ia^2\omega_c t/L^2)}{(1+ia^2\omega_c t/L^2)^{1/2}}$$

$$\times\ \mathrm{Exp}[ik_o x - i\omega_c(1 + a^2 k_o^2/2)t] + (k_o \rightarrow -k_o)\Big\} \qquad (17)$$

which represents two pulses traveling in opposite directions. The peak amplitude of each pulse is expected to travel with the group velocity given by (16). Although the modulation envelope remains Gaussian shape, its width is not constant, but increases with time. The width of the envelope is

$$L(t) = [L^2 + (a^2\omega_c t / L)^2]^{1/2} \qquad (18)$$

This expression demonstrates that for a given elapsed time t, the sharper the envelope, the greater is the dispersive effect. For initial pulse width $L \gg a$, there will be an insignificant change in pulse-shape even after a finite time spent in the medium. The second term in Eq.(18) indicates that at long times, however, the width L(t) of the

Gaussian pulse increases linearly with time. An example of how rapidly a sharper pulse spreads as a function of time compared with a wider one is portrayed in Fig.1 (Ref.13). On the left side of this figure, is shown a pulse width $L \gg 1/k_o$ i.e., the initial pulse width is much larger compared with the wavelength; hence it does not spread rapidly with time. On the other hand, the pulse having an initial width $L \ll 1/k_o$, shown on the right side of Fig.1, spreads very rapidly.

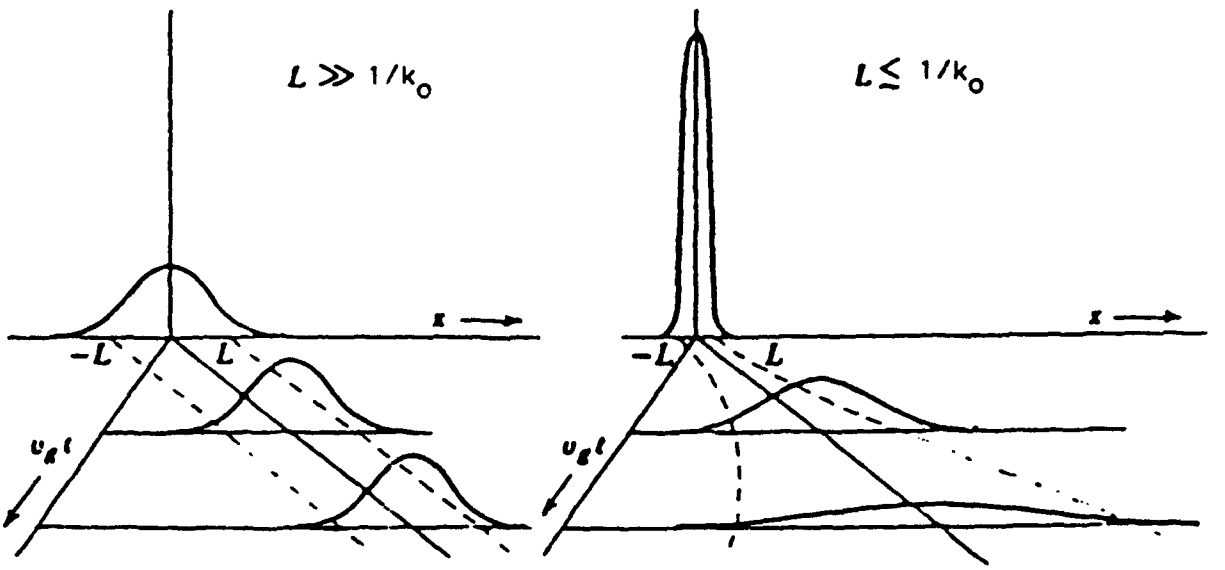



Fig. 1. The broadening of electromagnetic pulses having two different initial pulse widths as they propagate through a dispersive medium. (Figure is taken from Ref.13. )

Even though the above results are obtained for a special choice of initial pulse (Gaussian) and dispersion relation (approximation of plasma), their implications are quite general in nature. The spreading of a pulse however, can be reckoned by noting that a pulse with initial spatial width $\Delta x_o$, must have within it a spread of wave numbers

$\Delta k \sim 1/\Delta x_o$ . This means that the group velocity evaluated for various values of k within the pulse, has a spread in it of the order

$$\Delta v_g \sim w'' \Delta k \sim w'' / \Delta x_o \tag{19}$$

where $w'' = dw'/dk = d/dk(dw/dk)$

At a time t, this indicates that a spread in position is approximately equal to $\Delta v_g . t$. Combining the uncertainties in position by taking the square root of the sum of squares, the width $\Delta x(t)$ at time t, can be given [14] as:

$$\Delta x(t) = [(\Delta x_o)^2 + (w''t/\Delta x_o)^2]^{1/2} \tag{20}$$

Eqns. (18) and (20), therefore, agree exactly if we put $\Delta x_o = L$. The above expression (20) represents more general result that if $w'' \neq 0$, a narrow pulse spreads rapidly because of its broad spectrum of wave numbers.

b. Evolution of Precursors:

If the conditions of the medium as well as the excitation field are right, the transient precursors appear just before the steady state signals show up. The formation of these precursors exclusively depends on the intricate nature of the medium and on the proper excitation field. Theoretical studies conclusively show that the precursors will be formed if the medium is a dispersive one and also the exciting field be a pulsed source with an extremely fast rise-time [6]. In fact, the origin of the fields is realized through the consideration of the

principle of superposition of monochromatic plane waves, each having a certain amplitude and a definite phase relation with respect to the other monochromatic component within the pulse envelope, and thereby traveling with its own characteristic velocity.

It was Lord Raleigh [15] who first defined the group velocity during its characterization in sound wave. Ever since, this definition of group velocity has been applicable in virtually every wave analysis. Lord Raleigh considered that the group velocity would correspond to the velocity of signals or energy propagation. But it was later found that the group velocity could become larger than c, or even negative within an absorption band of a dispersive medium. This immediately raised a serious contradiction with the theory of relativity which states that no velocity can exceed the speed c. Sommerfeld took this problem with great interest and accurately demonstrated that the signal does not always propagate with the group velocity and showed that even though group velocity may be greater than c in some cases as pointed out earlier, the signal velocity is always less or equal to c. Sommerfeld introduced [2] the general mathematical method to discuss the wave propagation in a dispersive Lorentz medium and showed that the front of the signal could progress with c. The theory indicates that the signal is profoundly distorted in a dispersive medium. The medium is initially at rest, then the front appears with the speed c, but this front corresponds to infinitely small fields and electronic motions, both of which gradually build up. Although Sommerfeld did not obtain the complete physical picture of this complicated nature of the signal propagation, his classic theory provided a precise formulation. According to his theory, the signal does not retain its original shape

after passing through a certain depth in a dispersive medium, but on the other hand, very weak signals appear at first which are called precursors. Brillouin extended this important work and discussed the problem in great detail with a complete answer as to the physical nature of the precursors.

Brillouin [3,4] first introduced the mathematical method of steepest descent path in his analysis of the signal propagation through a dispersive Lorentz medium, and obtained asymptotic behavior of the signal. In using this method, the contour integration containing a complex-valued phase function is deformed through the saddle points of such a function along the path of the steepest descent. Therefore, the main contribution to the integral is due to the immediate region about the dominant saddle point as the point of observation moves to infinity with the field. Using the saddle point technique, Brillouin also showed that the signal velocity is smaller than the velocity c, and is equal to the group velocity except in the region of absorption where the group velocity can be greater than the velocity c or even negative. Thus the raised contradiction with the theory of relativity is resolved once again. According to Brillouin's analysis, the appropriately chosen signal after penetrating a certain depth of the dispersive medium becomes distorted and it does not arrive suddenly, but there is a quick and continuous transition from the very week intensity of the precursors to that of the signal. The first precursors known as Sommerfeld precursors, arrive with a velocity equal to the velocity c, and very small period which gradually increases until it reaches the characteristic period of the oscillating electrons of the medium. The second precursors named after Brillouin, arrive with velocity smaller

than the velocity c, and very large period which decreases until it is equal to the characteristic frequency of the electrons. The amplitude of both the precursors is initially very small, but increases rapidly as their periods approach to that of the signal, and then decreases due to the damping of the medium. These precursors can also overlap at the transition regions. Finally the signal arrives with signal velocity, but is still deformed for a short time thereafter due to the overlapping of precursors. The entire evolution of these precursors and the signal according to Brillouin, is shown in Fig.2 (Ref.4).

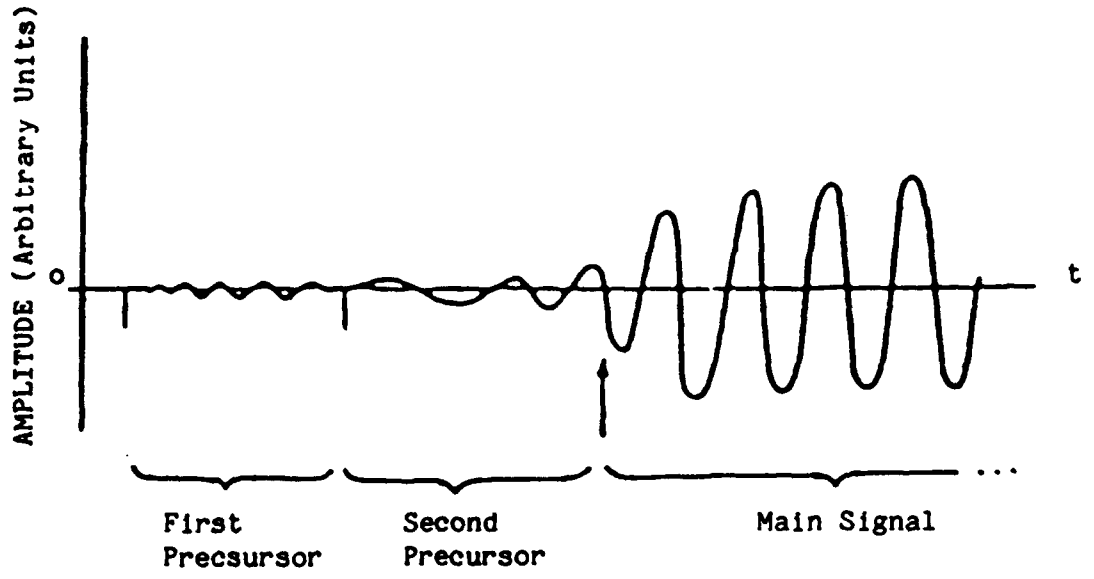



Fig. 2. Time variation of the entire field propagating through a Lorentz-type of dispersive medium. (Figure is taken from Ref. 4.)

Oughstun and Sherman [10] have recently extended the classic analysis of Sommerfeld and Brillouin and provided more accurate description of the entire precursor field evolution and signal arrival for an appropriate pulse propagating through a dispersive Lorentz

medium. Their result is qualitatively similar to that predicted by Brillouin, but the quantitative improvements are quite significant. In their analysis, they have shown by the numerical techniques that Brillouin's approximations for saddle-point locations break down in certain space-time regime. They have also derived more accurate analytic approximations which describe the saddle-point behavior determining the pulse dynamics. Their results provide a correct representation of the dynamic evolution of the Sommerfeld and Brillouin precursors over the entire time range. Unlike Brillouin's prediction, their analysis shows that the initial amplitude of both precursors is very large and then both decrease steadily. This modified dynamic behavior of the entire field is shown in Fig.3 (Ref.10).

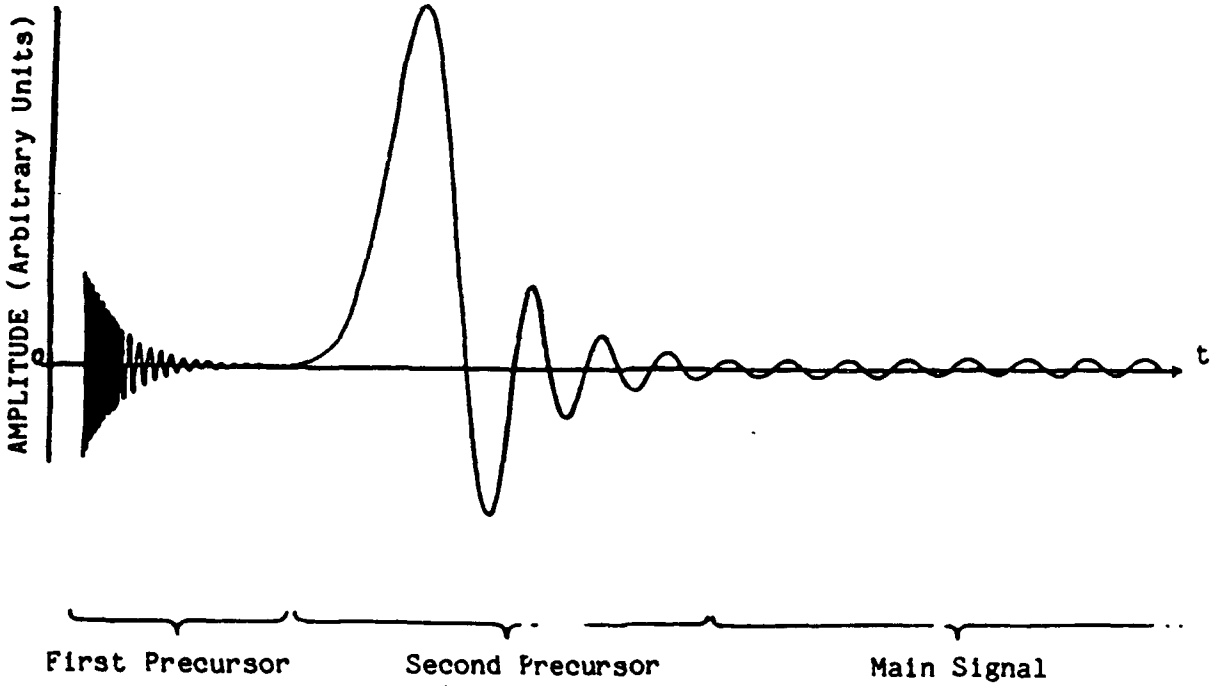


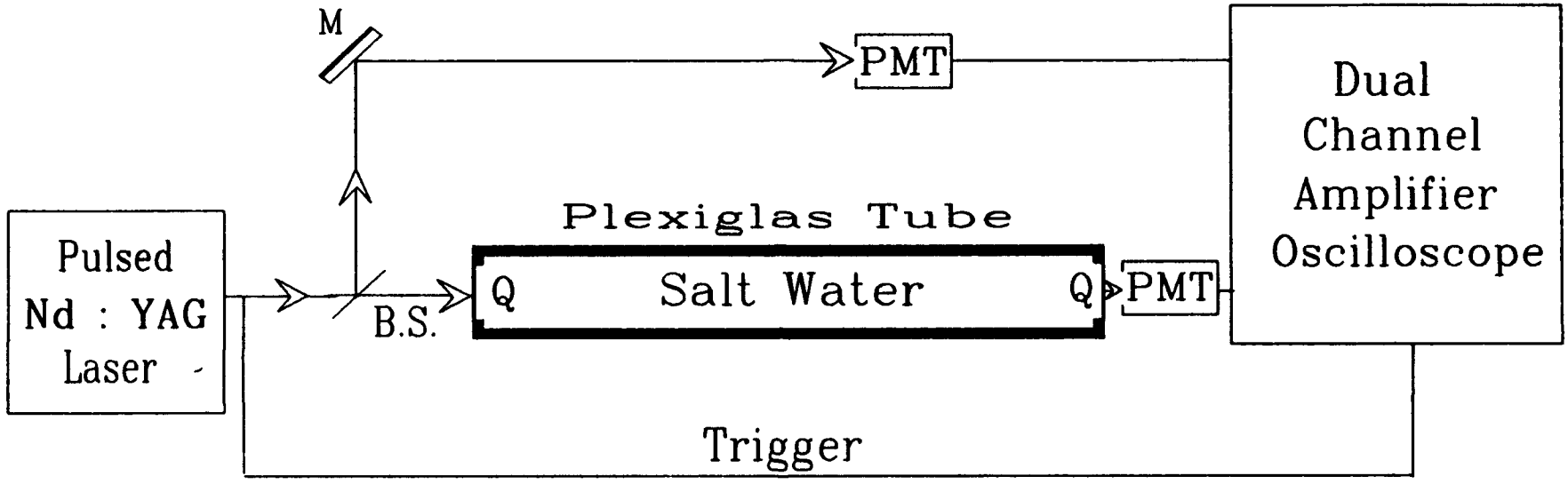

Fig. 3. Dynamic evolution of the entire field propagating through a Lorentz medium. (Figure is taken from Ref. 10.)

## IV. DESCRIPTION OF THE EXPERIMENTAL DESIGN AND SIGNAL ANALYSIS:

In order to demonstrate the dispersion effect of salt water on optical pulses an experiment can be easily performed. The schematic of this experiment is shown in Fig.4. A Q-switched, pulsed Nd:YAG laser with a pulse- width of 10 ns will be used as an exciting light source. This laser operates at its fundamental mode at 1064 nm. Using a frequency doubler, we can achieve a visible light at 532 nm. The laser intensity even after frequency doubling will still remain sufficiently high for our experimental purpose. The incident light is then split by a beam splitter B.S., about 95% of the beam going through the salt water column and only 5% will be sent in the air to a light detecting Photomultiplier tube (PMT) via a high reflective mirror M. The salt water which is a dispersive medium is contained in a plexiglas tube at both ends of which are mounted anti-reflection coated quartz windows Q. These quartz windows have negligible dispersion effect on the visible light. An identical photomultiplier tube is placed behind the other end of the tube to detect the distorted profile of the laser pulse which is caused by the dispersive nature of the salt water. The signals from both PMT's are sent to a sensitive dual channel amplifier oscilloscope which is triggered by the same exciting light source as shown in the schematic.

According to Cocking [16], water containing about 3.5% by wt. mineral salts has negligible effects on absorption in the visible region. A simple calculation indicates that the transmittance of a light at 532 nm is as high as 40% even after propagating through a 5 m long path of salt water. The average power of the second harmonic of

# Experimental Design to Demonstrate the Dispersion Effect of Salt Water on Optical Pulses




PMT = Photomultiplier Tube
M = High-reflective Mirror
B.S. = Beam Splitter
Q = Anti-reflection-coated Quartz Windows

Fig. 4

the Q-switched Nd:YAG Laser is about 0.5 W and the pulse width 10 ns. The peak power of this laser is over 50 MW. Therefore the intensity of the laser before it enters the photomultiplier tube is significantly high. It might even be necessary to attenuate the incident laser intensity in order to avoid overloading the signal analyzing electronic devices. Hamamatsu 1P28A photomultiplier tubes are suitable for signal detection in this study. For the detection of high-speed and pulsed light signals, a coaxial cable will be used to make the connection between the photomultiplier tube and the electronic apparatus e.g., oscilloscope. Since commonly used cables have characteristic impedances of 50 or 70 ohms, this cable will need to be terminated in a pure resistance equivalent to the characteristic impedance to provide impedance matching and ensure distortion-free transmission for the signal waveform. When a matched transmission line is used, the impedance of the cable as seen by the photomultiplier tube output will be the characteristic impedance of the cable and will not depend on cable length or frequency. Therefore, the cutoff frequency is then determined only by the photomultiplier tube or the oscilloscope amplifier characteristics. The rise time of these PMT's is 2 ns and the average anode current would be 0.1 A at operating 1000 dc voltage. The anode dark current of the PMT and the resulting noise are critical factors in determining the lower limit of light detection. But the dark current of these PMT's is only 2 nA. Therefore, the resulting noise is negligibly small and will be of no problem in detecting our signal which will be much larger than the expected noise.

In order to observe the waveform of high-speed pulses using an oscilloscope, a low load resistance will be necessary. Since, however,

there is a limit to the oscilloscope sensitivity, an amplifier is commonly used. A Tektronix 7834 storage oscilloscope will be used to display the signal which can finally be photographed using a Tektronix C-5C oscilloscope camera. The time resolution of this oscilloscope is 1 ns/div and time constant is less than 1 ns. Therefore, the laser pulses having pulse width of about 10 ns will be well resolved and can be analyzed without having any distortion in the signal. The incident laser at 532 nm will have a definite spatial as well as temporal spread. The initial temporal spread which will be detected by the oscilloscope is about 10 ns at its full width and half maximum (FWHM). According to the theory, the optical pulse of such an exceedingly narrow width is expected to spread both spatially and temporally, as it propagates through salt water This dispersion effect can easily be detected and observed using above mentioned electronics apparatus. Because of high sensitivity of the PMT at the desired wave length and high resolution of the oscilloscope, signal to noise ratio is expected to be significantly high and hence the signal illustrating the dispersion effect will be much greater than the expected noise in the measurements.

## V. RECOMMENDATIONS:

The dispersive property of salt water will hopefully be demonstrated with the funding from Mini Grant Program of AFOSR, and also with the help from personnel at the Laser Laboratory here at the Brooks AFB, San Antonio. In this experiment, the plexiglas tubes of at least three different lengths e.g., 2 m, 3 m and 5 m are recommended in order

to observe significant dispersion effect of salt water on pulses from Nd-doped YAG laser. After completing the proposed experiment according to our design, we shall be able to perform another related experiment which will also demonstrate the dispersive nature of salt water on laser pulses. In this experimental set up, we will obliquely send the incident laser pulse at a small angle with the normal to the front surface of plexiglas tube containing the salt water. The distorted signal from the other end of the tube will then be sent through a quartz prism for further dispersion. The signal after passing through the prism is expected to be dispersed enough so that the various components of the output signal can be detected by a sensitive photomultiplier tube which will be accurately positioned to cover the entire range of the optical spectrum. This will also decisively demonstrate the dispersion effect of salt water on optical pulses.

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FINAL REPORT


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| Date: | 04 August 88 |
| Contract No: | F492620-87-R0004 |

# LITERATURE SEARCH ON NUTRITION AND THE RELATION TO TACTICAL AIR COMMAND PILOTS, G-TOLERANCE AND ENERGY OUTPUT

By

Sonia Hart Sawtelle

Studies have shown that irregular and insufficient caloric intake may influence the nutritional state of pilots causing hypoglycemia, reducing G-tolerance and even provoking premature G-induced loss of consciousness. The literature in acceleration physiology has noted that nutrition does play a role in G-tolerance and G-stress.

Extensive library research was done to provide the groundwork for nutrition and the Tactical Air Command pilot; nutrition and G-tolerance; energy output of fighter pilots; special diets for fighter pilots; current dietary recommendations for the Tactical Air command pilot; hypoglycemia and it's relation to G-tolerance; the relation between Tactical Air Command pilots and specific athletic groups of similar energy outputs; and the possibilities of diminishing the fatigue factor of pilots through improved nutrition.

The significance of the literature is discussed and recommendations are suggested for further study.

## 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 experiences was rewarding and enriching because of many different influences. Dr. Larry Krock provided me with support and was invaluable in overcoming many technical roadblocks. The concern and constant support of Dr. William Storm, Dr. Russell Burton, Dr. W.C. Alexander, and Dr. Richard Miller was greatly appreciated. Sgt. Robert Ivy and Mrs. Irma Giesberg provided a truly enjoyable working atmosphere. Ms. Janet Wiegman was a constant source of inspiration, support, and encouragement. The entire personnell of the Crew Technology Division were a tremendous and positive influence to my experience.

## I. INTRODUCTION

G-tolerance is crucial for pilots flying modern high performance aircraft. Tolerance to repeated acceleration stress is important to these pilots. Multiple sortie surges over consecutive days and perhaps weeks during conflict will severely tax the physiology and psychology of pilots. The body's energy stores may be depleted by the strenuous physical work induced by straining maneuvers. Irregular and insufficient caloric intake may influence the nutritional state, reducing the G-tolerance and even provoking premature G-induced loss of consciousness. (1)

It is desirable that all negative factors influencing G-tolerance be reduced as much as possible before discussing methods of improving G-tolerance. All of the negative factors that could affect G-tolerance should be considered. These negative factors affecting G-tolerance could include: heat stress, dehydration, hypoglycemia, hypocapnia through hyperventilation, hypoxia, fatigue, sleep deprivation, alcohol, medication, infection, etc. These negative factors may impede flight safety and effective operation of the aircraft (1).

The Crew Technology Division at USAF School of Aerospace Medicine at Brooks AFB is concerned with G-tolerance and the factors that might affect it such as: tilt of the seat, straining maneuver, heat stress, fitness level and nutrition.

### Hypoglycemia

On occasion, especially before G-loss of consciousness was found to be a relatively normal and common occurrence for fighter pilots (before 1980), the nutritional status of pilots was considered to be a possible cause of

an accident involving G-loss of consciousness. A medical reason for G-loss of consciousness had to be found and hypoglycemia appeared to be a likely candidate, although the effect of low blood sugar on high G-tolerance has never been well measured(9). The probable reason for this concern about hypoglycemia on G-tolerance is that faintness is commonly associated with hunger, even though reduction in orthostasis or G-tolerance have not been statistically correlated (2).

Stewart (9) showed that in some instances a reduction of the blood sugar may lead to unconsciousness during positive acceleration in aircraft, and he suggested that the cause may be a lowering of the substrate supply to the brain.

In 1956 and 1957, known cases of unconsciousness in flight were analyzed and hypoglycemia was a significant finding (7).

The significance of these results in flight is more difficult to access, but there is considerable evidence that missed meals or hurried snacks are a common precursor to lapses of consciousness in flight, and hypoglycemia may, therefore, be a significant factor in aircraft accidents (6).

It would be of interest to correlate the time at which accidents or losses of consciousness in flight occur more closely with the time of the last meal and the predicted level of blood sugar. On the evidence available, which is limited, it would seem that a fairly long period since the last meal presents less of a hazard than does a small and inadequate snack taken a few minutes before flight (6).

## Current Dietary Recommendations For Aircrews

The current diet that is recommended for the Tactical Air Command pilot is one that contains 50-60% complex carbohydrates, 20% fats and 20% protein. It is also recommended that pilots should choose their diets from the four food groups (12):

1. Two servings from the milk group.
2. Two servings from the meat group.
3. Four servings from the fruit and vegetable group.
4. Four servings from the bread and cereal group.

Other suggestions include:

1. Eat before flying
2. Choose nutritious, easily digested foods
3. Allow plenty of time for meals
4. Eat breakfast
5. Don't eat bulky, greasy, gaseous, or indigestible foods
6. Don't drink alcoholic beverages before flying
7. Don't overeat
8. In-flight meals are needed to maintain efficiency during flight, maintain alertness and maintain the powers of concentration.

The recommendations further state that long periods without eating and insufficient fluids can cause unnecessary fatigue, slower reactions, inability to concentrate, and in extreme cases blurred vision (12).

## Energy Output of the Fighter Pilot

The high G environment of the modern fighter and attack aircraft represents a significant departure from that found in the older fighter. Thrust available in today's aircraft enable them to not only attain, but to sustain the G-loads the older planes could only hold for short periods of time. Subsequently, modern aircrewmembers must be able to fly and fight in a extremely demanding physical environment (3).

Currently, the only compensating mechanism available are the anti-G suits, the tilt of the seat and the straining maneuver. Performing the straining maneuver under G-load is very physically taxing. In a combat environment, he may also be expected to fly three or four sorties per day. The potential for fatigue derived from the combat arena is enormous (13).

Muscle fatigue will occur after continued use of the lower extremities and abdomen. Long term heavy resistance exercise can induce some effects favoring regulation of the blood pressure during G-stress. It has been demonstrated that an improved G-tolerance is in association with a moderately intense strength training program (13)

The physical demands have been compared to those of a weight lifter, body builder, or sprinter. The type of activity consists of repeated bouts of high intensity, short duration work.

An athlete's (pilot's) energy requirements vary depending on weight, height, sex, age, metabolic rate and on the type, intensity, frequency, and duration of the activity. Daily routine training can increase the energy needs of the athlete (pilot) two or three fold over the non-training requirements. Normally hunger sensations support the need for additional calories. However, the intense, emotional stress of training and combat combined with hectic travel schedules and potential lack of nutrition knowledge, may mean athletes (pilots) fail to consume sufficient and essential nutrients (4).

## Special Diet To Improve Performance

Studies of the effects of various nutrients on athlete's (pilot's) performance or related activities have been underway for many years to provide some theoretical basis for relating dietary modification to improvement in physical performance, but there is need for further research (10).

Diets that facilitate the best performance by athletes (pilots) must contain adequate quantities of energy, protein, fat, carbohydrates, vitamins, minerals and water. The quantity of calories consumed must be increased according to energy expenditure during the regimen of physical conditioning, training, and combat (10).

What is the proper role of nutrition in achieving top performance? Many sport's authorities suggest that the best nutritional preparation for peak performance is simply a well-balanced diet. The presence of an adequate

diet is an obvious requirement for successful competition (combat). A fundamental objective for athletes (pilots) would be to adjust their diets to the energy requirement for their "sport". The basic nutritional prescription for athletes is simply a normal diet, regular meals, ample fluids, and a variety of foods and caloric intake sufficient to meet the energy demands.

Carbohydrates are a major source of energy during short-term, exhaustive work; both fats and carbohydrates are the sources of energy during steady-state activities. Studies conclusively demonstrate that diets lacking in carbohydrates have severe effects on work performance (10).

## Nutrition Education

Although nutrition information is widely available to athletes (pilots), few formal studies have focused on their food habits to determine the relationship of eating patterns to nutrition knowledge and to the energy demands of the particular activity (10).

## II. OBJECTIVE OF A FUTURE RESEARCH EFFORT

With the review of the literature in mind, one would realize that currently there isn't any information on the diet of the Tactical Air Command pilot. There is no awareness of the pilot's nutritional knowledge.

The purpose of a research effort would be to do a dietary analysis to collect information regarding the type (protein, fats, and carbohydrates) and amounts (calories taken in) of food Tactical Air Command pilots consumer. The basis for gathering this information would be to:

1. Describe the current dietary habits of the Tactical Air Command pilot.
2. Calculate if the diet is adequate for the type of work that the Tactical Air Command pilot must perform during training and combat.
3. Relate the dietary intake of Tactical Air Command pilots to the diet that is currently being recommended to Tactical Air Command pilots.
4. Decide if nutritional guidance is necessary for this population.
5. Relate the dietary intake of Tactical Air Command pilots to body builders, weight lifters, and sprinters who have similar energy outputs.

## III. PROCEDURES

Dietary analysis surveys for three day or seven day diet recall would be sent to specific Tactical Air Command Squadrons (Holloman, Red Flag, Reserve alert Fighters, etc). The proposal to do this study would come from funding from the Mini Grant Program. Recordings would be made on standard dietary forms. Detailed instructions would be given to voluntary participants. Each volunteer would be instructed about the recording of food intake. Each volunteer pilot would be asked to record everything he ate or drank during the survey period. An interview by a trained investigator would also be included as part of the recall.

Vitamin and mineral supplements would be noted, but would not be included in the dietary analysis.

### Computer Program

The Prucal Prudent Diet program would be used to analyze the respondent's dietary record. Total number of calories, protein, fats, carbohydrates, Vitamin A, Vitamin C, Vitamin B-6, calcium, thiamin, sodium, iron, simple sugars, unsaturated fats and saturated fats would be calculated for one day.

## IV. RECOMMENDATIONS

1. It is recommended that a three day or seven day dietary analysis of Tactical Air Command pilots be studied.
2. It is recommended that the dietary analysis be related to age, sex, type of plane flown, and number of hours flown.
3. It is recommended that a comparison be made between the diet of the Tactical Air Command pilot and his centrifuge experience being that of endurance or tolerance.
4. It is recommended that a comparison be made between the MRE (Meals Ready to Eat) and the nutrition and that is being recommended.
5. Discern if improved nutritional guidance is needed for the Tactical Air Command pilot.
6. It is recommended that the present diet of the Tactical Air Command pilot be compared to the diet that is currently being recommended.

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FINAL REPORT

# A SMALL INERT-GAS GENERATOR


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Date: 10 Aug 88

Contract No: F49620-87-R-0004

# A Small Inert-gas Generator

by

Paul O. Scheie

## ABSTRACT

A small, inert-gas generator (SIGG) was assembled using type 4A molecular sieve subjected to pressure swing adsorption. Properties of cylindrical beds in tubes 1" x 16", 24", and 36" were studied at 20, 30, and 40 psig, with flow rates of 150, 300, 500, and 1000 cc/min, and cycle times of 4, 8, 12, and 16 sec. A variety of purge flows was used. Gas which was up to 99.9% inert was generated, and a mathematical model was formulated to describe the results under conditions of no purge flow. For a given bed size, the constraints for low oxygen content in the product gas included a cycle time of at least 8 sec, a delivered volume less than the void volume of a bed, and a ratio of product flow to inlet pressure of less than 20 cc/min-psig.

## Acknowledgements

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this project. Universal Energy Systems is to be commended for the well-run and helpful administrative aspects of the program.

My experience was educational, enriching, rewarding and fun. Everyone at Brooks with whom I came in contact was most friendly and helpful. Ken Ikels gave encouraging supervision to the project and set the tone for a splendid working atmosphere and a successful collaboration. Cherie Noles provided unerring and often anticipatory assistance as well as computer expertise, all with infectious cheerfulness. Capt. George Miller is gratefully thanked for suggesting the project. He and Clarence Theis both were always willing to discuss any aspect of the project and offer useful words of experience. Col. John Bomar's interest in the project was influential in getting it launched.

## I. INTRODUCTION:

The Air Force has particular interest in both oxygen-rich and oxygen-depleted gas. Oxygen-rich gas is used by personnel in aircraft at high altitudes, while oxygen-depleted gas is used in aircraft to cover and replace fuel in tanks as they empty in order to lower combustibility in the event that a projectile penetrates a tank or that a crash landing occurs.

Conventionally, these objectives have been met by carrying tanks of compressed gas. However, several advantages ensue when dependence upon such tanks is reduced by including on-board generators capable of providing the desired gas from available air. On-board oxygen generating systems (OBOGS) as well as on-board inert-gas generating systems (OBIGGS), both of which utilize molecular sieves and pressure swing adsorption, currently are operational in some aircraft. A combined unit may be even more advantageous.

The Crew Systems Branch, Crew Technology Division, School of Aerospace Medicine, Human Systems Division, Brooks Airforce Base, Texas, has been intimately involved with the OBOGS project. The use and properties of the appropriate molecular sieves used with pressure swing adsorption have been studied and are documented in the literature. On the other hand, there is little information in the open literature concerning the OBIGGS. It was with this in mind that an investigation of the performance of molecular sieve type 4A in a pressure swing adsorption apparatus was undertaken.

Most of my own background does not pertain specifically to this project, although my more recent research interests have centered on the adsorption of liquids and gases on certain solids.

## II. OBJECTIVES OF THE RESEARCH EFFORT

My assignment was to assemble a small inert-gas generator (SIGG), modeled after the Small Oxygen Generator (SOG) previously described (7), using type 4A sieve material instead of the 5A and 13X types used in the SOG. Whereas 5A and 13X preferentially remove nitrogen from inlet air leaving an oxygen-rich product, type 4A reportedly removes the oxygen, leaving the product oxygen-depleted. However, little information is available concerning expected or actual performance. There are requirements listed for specific aircraft indicating that product gas with less than 2% oxygen would be satisfactory.

The SIGG utilizes pressure swing adsorption for which there are 6 important parameters: inlet pressure, exhaust pressure, product flow rate, purge flow rate, cycle time, and size of the beds of molecular sieve. Initially, a suitable combination of values for these parameters had to be found, after which performance (percent Oxygen in the product gas) could be studied as one parameter at a time was varied.

## III. RESULTS

a. Initial results indicated that the least oxygen appeared in the product gas when the sieve beds were at least 16 inches long, inlet pressures

were greater than 20 psig, product flows were less than 500 cc/min, cycle times were greater than 4 sec, and purge flow rates were very small.

These results suggested that relevant properties of the SIGG could be obtained by undertaking a series of experiments that would include all combinations of the following variations in the different parameters:

Bed length: 16, 24, 36 inches

Inlet pressure: 20, 30, 40 psig

Exhaust pressure: 1 atm

Product flow rate: 150, 300, 500 cc/min

Purge flow paths: 0, 80, 320, 640 inches of microbore tubing

Cycle times: 4, 8, 12, 16 sec

Some additional experiments were performed with an exhaust pressure of 1/2 atm, as well as some with a product flow rate of 1000 cc/min.

Product gas concentrations were measured on a Perkin Elmer Mass Spectrometer. For each experiment 5 measurements at 10 sec intervals were recorded after a steady state had been attained. These values, together with the other parameters, were stored on a Zenith Z-100 computer

Minimum oxygen concentrations from the three bed lengths were 1% from the 16 inch beds, 0.5% from the 24 inch beds and 0.1% from the 36 inch beds. It was found that the volume delivered by each bed during a 1/2 cycle - that is, the product volume + the volume used to purge the other bed - was an important factor in determining the concentration of oxygen in the product. The oxygen concentration increased when the delivered volume exceeded the void volume of the bed (void volume is the volume external to

the sieve particles). In addition, low oxygen concentrations were associated with cycle times of 8 sec. or greater, and a ratio of product flow rate to inlet pressure of less than 20 cc/min-psig.

b. It was found that each experiment with no purge flow could be described mathematically with equations that contained 4 constants, one each for nitrogen and argon, and two for oxygen. The percent of nitrogen, oxygen, and argon in the product gas corresponded to a simple exponential decrease with time of the amount of nitrogen and argon in the vol delivered. The amount of oxygen decreased with a more complex exponential, one that increased less than linearly with time. The constants for nitrogen and argon did not vary appreciably from experiment to experiment while those for the oxygen did show considerable variation. Unfortunately, the uncertainties in the measured gas concentrations were such that a systematic investigation of these constants was not possible. They were consistent with a model that suggests the argon simply diffused into the sieve material (and presumably out again), that nitrogen did the same, with some slight adsorption. Oxygen, on the other hand, was adsorbed more than ten times as effectively as the nitrogen, with the accessible adsorption sites becoming saturated with time.

## IV. RECOMMENDATIONS

a. The results clearly suggest that a full-scale unit should be constructed to determine whether its performance characteristics are similar to those of the SIGG.

b. It would be interesting to obtain an OBIGGS unit and determine

how its construction and performance compare to the SIGG and to a scaled up unit.

c. Some thought might be given to utilizing the SIGG unit in front of a cryogenic unit that can provide a small, steady supply of liquid nitrogen for use in cooling superconducting equipment that seems a likely possibility in the not too distant future.

d. The analysis of the data from the SIGG was somewhat different from that of other pressure swing adsorption gas separators. It was encouraging that this analysis resulted in such simple constraints for good performance (cycle time, delivered volume, and the ratio of product flow rate to inlet pressure), and it was remarkable that relatively simple mathematics were able to provide satisfactory descriptions for the expermiments where no purge flow was present. It would be interesting, and perhaps instructive, to apply a similar analysis to other sieve materials, as well as to study the variations in the mathematical constants used to describe the results in order to more fully ascertain the physical significance of each.

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FINAL REPORT

# HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) DETERMINATION OF HIGH ENERGY PHOSPHATE POOL


| | |
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| Date: | August 12, 1988 |
| Contract No.: | F49620-87-R-0004 |

# HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) DETERMINATION OF HIGH ENERGY PHOSPHATE POOL

BY

Sharon T. Williams

## ABSTRACT

A High Performance Liquid Chromatography (HPLC) procedure was performed for the analysis of the high energy phosphate nucleotides in platelet rich plasma (PRP) fractions of various subjects. The analytical procedure involved the extraction of platelets from whole blood, centrifugation, aspiration of the supernatant, and a freeze-thaw cycle. Finally, the samples were analyzed for the nucleotides using the HPLC. At present, only one compound was evaluated for use as an internal standard. This compound was 3-(4-hydroxyphenyl) propionic acid. We found that the retention time of this internal standard was too close to one of the nucleotides being analyzed.

The nucleotide composition was determined in the PRP of various subjects. Concentration levels of high energy phosphates in small animals was not determined as originally planned due to lack of equipment (under repair). Therefore plasma was used as a substitute for the biological unknown. We used the Rao et al. [9] HPLC procedure for analyzing the concentration of the adenine nucleotide phosphates from human blood. In the future we hope this HPLC procedure will

provide the specificity and sensitivity needed for the quantitation of these nucleotides and thus brain energy depletion in animal models before and after G induced Loss of Consciousness (G-LOC).

## ACKNOWLEDGEMENTS

I would like to thank the Air Force Command and the Air Force Office of Scientific Research for sponsorship of this summer research (1988). I would also like to thank Dr. Paul M. Werchan for his assistance and guidance throughout this research project. Also, appreciation is expressed to Dr. F. Parker, Ms. E. Forster, and Mrs. J. Barber for their cooperation. I would also like to thank Mr. Garcia and Mrs. Baskin for their clerical assistance.

Finally, I would like to thank my husband, Derald, for his support and encouragement throughout this summer. This has been a very fruitful, worthwhile and constructive experience.

## I. INTRODUCTION

I received the M.S. in Biochemistry from Southern University studying the effects of pesticides on lipid metabolism. In the summer of 1984, I investigated the metabolism of arachidonic acid with animal lungs at Tulane Medical Center in New Orleans.

In the summer of 1987, at the Clinical Pathology Laboratory of USAF School of Aerospace Medicine, I was involved in the development of a gas chromatographic procedure to analyze the omega-3 and other polyunsaturated fatty acids contents in serum lipid fractions of patients with and without coronary artery disease (CAD).

The research problems at the Crew Technology Division of the School of Aerospace Medicine was to determine the best HPLC assay to analyze the concentration levels of adenine nucleotides in biological samples. This same assay will be used to determine brain energy depletion in animals before, during and following G-LOC. Because of my experience in biochemical and medical research, I was assigned to work on this problem under investigation at the USAF School of Aerospace Medicine at Brooks AFB, Texas.

## II. OBJECTIVE OF THE RESEARCH EFFORT

The goal of this summer research was to determine if the concentration of brain high energy phosphate is

diminished significantly before G-LOC. G-LOC is particularly hazardous to pilots of high performance aircraft because of the length of physical and mental debilitation that follows (12 to 15 seconds of absolute incapacitation and 15 seconds of relative incapacitation). My objectives were

1. Determine the proper extraction technique for metabolites from brains which have been fixed by using microwaves.
2. Determine the best HPLC assay for the adenine nucleotides.

Based on other models of cerebral hypoxia, it is hypothesized that a significant depletion of high energy phosphates that are necessary for normal neuronal function will occur during a high G-level exposure. These metabolites will be extracted from brain samples of small animals (rats).

## III. DEVELOPMENT OF ANALYTICAL METHODS

Intracellular nucleotides contribute significantly to the physiological and biological regulatory functions in tissues and cells. In the past, platelet adenine nucleotides have been assayed in several different ways [4-8], including a firefly luciferase [9], an enzymatic method [10], a fluorimetric procedure [11], and a column chromatography and HPLC method [12]. Several years ago ,

Rao, White, Jacimowicz, and Withop described a method using ion-exchange HPLC for separating and quantitating the adenine nucleotides of human blood platelets [13].

We had planned to determine the proper extraction techniques for the same nucleotides mentioned above. However, due to inoperative equipment, we decided to utilize the Rao et al. methods for analyzing these nucleotides [20]. By using human blood platelets, we feel that the Rao HPLC assay is one of the best for determining the adenine nucleotides. Our primary concern is the brain metabolites because cerebral ischemia has been found to severely depress brain energy metabolism and consequently neuronal function. Also, high energy phosphates are used as an index of viability of a brain that has suffered ischemia. Adenosine triphosphate (ATP) is the predominant free energy source of adenosine and cyclic adenosine monophosphate (C-AMP) [14-16].

The brain has a very limited storage supply of oxygen and glucose. When deprived of an exogenous source (cerebral ischemia), these supplies soon are depleted and loss of consciousness follows (within 10 seconds) [17].

Although the total brain energy state is restored to a near normal level within a relatively short time following recirculation, the reduced adenine nucleotide pool during ischemia shows only partial recovery, indicating a breakdown to related nucleosides and bases [18-19].

A precise and practical procedure for the determination

of these nucleotides is described as follows:

Preparation of Internal Standard Twenty milligrams of 3-(4-hydroxyphenol) propionic acid (Sigma Chemical Co.) was placed in a 25 ml volumetric flask and dissolved in 20 ml of 2N perchloric acid to produce a concentration of 1 mg/ml (stored in 0°C freezer).

Preparation of Adenosine Nucleotide Standards Twenty mg portions of cyclic adenosine mono-phosphate, adenine, adenosine di-phosphate, adenosine tri-phosphate (Sigma Chemical Co.), were placed in separate 25 ml volumetric flasks and dissolved in 2N perchloric acid to produce a concentration of 1 mg/ml (stored at 0°C).

Into a 100 ml volumetric flask was added 100 ul of the nucleotide standard solution and 99.9 ml of 2N perchloric acid to produce a 1 mg/ml concentration. One hundred ul of this solution was added to another 100 ml flask and filled to the mark with 2N perchloric acid to produce a concentration of 1 ng/ml.

Extraction of Nucleotides Blood for these studies was obtained from normal donors. The blood was drawn into an anticoagulant tube which contained ethylenediaminetetracetic acid (EDTA) to prevent coagulation. Nucleotides were determined from platelets obtained from 1 ml samples of fresh PRP. One ml of blood was centrifuged at 2,140 RPM for 15 minutes to obtain platelet pellets. The supernatant (plasma) was aspirated and the platelets were precipitated with 100 ul of cold 2N perchloric acid. Ten ml of the

internal standard was added and mixed by gentle inversion.

The samples were again sedimented in the centrifuge (2,140 RPM) for 2 minutes at room temperature. The PRP was aspirated, placed in fresh siliconized tubes and neutralized with 5N KOH to a pH of 5.5-8.0. The neutralized samples were subjected to a freeze-thaw cycle to achieve complete precipitation of the salt. To sediment the salt generated during neutralization, all of the samples were centrifuged once more for 2 minutes. The clear neutral extracts were separated and 10 ml samples of the extracts were injected into the HPLC for the separation of nucleotides.

HPLC Instrumentation The HPLC determination of nucleotides utilized a Waters Associates Model 840 which enables one to set up and run HPLC analyses quickly and efficiently on up to four HPLC systems simultaneously. The 840 supports, through interface modules, the operation of a 730 Data Module, a 510 Pump, a 481 Variable Wavelength UV Detector, a TCM Column Heater and a 710 B Sample Processor (Waters Associates, Milford, Ma.). The detector wavelength employed was 254 nm at a sensitivity setting of 0.001 absorbance units full scale (AUFS). At a flow rate of 1.0 ml/min and a mobile phase consisting of acetonitrile and water (20:80) and 1 vial of 0.005M tetrabutylammonia phosphate (PIC A Reagent: Waters Associates). Separation was accomplished on a micro Bondapak c18 column (3.9 mm I.D. x 30 cm steel columns: Waters Associates).

## IV. PERFORMANCE OF HIGH ENERGY PHOSPHATE ANALYSIS

The results of the analysis of the concentration levels of high energy phosphate in human platelets are shown in Figure I. These samples contain a substantial quantity of adenosine diphosphate (ADP), small quantities of adenosine and adenine, but minute amounts of C-AMP and ATP. In this present study, a mixture of various nucleotides were analyzed using the conventional micro (M) Bondapak C18 column. Solvent elution was isocratic and the flow rate was 1 ml/min. Complete separation of all the nucleotides at ambient temperature was achieved in approximately 20 minutes. Tables I and II gives the retention times of the nucleotide standards and human platelet nucleotides respectively. The identification of the individual peaks was done by comparison of retention times of standards added to the platelets extract and assayed under identical conditions.

## V. RECOMMENDATIONS

Even though platelet adenine nucleotides have been assayed in several different ways, a simple, rapid and efficient method is still unavailable [4-8]. Unfortunately, no information was provided regarding reversed phase separation of platelet nucleotides and the linear gradient methods described took a considerable length of time for

complete separation of all the nucleotides [20].

An important recommendation is that future analysis be done with the use of radially compressed columns. It has been reported that the time required for each analysis could be further reduced by using this type of column. This method has proven to be fast and efficient and provides excellent separation of platelet nucleotides [12,13,21]. Also the results lead to the recommendation that reversed-phase chromatography using isocratic elution be employed in future studies.

Due to the limited time alloted for this project, an appropriate statistical analysis of the results needs to be done. In essence, the mean values for each nucleotide should be determined.

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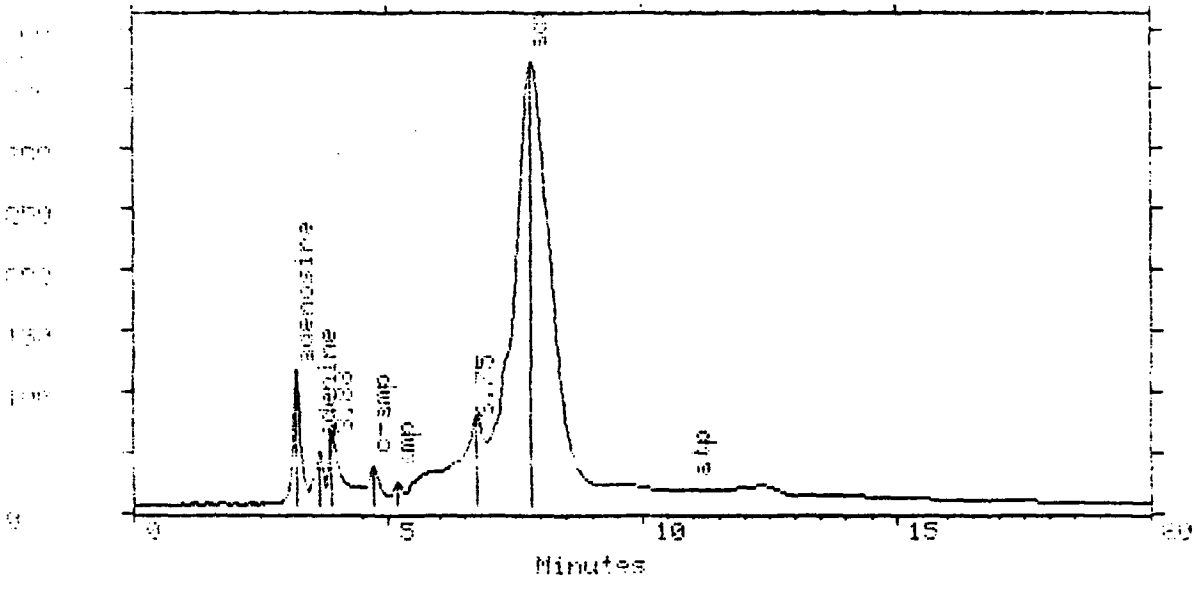



Fig.1 A typical chromatogram for the nucleotides standards

TABLE I. Retention Times of Nucleotide Standards

| Nucleotide Standards | Retention Times (min) |
|---|---|
| Adenosine | 3.13 |
| Adenine | 3.39 |
| Cyclic Adenosine mono-phosphate (C-AMP) | 4.38 |
| Adenosine mono-phosphate (AMP) | 5.30 |
| Adenosine di-phosphate (ADP) | 7.64 |
| Adenosine tri-phosphate (ATP) | 10.21 |

Separation of various nucleotide standards from a mixture by isocratic elution using a micro Bondapak C18 column. Complete separation of all the nucleotides at ambient temperature was achieved in approximately 20 minutes. Chromatographic column conditions: 3.9 mm I.D. x 30 cm; Micro Bondapak C18; solvent: acetonitrile-water-Pic A; detector: UV 254 nm; samplesize: 10 microliters.

TABLE II. Retention Times of Human Platelet Nucleotides

| Human Platelet Nucleotides | Retention Times (min) |
|---|---|
| Adenosine | 3.21 |
| Adenine | 3.65 |
| Cyclic Adenosine mono-phosphate (C-AMP) | 4.50 |
| Adenosine mono-phosphate (AMP) | 5.33 |
| Adenosine di-phosphate (ADP) | 7.83 |
| Adenosine tri-phosphate (ATP) | 10.50 |

Separation of human platelet nucleotides by isocratic elution using a micro Bondapak C18 column. Complete separation of all the nucleotides at ambient temperature was achieved in approximately 20 minutes. Chromatographic column conditions: 3.9 mm I.D. x 30 cm; micro Bondapak C18; solvent: acetonitrile -water-PicA; detector: UV 254 nm; sample: 10 microliters.

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FINAL REPORT


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| Contract No: | F49620-87-R-0004 |

# Chemiluminescent Probes Based on Luminol and Luminol Derivatives

by

John R. Wright

## ABSTRACT

Luminol reacts with 3-aminotyrosine (3AT) in the presence of nitrite ion to form brown, melanin-like products which have potential value as biological probe substances. The reaction yield is optimal when the 3AT/luminol molar ratio is about 1.6, and zone electrophoresis at pH 6 reveals two products which are unique to this reaction mixture. One product is neutral/insoluble and the other is an anion. Both are chemiluminescent. In related work it was found that colloidal copper(II) hydroxide effects a temperature-sensitive catalysis of the luminol/$H_2O_2$ chemiluminescent reaction suitable for thermal dosimetry (i.e., for measuring the spatial distribution of RF energy deposition). The empirical equation for luminosity as a function of temperature ($^oK$) is $I/I_o = e(-19,400/RT)$, where R = 1.987 cal/$^oK$-mol. An analogous dosimeter based on cobalt(II) produces a bright, transient chemiluminescent reaction when $CO_2$ is introduced. Also, diazotizing reactions with luminol alone produce a product which chemiluminesces brightly with alkaline peroxide and no activating metal ions.

## I. INTRODUCTION:

Chemiluminescent reactions (hereafter, CL) have found numerous practical applications ranging from immunological labeling methods to probes for detecting the presence of free radicals (1,2). These are potentially very sensitive methods, being limited only by background light levels and counting times if photon-counting detectors are used. A number of different CL reactions have been characterized (1,3), but perhaps the most important of these is the CL reaction of 5-amino-2,3-dihydro-1,4-phthalazinedione (known as luminol) initiated by free radicals.

This project has been concerned with a chemical study of potentially useful probe substances based on brown, melanin-like reaction products which form when mixtures of luminol and 3-aminotyrosine (hereafter, 3AT) react with sodium nitrite. These substances appear to be excellent CL probes for detecting free radicals generated catalytically by the green hemoproteins (4,5). Insoluble versions of these products might also be used as CL probes for measuring the phagocytic activity and free radical-producing capacity of macrophages.

The project also examined a special application of luminol itself, namely as a temperature sensitive probe for imaging the spatial distribution of RF energy deposition in target phantoms.

## II. OBJECTIVES OF THE RESEARCH EFFORT

As defined in the pre-summer visit, the main objective

of this project was to be a chemical structure characterization of the melanin-like, CL active product which forms when 3AT, luminol and nitrite ion react in a mixed DMSO/water solvent. An additional objective has been to optimize the yield of this substance. When it became clear that the product consisted of more than one component, attention had to be focused on identifying methods for effectively resolving and purifying the components of the product mixture, and a major portion of the summer's work was addressed to that problem. Nevertheless, the ultimate goal in this work is a structural characterization, to the extent possible.

During the project a secondary objective of finding a CL-based thermal dosimeter suitable for imaging RF energy depositions was defined. This goal was based on a visible need.

## III. MATERIALS AND METHODS:

The luminol used in this study came either from J. T. Baker or Sigma Chemical Co., and 3-aminotyrosine was obtained from Sigma Chemical Co. The 3% hydrogen peroxide used here bore the label "Swan". All other chemicals used in this work came from containers with labels indicating analytical reagent purity. All synthesis protocols were carried out in open beakers and similar containers. No attempt was made to exclude air from the reaction mixtures.

Luminometric measurements of the light intensity produced by CL reactions were carried out using a Turner TD-

20e luminometer. In most cases the instrument was thermostated at 37 $^{o}$C. Luminous intensity values for samples originating at other temperatures were obtained by plotting time curves and extrapolating back to the zero point in time. Electron spin resonance spectra were recorded using a Varian E-line spectrometer operating in the 9 GHz band. The instrument was not set up for cryoscopic or flow measurements, and the measurements were thus at room temperature in capillary cells. Nuclear magnetic resonance spectra were obtained using the SEOSU 4.7 Tesla superconducting multinuclear facility at Durant, Oklahoma. Protons resonate at 200 MHz in this field. Absorbance measurements in the visible spectrum were carried out by means of a Bausch and Lomb Spectronic 2000 using polystyrene cells.

The zone electrophoresis medium used in this work was Gelman Sepraphore III (cellulose acetate). The chromatographic stationary phases - Sephadex G-25 and G-50 and DEAE Sephadex - were obtained from Sigma Chemical Co.

## IV. THE LUMINOL/3-AMINOTYROSINE/SODIUM NITRITE REACTION:

Mixtures of luminol, 3AT and sodium nitrite react in a mixed DMSO/water solvent to produce brown water soluble and insoluble products (6) which have been named by the acronym DALM (diazoluminomelanin, since 3AT is closely related to the biological substrates which are converted into melanin). Samples prepared prior to this project were found to be CL active, and they are stable to air in the dry form. Two

questions have been raised concerning these substances. First, one wonders if they are just mixtures of luminol and a brown derivative of 3AT, or if covalent linkages are involved. If the latter situation is the case, the second question focuses on the structural chemistry of the products.

To gain insight concerning the first question, solutions of 10 mM luminol in DMSO, 10 mM 3AT in water and 100 mM $NaNO_2$ in water were prepared. These were mixed in various proportions (but with the DMSO/water ratio constant) in a set of controlled experiments to find the main effects. It was observed that no color is formed if nitrite is omitted. It was also noted that luminol and nitrite do not react under the conditions of the color-forming reaction; however, if the medium is acidified by adding acetic acid, they do (see VII, below). An orange-brown pigment forms when 3AT and nitrite react in the absence of luminol, but if luminol is included, the product becomes substantially darker. Fig. 1 shows the effect of varying the 3AT/luminol molar ratio with 3AT + luminol at 10 mM and nitrite at 20 mM.

DALM may be precipitated by adding a 20-fold excess of acetone five minutes after starting the reaction. After centrifuging the precipitate and discarding the supernatant, this material may be redissolved or resuspended in a buffer and applied to chromatographic columns or electrophoresis strips. The best separations were effected by means of zone

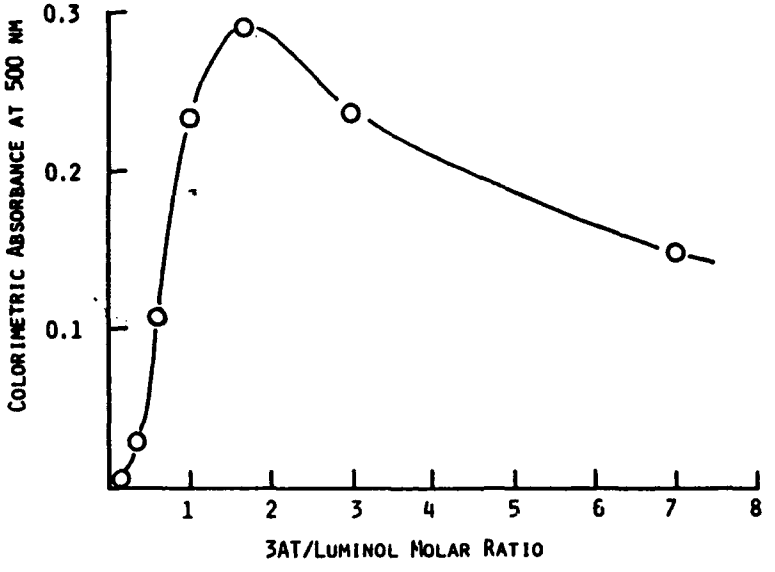



FIGURE 1 THE EFFECT OF REACTANT RATIO ON PRODUCT YIELD

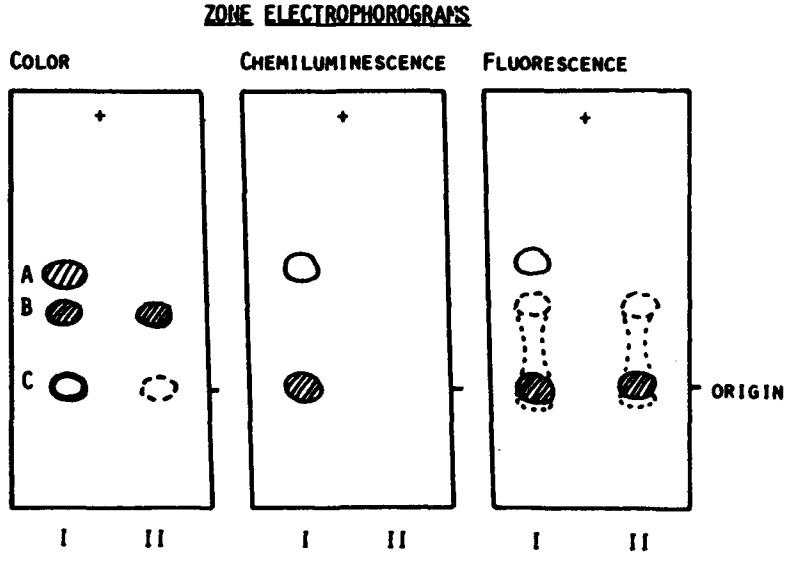



FIGURE 2 TRACK I (LUMINOL/3AT/NITRITE); TRACK II (3AT/NITRITE)

electrophoresis in a 0.1 M, pH 6 acetate buffer. These electrophorograms could be inspected for visible pigments or long or shortwave UV-induced fluorescence. CL bands could be detected by soaking a fresh strip in alkaline hydrogen peroxide and, after removing excess fluid, blotting this against the electrophorogram. The CL bands could be visualized in a dark room; also, by "eclipsing" a band with a narrow spatula, its position could be located when the lights were turned on. Fig. 2 shows sketches of the results with nitrite/3AT/luminol and nitrite/3AT reaction mixtures.

These results indicate that electrophoretic component B (orange-brown) is a product derived from 3AT alone, but the brown anionic component A is unique to the complete reaction mixture, strongly indicating that it is a covalent product of luminol and 3AT. Component A shows blue fluorescence (as luminol does), and it is CL active. Component C (brown) is electroneutral/insoluble and it remains at the origin. The fact that this zone shows strong CL activity and blue fluorescence is ambiguous since luminol itself is electroneutral at pH 6 and is expected at the origin.

Component A may react to form C. When acetone is added to the reaction mixture at the five minute point, an insoluble haze forms immediately. If this is centrifuged at once, redissolved in pH 6 buffer and subjected to electrophoresis, there is relatively more of the A-component and less of C; however, if after adding acetone time is allowed for a flocculent precipitate to develop (about 20 min.), the relative proportions of A and C are reversed. An

alternative interpretation is that A is more insoluble and precipitates first.

Other, larger-scale purifications have been attempted. DALM adsorbs on Sephadex G-25 and G-50 columns and elutes following the salt fraction even when the ionic strength of the eluent is larger than what is normally required for molecular weight separations. This stationary phase tends to produce smeared bands even at low flow rates. The best column separations were achieved on DEAE Sephadex in a 0.1 M, pH 5 acetate buffer, consistent with the anionic character of component A. The order of elution from DEAE Sephadex is approximately the inverse of electrophoretic mobilities at pH 6.

Other forms of DALM were prepared. If at the five minute reaction point 0.1 M, pH 5 acetate is added instead of acetone (equal volumes of buffer and reaction mixture), an insoluble DALM is formed which slowly precipitates over a 48 hour period. Both 3AT and luminol are reactive under acidic conditions, and a branched polymer would not be surprising. Part of this precipitate is not soluble in DMSO, and it is noted that the DMSO-extracted solid was CL active. Since luminol is very soluble in DMSO, any remaining reactant should have been extracted.

DALM prepared as described in the last paragraph is water insoluble, but the bulk of it dissolves in 1 N NaOH. Luminol behaves similarly due to the removal of azine protons (ionization) in the alkaline environment, and this

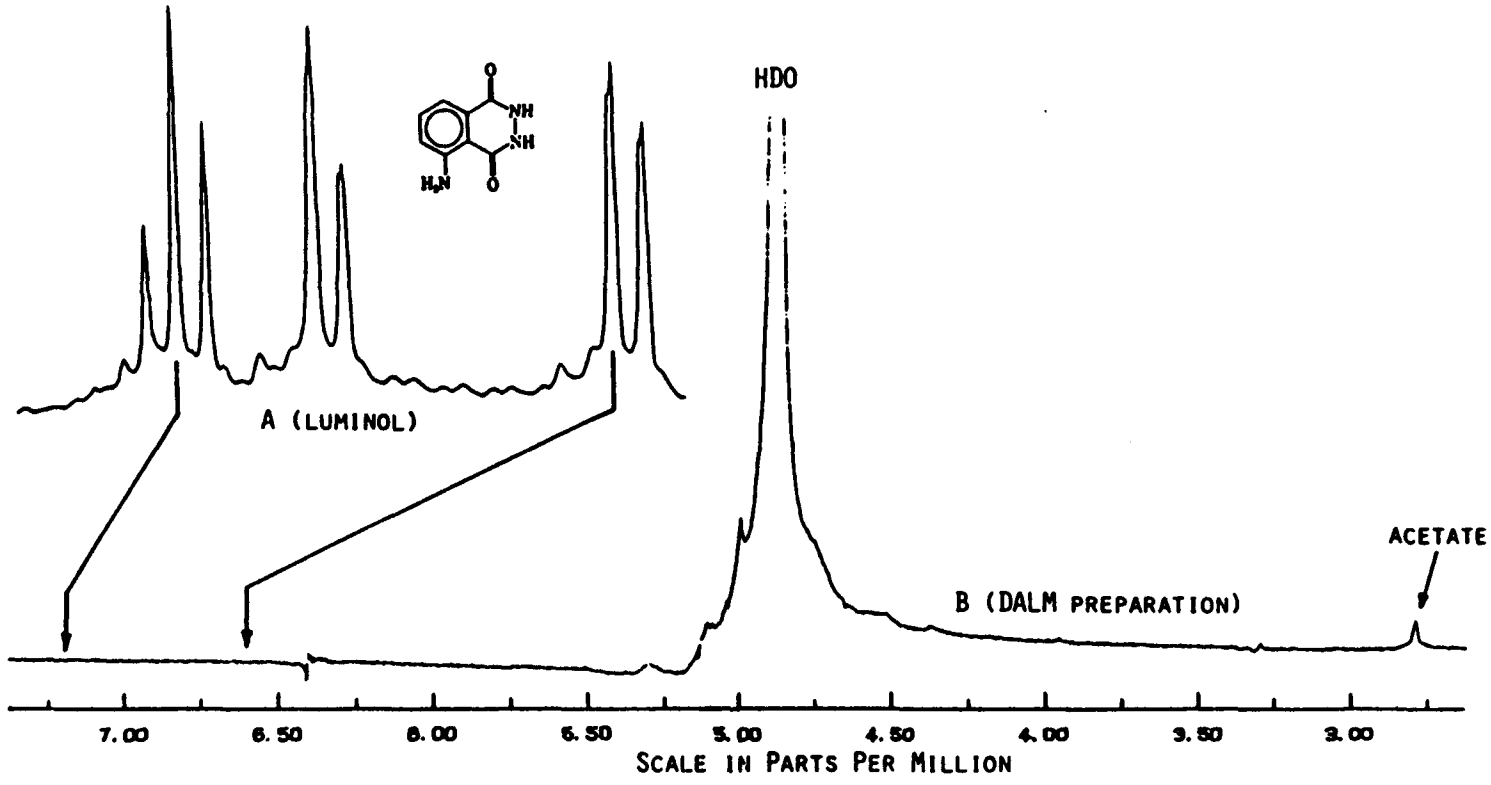



FIGURE 3 THE FOUR HYDROGENS SHOWN IN THE LUMINOL STRUCTURE EXCHANGE WITH THE SOLVENT. THE NMR SPECTRUM (A) IS DUE TO AROMATIC HYDROGENS (THREE, NOT SHOWN).

could reflect the presence of the luminol moiety in the DALM structure. For nmr purposes, the best solvent is $NaOD/D_2O$. A 200 MHz proton spectrum of luminol dissolved in $NaOH/D_2O$ (the fully deuterated solvent was not available) is shown in Fig. 3a, and Fig. 3b is the recording of DALM in the same solvent. The resonances are broadened beyond detection, either due to molecular size or paramagnetism or b It is noted that dried DALM preparations present an esr spectrum. Only a trace of the acetate buffer is visible, and the luminol resonances are conspicuously absent. Based on the measured noise level and a reference signal intensity, free, unreacted luminol would have been detectable at 0.003 M (or 0.057 wt. percent). However, later experiments showed that the uncatalyzed luminol CL reaction could be visualized at an order of magnitude less concentration. The nmr experiment needs to be repeated in $NaOD/D_2O$, and with longer signal averaging times.

Taken collectively, the foregoing evidence suggests that DALM contains the luminol and 3AT structures in a covalent linkage.

## V. A CHEMILUMINESCENT THERMAL DOSIMETER FOR MAPPING RF ENERGY DEPOSITION:

A CL dosimeter suitable for mapping spatial distributions of RF energy deposition in target phantoms is needed for the USAF Quantitative Luminescent Imaging System (QLIS). The dosimeter must meet several criteria, and these include the following: 1. the CL intensity must be

temperature dependent, 2. the light intensity should not be excessive (i.e., compatible with the sensitive video imaging system), 3. it should last long enough to allow measurements at a comfortable pace, 4. the dosimeter should be able to approximate biological ionic strength conditions, 5. it should be simple to prepare and 6. it should not be toxic.

The literature on luminol probe applications is extensive (1,2), and virtually all of the reported methods use an activating substance, usually a transition metal ion, to promote the luminol CL reaction (1,7,8). This suggested that if the activator were at a lower concentration, the CL intensity might be reduced to an acceptable level and the reaction might also last longer.

Several reacting mixtures were considered and tested, but most of these did not behave in a useful way. However, a dosimeter based on $Cu(OH)_2$ under conditions which do not lead to a precipitate of cupric hydroxide appears to meet most of the above-stated criteria.

Preparation of the dosimeter is as follows: A 2.0 mg sample of luminol is dissolved in 600 microliters of 1 N NaOH, and this is diluted and stirred with pure water to obtain a total volume of 20.0 mL (solution A). In a second step, a 90 microliter aliquot of 2% (w/v) aqueous copper sulfate (prepared from the hydrated salt, $CuSO_4$ $5H_2O$) is mixed thoroughly with 20.0 mL of 3% aqueous $H_2O_2$ (solution B). Solutions A and B are then combined with immediate, thorough stirring. The final solution is amber-colored. It effervesces $O_2$ and produces a dim CL throughout the solution

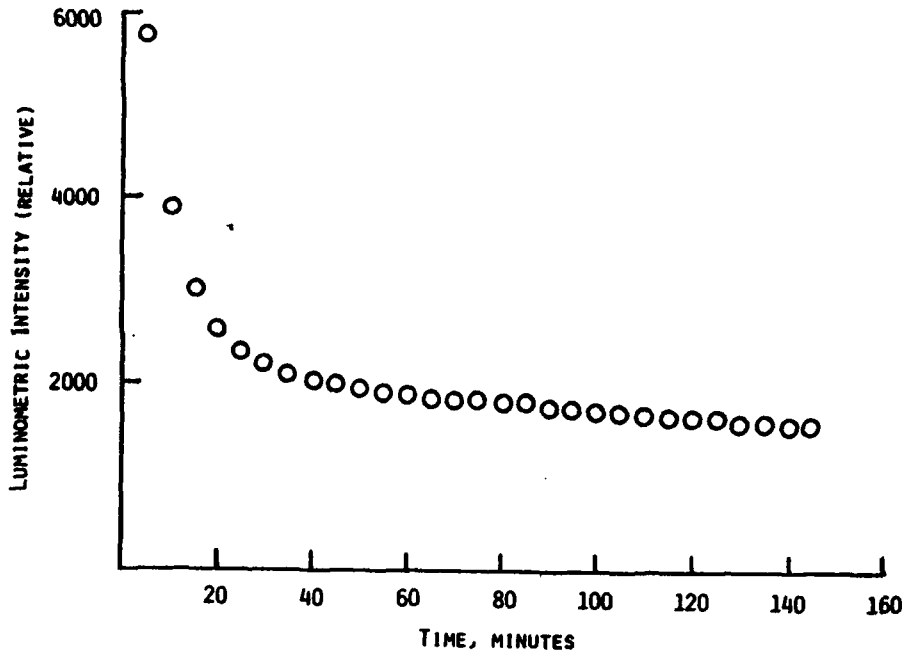



FIGURE 4 TIME DEPENDENCE OF DOSIMETER LUMINOSITY

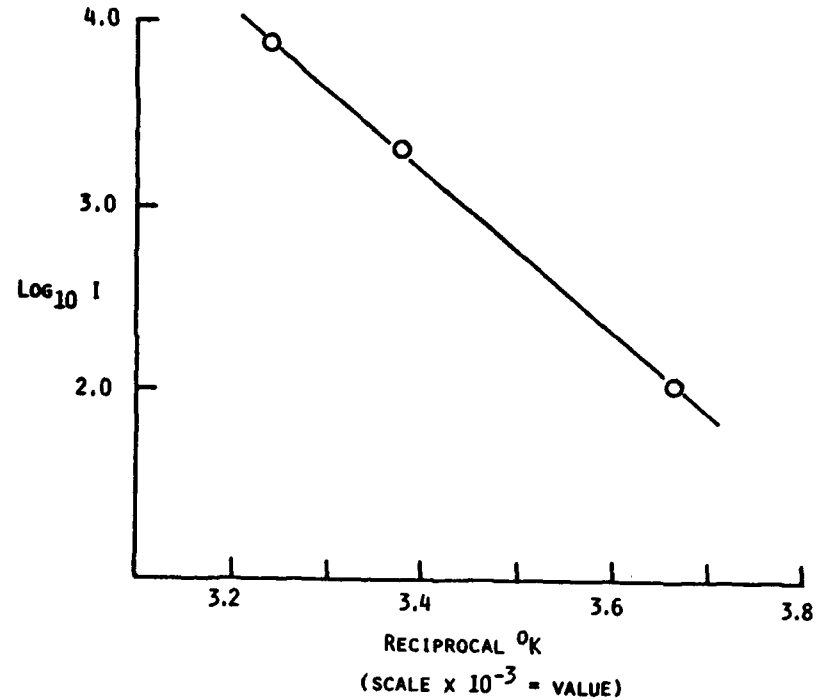



FIGURE 5 THE TEMPERATURE DEPENDENCE OF DOSIMETER LUMINOSITY

volume lasting upwards of 6 hrs. The composition of the final solution is mostly water with 0.015 N NaOH, 0.005% luminol, 1.5% peroxide and a trace of copper colloid (as $Cu(OH)_2$ - the amber color is due to peroxide interacting with this catalyst). NaCl or KCl may be added to increase ionic strength. At high salt concentrations the catalyst becomes insoluble.

A time curve for the dosimeter operating at 37 $^{o}C$ is shown in Fig. 4. The CL intensity decays exponentially for about an hour, then diminishes linearly for at least another hour. It is suggested that dosimeters be aged for an hour before using.

The CL intensity of this reaction is temperature dependent, and it obeys the Arrhenius relationship, as shown in Fig. 5. The empirical equation for CL intensity (I) is $I/I_o = e(-19,400/RT)$, where R is 1.987 cal $^{o}K^{-1}$ $mol^{-1}$ and T is in $^{o}K$. The pre-exponential factor ($I_o$) is a relative value which depends on the sample volume and photometer characteristics; thus the fractional form is reported here.

## VI. LUMINOL CHEMILUMINESCENCE ENHANCEMENT BY DISSOLVED CARBON DIOXIDE OR CARBONATE ION IN THE PRESENCE OF COBALT(II):

During the process of developing a thermal dosimeter, Co(II) hydroxide (as the colloid) was tried as a CL activator, since the literature reports it as an efficient activator of the luminol-persulfate system (8). The reaction mixture used here is identical to that described in

V., above, except that cobalt(II) sulfate hydrate replaces copper(II) sulfate hydrate. This solution indeed produced a more luminous reaction, but more interesting was the finding that its CL is very sensitive to $CO_2$, which is not observed for the copper-containing system.

If the exhaled breath is allowed to reach the reacting solution (by breathing on the surface or bubbling it through), or if carbonated water is added, a transient CL enhancement lasting about 5 s takes place. This is a vivid outburst which approaches illumination quality. A less vivid enhancement is seen if a carbonate or bicarbonate salt is added to the mixture. In the latter cases the reaction is not transient.

"Carbonate enhancement" effects in the luminol CL reaction have been reported in the literature (9,10,11). It has been speculated, reasonably, that since carbonate radicals are spin delocalized, electrically charged structures and therefore have longer lifetimes, the luminol CL reaction is favored by higher steady state concentrations of these radicals. Thus, the following kind of reaction is envisioned:

$$HO\cdot + CO_3^{2-} \longrightarrow HO^- + CO_3^{\cdot-}$$

However, since the initial effect of dissolving molecular $CO_2$ in the reacting mixture is so much more vivid than that produced by carbonate/bicarbonate solutions, a direct involvement of $CO_2$ should be considered:

$$HOO\cdot + CO_2 \longrightarrow H^+ + O_2 + CO_2^{\tau}$$

or:

$$HO\cdot + CO_2 \longrightarrow H^+ + \tfrac{1}{2}O_2 + CO_2^{\tau}$$

These are only speculations. Attempts using esr to detect steady-state radical concentrations in the CO(II) system have failed. A $CO_2$ effect is observed at the air/liquid interface when alkaline luminol/$H_2O_2$ solutions undergo a weak CL reaction in the absence of activating metal ions, and DALM behaves similarly. The Co(II) system promises to be a good model for exploring these properties.

VII. THE CHEMILUMINESCENCE OF DIAZOTIZED LUMINOL PRODUCTS:

In an acidic solvent luminol reacts with nitrite, presumably by diazotization, to form a red-brown product (example: 2.0 mg luminol in 1.0 mL DMSO combined with 4.0 mL 0.1 M, pH 5 acetate buffer is reacted with 8.0 mg $NaNO_2$; the darkening is virtually complete in 20 min.). If a portion of this mixture is rapidly mixed with a large excess of 50:50 (v/v) 3% $H_2O_2$/1 N NaOH, a bright, deep blue pulse of CL is observed for 2-3 s. The order of mixing with luminol is important. If the alkaline peroxide is added first, then nitrite after a 1 min. delay, no bright CL is observed. This seems to rule out nitrite in the CL activation of luminol itself, and the evidence is consistent with the formation of a diazo derivative or polymer of luminol. The red-brown material elutes as a single band from DEAE Sephadex in 0.1 M acetate at pH 5, and it is electroneutral on electrophoresis strips in a pH 6 acetate buffer, which

appears to rule out a cationic diazonium species. The substance decomposes slowly in 1 N NaOH with a half life of about 30 minutes.

No further chemical characterization of this substance has been possible. It is noted that the literature describes a liquid-state, luminol/$H_2O_2$ chemical laser (12), and this substance may be useful in a laser application.

## VIII. RECOMMENDATIONS:

Since chromatographic and electrophoretic separations have shown that at least two components of the DALM product mixture are unique to the complete set of reactants (luminol, 3AT and nitrite ion), a structural study is recommended. This would involve the purification of amounts of these substances sufficient for percent elemental analyses, molecular weight determinations, chemical degradations (and degradation component purification) and spectroscopic characterization of degradation products by nmr, infrared, etc. Nmr studies of the intact DALM materials appears to be a fruitless effort based on experience gained during the summer project; however, a kinetic study of the formation reaction may be informative (see below).

The author searched the chemical literature for references to nmr studies of luminol and its derivatives, but none were found. Even the large Sadtler collection of nmr spectral reference data omits luminol in spite of the importance of this compound. This seems surprising in view

of the fact that nmr chemically induced dynamic nuclear polarization (CIDNP) studies (13,14) of the luminol CL reaction might provide new insight into its mechanism, which is enshrouded in some doubt. A search of the CIDNP literature, which only goes back to 1967, produced no luminol papers. It is thus recommended that $^{1}H$ and $^{13}C$-nmr CIDNP studies of the luminol CL reaction be carried out. This could include studies of the catalyzed systems (provided paramagnetism doesn't interfere) and compound formation reactions described in this report since all appear to involve free radical intermediates.

Acknowledgements

I am indebted to the Air Force Systems Command and the Air Force Office of Scientific Research for the funding of this project. I am also equally indebted to Universal Energy Systems for their well-organized supporting measures for the past ten weeks. Major Johnathan L. Kiel, the USAF Researcher to whom I was assigned, has been a source of support and much wisdom. He is to be commended for maintaining a good working environment and showing respect for those who are under his supervision. Dr's. Jill Parker and David Erwin also supported me on many occasions, and I would have wasted a great amount of time finding information, chemicals, equipment, supplies and the like without continual help from TSgt David Simmons, TSgt Chris McQueen and A1C Gerald O'Brien. Beth Toth and Yvonne Garcia aided in the preparation of this report.

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1988 USAF-UES SUMMER FACULTY RESEARCH PROGRAM/

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FINAL REPORT

# PC - MAINFRAME INTERFACE FOR DATA ENTRY


| | |
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| Date: | 5 Aug 88 |
| Contract No: | F49620-87-R-0004 |

# PC - MAINFRAME INTERFACE FOR DATA ENTRY

by

David R. Cecil

## ABSTRACT

A microcomputer based data entry system was developed to enable researchers to enter their data sets directly to floppy diskettes. Two versions were created, one for hard disk drive PCs and the other for floppy drive only micros. Menus, on-line help, and error recovery were built in as was hard-copy documentation since prior computer knowledge on the part of the end-user researcher was not assumed.

In addition a conversion/interface computer program was designed and implemented for Statistical Consulting to convert the floppy diskette data sets into forms both readable by the statistical package SPSS-X and transmission acceptable to the mainframe VAX computers at WHMC.

## Acknowledgments

I wish to thank the Air Force Systems Command and the Air Force Office of Scientific Research for sponsorship of this research. Additional thanks go to Universal Energy Systems for their help in the administrative and directional aspects of this program.

I am very appreciative of the staff of Clinical Investigation Directorate at Wilford Hall Medical Center. CID's director, Col. John H. Cissik, provided me with encouragement and an excellent working atmosphere. Dr. Cliff Butzin, chief of the Research Consultation Division of CID, provided stimulating discussions about, support for, and direction to, the project. I am indebted to Lt. Col. Ron Letscher and Mrs. Velma Grantham for their generosity in allowing me freedom of use of their microcomputers. The entire staff of CID is to be commended for their friendliness and help during my work there, with special thanks going to Dr. Bill Ehler for many enjoyable luncheon discussions.

## I. INTRODUCTION:

My research interests over the past decade have been in the applied mathematics areas of numerical methods, combinatorics, graphics, and probability. With the advent of microcomputers (PCs) I have concentrated on developing computer software to assist me in these areas. I have also become aware of the needs of first-time computer users and have published a book in the area of debugging of computer programs.

The Clinical Investigation Directorate at WHMC on Lackland AFB conducts research in a very large number of areas. Of main interest to me were the areas of data processing & analysis, and simulation/modeling.

I was most interested in ascertaining how much micro-computers were involved in the data process/analysis area since PC usage frees up mainframe time and memory, and I was curious as to whether researchers were being encouraged to learn to use microcomputers since PCs can be, potentially, of so much use to them. Finally I wanted to see if simulation/ modeling using microcomputers was desirable &/or feasible, since large scale processes tax the resources of even large computers.

## II. OBJECTIVES OF THE RESEARCH EFFORT:

Most of the researchers at WHMC record their data by hand, rather than directly into a mainframe or microcomputer. Some reasons for this are lack of knowledge of how to use any kind of computer, apprehensiveness about the permanence and retrievability of the data, and uneasiness about the data's

security and openness to others.

My assignment as a participant in the 1988 Summer Faculty Research Program was to design a data entry system for researchers to directly enter their data on personal computer floppy diskettes. Little or no knowledge of computers was to be assumed on the part of the researchers. Ease of use, shortness of time to learn, and ability to handle large data sets were to be the hallmarks of the system.

After the data set was entered on floppies, I was to construct a method to interface the diskette format with that of the mainframe VAX computer in a form that was SPSS-X ready. In addition, the SPSS-X analyses were to be capable of being downloaded onto diskettes in a form compatible with commercial presentation software packages.

## III. Program Design Initialization and Implementation

Before a microcomputer based data entry program or system of programs could be designed, an analysis of available hardware and software was under-taken. The PC microcomputers used at WHMC are manufactured by Zenith with two series in use, the Z100 and the Z200.

The Z100 series PC is a two floppy drive, no hard disk, type of microcomputer. It is not 100% IBM compatible and, as is, cannot run many of the popular software programs (such as SuperCalc) that have been written for the IBM PC. The message Wild Interrupt appears whenever a noncompatible program attempts to execute. To run such programs a hardware emulator board (approximately $400.00) or special emulator software

(about $65.00) must be installed. The Z100s I used had no emulators. The DOS used in these machines is typically version 1.01, although later versions are available. The Z100 machines are being phased out.

The Z200 series Zenith PC is fully IBM compatible. It has one floppy and one hard disk drive. The Z200s I used did not have math-coprocessors. The DOS available is version 3.0 or later. These Pcs are available throughout WHMC. Even if a particular office does not have a Z200, other offices nearby have ones that are available. In addition the computing center has six machines that are always available (except during training sessions which have not yet begun).

Printers, especially the ALPS brand of 15" dot-matrix printers, are abundant. It was therefore decided to design hard-copy printout into the data entry program. This would provide the experimenter with a permanent record of data names, values, and the order in which they were entered. If error corrections &/or additions are required later this printout supplements the information stored on diskette.

It was ascertained that a variety of software programs and packages exist at WHMC that could be used to design a data entry system. SuperCalc3 Release 2.1 appears to be the prevalent package & the one that people feel most comfortable with. Version 3 does not provide any type of macro command but does support execute files. This means that black-box application programs could be written around SuperCalc3. Hence an end-user of these execute files needs no computer expertise nor knowledge of SuperCalc3 in order to perform quite complex

data entry procedures.

While black-box application programs can also be written in higher-order languages such as BASIC or FORTRAN, it was noted after consultation with the computer center at WHMC that entensively coded and tightly packed BASIC or FORTRAN programs are not easy to change at a later date either by the original program designer or by others.

The decision was made to write data entry programs around SuperCalc3; having one program (called CALC) that could be used by a PC with at least one disk drive & an IBM emulator (e.g. some of the Z100s), and another program (CALCHD) designed for PCs with a hard drive (the Z200s). The CALCHD program can handle much larger data sets and operate much faster than the floppy disk drive based CALC program.

Lines, boxes, fancy prompts & exotic graphics are possible if ANSI.SYS is present in the file CONFIG.SYS. This configuration file information is accessed as soon as a PC is booted up. If the ANSI file is absent then the PC must be rebooted after editing the CONFIG file to include ANSI. The PCs I examined had minimal CONFIG.SYS files installed. In lieu of renaming this file, introducing new CONFIG and AUTOEXEC.BAT files, & then reconstructing the original CONFIG file at the end of the data entry programs; it was decided to forego the use of fancy logos and graphics.

## IV. Program Anatomy and Special Features

The CALC/CALCHD programs were designed for use by experimenters with minimal knowledge of microcomputers. The

only assumptions make were that the end-user would know how to turn on and boot-up a PC, know where disk drive A was, and how to correctly insert a floppy diskette in this drive. To design around these assumptions quite a large number of batch, screen overlay, execute, calculate, print, & utility files had to be constructed, tested, and integrated together.

Overall program control is assigned to two files, CALC.BAT and CALCHD.BAT. The major difference in these is that CALCHD creates a subdirectory on the hard disk for file storage & fast retrieval and then, at the end of the data entry, deletes this subdirectory. Files KEY-FAKE.COM and RFD.COM are designed to minimize key stroke entry on the part of the end-user.

Two introductory (SCREEN1.OVL and SCREEN3.OVL) and three help (A.DAT, B.DAT, and C.DAT) files supply on-line information for the user. TICKER.COM furnishes an eye-catching prompt for the first two screens while SNAPSHOT.COM enables one-key access to the help data files.

Interfacing with SuperCalc3 is accomplished by a collection of execute (.XQT) files. Command file ONEKEY.COM provides immediate accessibility to the .XQT files through function keys F3 thru F8. Since ONEKEY and SNAPSHOT reconfigure the keyboard their effect must be eliminated and the keyboard returned to normal at the end of the program. The files INSTALL.COM and REMOVE.COM accomplish this task.

BEGIN.XQT executes at startup. It sets up the spreadsheet format and awaits variable names. VAR.XQT (accessed thru the F4 key) records these names and assists in the entry of the first patient's data. FIRST.XQT (thru F5) and DATA.XQT (thru

F6) set up data entry for the other patients. Data verification thru counting of non-blank, non-text entries is performed by END.XQT (thru F7), while KEEP.XQT (for CALC) and KEEP1.XQT (for CALCHD) save the data set to diskette (via F8). The F3 key accesses REDO.XQT, an error-handling file.

FILE1.CAL, used by END.XQT, is a large file of 38,784 bytes containing 500 cells of formulas used to count the number of numeric entries. This file limits the CALC/CALCHD programs to a maximum of 500 patients per data set, which seems to be about the maximum data size brought to Statistical Consulting.

Hard-copy printing is done by the P90.EXE file under the direction of PRNSCN.BAT. The printing is performed sideways (so called landscape printing) instead of the usual portrait mode. This makes for easier reading and inspection of large data sets. Data names as well as data values are printed.

## V. Program Usage

To be able to provide all the needed files and also to handle as large a data set as possible, CALCHD was designed as a two diskette set (similarly so with CALC). One diskette contains the executable, batch, and overlay files while the other (labeled DATA) is used for data storage and batch termination control. Data sets too large for PC memory &/or diskette storage (limitations are noted in the introductory overlay screens of the program) can be managed by breaking the data into multiple parts. Full instructions are given on-line during program execution.

Multiple copies of the two diskette CALCHD program & of the

two diskette program CALC were prepared and are available in Statistical Consulting. In addition to the introductory overlays and on-line help screens, handouts of sample sessions were prepared & are available. These mini-manuals give step-by-step instructions starting with PC bootup, going through program usage and data entry, and culminating with DATA diskette submission to Statistical Consulting.

VI. SPSS-X Readable Files

Some method of converting the DATA diskette file into a form acceptable to SPSS-X (the principally used statistical package at WHMC) must be devised. Both mainframe SPSS-X and its PC counterpoint SPSS/PC+ read standard ASCII files provided that each line has a maximum of 80 characters and ends with a carriage-return line-feed sequence, that the end-of-file is marked by Ctrl-Z, and that there exist no special format codes or other information specific to the program from which the files were created.

Programs such as Lotus 1-2-3, Symphony, PC-Calc, VisiCalc, and SuperCalc3 usually store their data in special binary format (using .WKS, Worksheet, for Lotus; .DIF, Data Interchange Format, for VisiCalc; and .CAL for SuperCalc3) which neither SPSS-X nor SPSS/PC+ can read. Some of these programs do have the capacity to create SPSS-X acceptable ASCII files through the use of an alternate storage form with a file extension of .PRN.

The CALC/CALCHD programs save data to diskette under the name RESEARCH.CAL and print out the file RESEARCH.PRN. Two

problems exist that make it undesirable to use RESEARCH.PRN as SPSS-X input. The relatively large file size takes longer to send to the VAX (with retransmission of incorrectly sent packets being quite common and consuming even more time), and missing data values are hard to spot in large data sets).

Comma separated value (.CSV) files resolve both difficulties. To illustrate comparable file sizes, a data set of 500 patients with 26 variables each was created. For convenience, each of the 500 X 26 entries was assigned a value of 2. The .CAL file was 152,704 bytes in length, the file with extension .PRN 126,208 bytes, and the .CSV file a measly 28,160 bytes. No display format or printing information is stored in a .CSV file. In contrast, .CAL files must record values (actual numbers or strings of characters), formats (for individual cells, rows, columns, and global conditions), and formulas (expressions assigned to cells specifying calculations or comparisons which are resolved into values).

A .CSV file consists of rows of data (numeric values &/or string items), each terminated by a carriage return and a line feed character. The data items in each row are separated by commas, with string data enclosed in double quotes. Comma separaters are of great value in visually inspecting data for missing values since ,, appearing anywhere in a .CSV file indicates a non-entered or missing data value.

Existing files with extensions of .WKS, .DIF, or .CAL can be converted into .CSV files acceptable to SPSS-X. This is accomplished by the Super Data Interchange program SDI.COM. A batch file CLIFF.BAT was written to enable SDI to convert

RESEARCH.CAL files saved by the CAL/CALCHD programs. The CLIFF.BAT and SDI.COM files are included on the DATA diskette of the program set. Dr. Cliff Butzin (or another qualified person) needs only supply a new appropriate name for the RESEARCH file. The CLIFF/SDI program combination saves the data under the new name with a .CSV entension. Hand-outs of sample sessions creating .CSV files were prepared and are available from Statistical Consulting.

## VII. PC-VAX Interfacing

After data entry by CALC/CALCHD and then conversion to .CSV form by CLIFF, the data must, somehow, be sent to the main VAX computer at WHMC. A communications protocol (i.e. the intermingling of control information with data & the resulting actions) is needed to enable a VAX and a PC to talk to one another without corruption of data (noise on the transmission line connecting the two computers) and with sychronization of the two (one computer can send data faster than the other can accept it).

Kermit is an error-correcting file transfer program for use over serial communication lines such as those provided by an ordinary telephone. Kermit is available at WHMC, both in a PC version for the Zenith 200 and in a mainframe version for the VAX. Kermit sychronizes the PC and VAX by way of 'packets'. A packet of control information includes a sychronization marker, a packet number, a length indicator, and a checksum. Lost or corrupt packets are detected and retransmission is requested.

The necessary Kermit files for use on the PC (KERMIT.EXE, KERMIT.SCN, and MSKERMIT.INI) are included on the DATA diskette. KERMIT1.BAT was written to automate the dial-in & connect sequence of Kermit. This batch file also appears on the DATA diskette. Step-by-step instructions, including a section on what to do when things go wrong, were prepared for using Kermit on a Z200 PC. The use of Kermit on the VAX as a server for the PC is also included. The server concept (only appearing in later versions of Kermit and, fortunately, implemented on the VAX) greatly simplifies data transfer since back-and-forth acknowledgement by VAX and PC is eliminated.

## VIII. Downloaded SPSSX Analyses

The .CSV data set, entered on the VAX by KERMIT, can now, or at a later date, be analyzed using SPSS-X. This may be performed at either a VAX type terminal or at any PC connected to the VAX. From the PC an SPSS-X program can be written, edited, ran, and the results saved to diskette using the B$ menu item of the VAX.

Some limited graphics are available in SPSS-X, but they are inferior to the graphics produced by many PC presentation packages such as Harvard Graphics, Perspective, or Microsoft Graph. An experimenter's data set can best be graphically protrayed using one of these PC packages with the .CSV data form produced by CLIFF/SDI rather than from the SPSS-X output of the VAX.

IX. RECOMMENDATIONS:

It is recommended that CALC/CALCHD be used whenever possible. Some possible ways to accomplish this are:

to distribute the sample session/mini-manuals to all researchers having protocols at CID,

to inform the new doctors & dentists as they arrive at WHMC of the program via the series of lectures given them by Statistical Consulting,

& to designate those that have already used the program as facilitators for their respective areas.

It is suggested that informational material on graphs and graphics (their uses and abuses & their implementation on PCs) be developed. The .CSV form of data produced by CLIFF/SDI is ready for input to many PC graphics packages. The few packages that are available at WHMC have been under-utilized. Information on the variety of available packages (graphics only, graphics/text presentation, & animation) and instruction in the presentation of graphs (which form best brings out the significance of the data, where to place labels & legends, how to eliminate chart junk, etc.) are especially needed. Details on the construction of SuperCalc3 graphs would be quite useful.

It is also suggested that more PC-VAX interaction be attempted. The combination of advantages of using the PC (no waiting time to use) and of the mainframe VAX (vast storage resources) have not been fully explored. More modems are needed as is a manual on B$ editing on the microcomputer.

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# FINAL REPORT

# ORAL HEALTH


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| Contract No: | F49620-87-R-0004 |

# ORAL_HEALTH

by

Jorge L. Sintes, D.M.D., Ph.D.

## ABSTRACT

This project describes the research activities performed by the author during his Summer Faculty Research appointment at Wilford Hall Medical Center, Benjamin Dunn Dental Clinic. Participation in various on-going research projects was part of the experience. These research activities included: a) a review of the literature related to "**Effects of smokeless tobacco and food intake on the oral mucosa of Air Force basic trainees**", b) participation in "**Resistance form created by 11 pin types in complex amalgam restorations**" and c) participation in "**Finishing techniques for composite resin**". In addition, the author submitted a protocol entitled "**Effects of biological stressors on salivary cortisol levels and subsequent bioavailability of salivary proteins and calcium**" to the WHMC Institutional Review Committee for approval. Collection of salivary samples from various patients with Graves' disease has been accomplished.

## ACKNOWLEDGMENTS

My experience was rewarding and enriching because of many different influences. Col. John O. Burgess provided me with support, encouragement, and a truly enjoyable working atmosphere. The help of Drs. James Jacobson and Uwe Fohlmeister was invaluable in procuring patients for the stress hormones study. I greatly appreciated the concern of the professional as well as of the technical staff in the Department of General Dentistry at the Benjamin Dunn Dental Clinic. The pleasantness of the staff at the Oral and Maxillofacial Surgery Clinic was deeply appreciated. The technical support of Ms. Rita Smith, Library Services, clearly added to every aspect of this research experience. Finally, I want to thank all the personnel at C.I.D., especially my local focal point, Col. John H. Cissik and Drs. Patterson and Bradley, for their constant support and Mrs. Velma Grantham for the editing of this report. I was also rewarded by the support of Mr. Bobby Larry, Sophomore Dental Student, and Mr. Antoinne Able, Senior Medical Student, Meharry Medical College, for their interest in the advancement of Oral Health Research.

I. **INTRODUCTION:**

The author is a trained dentist with a Masters degree in Public Health and a doctoral degree in Nutritional Biochemistry and Metabolism with a minor in Oral Biology. Being Chairman of a Department of Preventive Dentistry and Community Health, I have diversified interests and can work in all areas related to Oral Health. The research interests of the Department of General Dentistry at Wilford Hall Medical Center are quite diversified and overlap my training in Preventive Health. Thus the opportunity for collaborative research activities between myself and the on-going research activities in the above mentioned department.

Because of my research training, expertise and interest in nutrition, I was able to interact with other health care professionals while at Lackland Air Force Base. Prior to my arrival and during my pre-summer visit, I was able to meet with various researchers, primarily Endocrinologists, and discuss with them the possibilities of developing a protocol addressing **stress hormones**. As a result of that initial contact, upon my arrival I was able to develop an exempt protocol addressing the effects of biological stressors on salivary cortisol levels and subsequent bioavailability of salivary proteins and calcium.

II. **OBJECTIVES OF THE RESEARCH EFFORT**:

A. To collaborate in a research project related to the effects of resistance of various types of pins used in dentistry.

B. To collaborate in a research project related to finishing techniques of various composite resins.

C. To develop a research protocol in the field of salivary endocrinology and to conduct an independent research effort in the field of salivary endocrinology.

D. To lecture in the field of nutrition and oral health in the General Dentistry Residency Program at Wilford Hall Medical Center.

E. To review the literature related to the effects of smokeless tobacco and food intake.

F. To collaborate in a research project related to the effects of bases and liners in dentistry as they affect bonding of the material to dentin and amount of fluorides released over a period of time.

G. To review and update a questionnaire for diabetic patients.

H. To collaborate in a research project related to the effects of cold sterilization on the accuracy of dental casts.

I. To collaborate in a research project related to the evaluation of different etching techniques for the Maryland dental bridge.

III. **Resistance form created by 11 pin types in complex amalgam restorations.**

The purpose of this study is to determine through the use of a uniform displacing load the resistance obtained in complex amalgams with different pin types.

Extracted mandibular molars that were both caries and restoration free were selected and stored in tap water except for specimen preparation and testing. The occlusal surface of each tooth was reduced with a model trimmer to provide a flat surface approximately 3mm above the cemento-enamel junction. The roots of each tooth were notched for retention and embedded in an acrylic base in a 1" by 1" aluminum cylinder. Acrylic was extended 2mm below the cemento-enamel junction. The 110 teeth were ranked and ordered by bucco-lingual size and divided into 11 groups of ten teeth each in order to distribute variations in bucco-lingual dimensions throughout the groups. Using the appropriate twist drill, channels for the pins were prepared at least 0.5mm inside the dentino-enamel junction oriented parallel to the adjacent external surface of the tooth. Six 0.6mm self-threading pins were inserted in each tooth using the manufacturer's directions. After insertion, pins were cut to leave 2mm of pin extending above the cut surface of the tooth as measured with a 2mm guide. After the resistance features were prepared for each group, a matrix band was placed and silver amalgam (Dispersalloy, regular set, pre-capsulated, Johnson & Johnson Dental Products) was prepared according to the manufacturer's recommendation and condensed into the preparations using hand and mechanical condensation.

Presently the samples are being inserted into a fixture on the bottom plate of the Instron Testing Machine (Instron Corp.) and a test load will be applied to each sample at a

constant crosshead speed of 0.05" (1.27mm) per minute until the sample fractures. Load diagrams will be charted by the Instron Machine.

The experimental design of this study included the following: 1) types of pin material and 2) size of pins. These will be subjected to a two-way analysis of variance with a control. Significant differences will be evaluated using a Duncan's Multiple Range test.

IV. **Finishing techniques for composite resin**

The purpose of this study was to determine the ideal series of finishing instruments for dental tooth colored restorative materials and to compare the surface smoothness, as measured by a surface analyzer and the scanning electron microscope, of six different composite resins obtained by finishing each composite with a series of finishing instruments.

Six materials were chosen for this study and six different finishing methods will be evaluated for each composite resin. The materials used were Heliomolar (Vivadent Inc.), Herculite XR (Kerr), Ful Fil (L.D.Caulk Co.), Visiomolar (Premier Dental Product Co.), P-50 (3-M Co.) and Estilux (Kulzer). Class V cavity preparations measuring 6 x 8 were made in different surfaces of extracted maxillary and mandibular teeth. Each of the six composite restorative materials were placed in 60 cavity preparations with all enamel walls. The enamel walls were etched for sixty seconds, a bonding agent applied and the resin polymerized

through a matrix strip for sixty seconds. Only half of the composite will be finished so that the surfaces produced by the finishing devices can be compared with the surface formed against the matrix band. The surface in contact with the matrix band will therefore serve as the control surface and the finished surfaces will be compared to the control. The specimens will be finished by six different finishing methods. For all finishing procedures, the manufacturer's directions will be carefully followed. Presently the 360 samples have been completed and are ready for polishing. This will be accomplished within the next month.

Roughness averages obtained for each composite will be evaluated for significance with a one-way analysis of variance. If a significant variance between the means exists, a Duncan's Multiple Range Analysis will be performed. The S.E.M. photographs will be evaluated using a Chi Square analysis.

V. Development and submission of an exempt protocol entitled **Effects of biological stressors on salivary cortisol levels and subsequent bioavailability of salivary proteins and calcium.**

The purpose of this project was to seek to test the hypothesis that severe and/or chronic biological stress, such as hypo and/or hyperthyroidism, adrenal insufficiency and steroid excess can create oral conditions conducive to increased dental caries.

Psychosocial stress, such as, death of spouse, divorce,

retirement and change in living conditions and biological stress, such as, injury and illness are among the most common biopsychosocial stressors giving rise to a series of well defined stress reactions in most individuals. In general, stressors can cause production of hormones which decrease lymphocyte number and/or function, increase cellular release of proteins, and increase blood sugar by gluconeogenesis of amino acids released from these proteins. No studies exist which relate the effects of biological stressors such as the above mentioned conditions with salivary calcium-binding concentrations, salivary calcium, total proteins and amylase as part of studies of any ethnic group. Therefore, we propose associating the biological effects of conditions such as hypo and/or hyperthyroidism, adrenal insufficiency and steroid excess with changes in salivary endocrinology, calcium bioavailability in saliva, salivary calcium-binding proteins and affinity and total protein in saliva in a population of patients at Wilford Hall Medical Center.

Participants in this study are patients that are receiving or will receive treatment in the Endocrinology Section of the Department of Medicine at Wilford Hall Medical Center and who would undergo a diagnostic test as part of their standard evaluation as medically indicated.

The following metabolic disorders are being studied:

a. various degrees of suspected hyperthyroidism,

b. suspected hypothyroidism,

c. adrenal insufficiency and

d. steroid excess.

Pure parotid secretions are being collected utilizing a Lashley cup connected to a disposable plastic tubing with a three-way stopcock attachment. Using a cotton applicator, a 2% citric acid solution is applied to the sides of the patient's tongue to stimulate saliva secretion. The total collection time is 10 minutes and the initial two minute secretion is discarded.

The following biochemical and physiological measurements will be performed on the saliva samples:

a. endocrine measurements - for assaying cortisol from the collected samples, the samples were frozen at -20° C. Samples will be thawed, centrifuged at 1200 x · g to remove particulate and assayed. A sample size between 10 and 50 microliters will be taken for assaying using the radioimmunoassay standardized kits obtained from Diagnostic Products Corporation. All assays will be run in duplicates.

b. biochemical measurements - total protein will be assayed using the Bradford coumassie blue protein assay (bovine serum albumin will serve as protein standard) and converted to equivalents of salivary protein using the methods described by Rabinovitch and coworkers. Calcium will be assayed following the Pierce colorimetric procedure (calcium chloride standards ranging from 0.25 - 1.5 mM will also be run). Amylase will be assayed following the methodology specified in Calbiochem kits for hydrolysis of amylopectin azure. Calcium binding will be measured by a

micro modification of the competitive Chelex resin assay.

All findings from salivary analysis will be statistically tested by a two-way analysis of variance (biological condition versus type of measurement). If this analysis is significant, **a posteriori** test using Duncan's multiple range will be conducted to determine which groups have statistically significant differences from the others. The null hypothesis is that there will be no statistically significant difference between any of the groups.

The remaining saliva collections will be performed by Mr. Antoinne Able during the month of August. Upon arrival of all endocrinologic and biochemical kits at C.I.D., the stored saliva samples will be assayed. The interpretation and analysis of the data will be performed by the principal investigator at his laboratory facilities at his parent Institution.

VI. To lecture to the General Practice Residents of the Department of General Dentistry at Wilford Hall Medical Center.

A two-hour lecture presentation entitled **Nutrition and Oral Health** was offered to the Senior Residents of the General Dentistry Program on June 29, 1988. With the collaboration of the Associated Milk Producers, Inc. of Greater San Antonio a brochure and literature were presented during this period. The lecture highlighted the development of a nutrition support program in an oral health facility. A step-by-step program entitled **Oral Health Nutrition**

**Assessment** was discussed and various oral health type patients were clinically managed throughout the lecture presentation. A total of 12 residents, 2 faculty members of the Department of General Dentistry, a dental student, a medical student, 2 nutritionists from the Associated Milk Producers, Inc. local office and a nutritionist from the School of Dentistry at the University of Texas Health Sciences Center were present during the discussion.

VII. To review the literature related to **The effects of smokeless tobacco and food intake.**

Smokeless tobacco was used by at least 12 million people in the United States last year. The use of smokeless tobacco is increasing, especially among male adolescents and young male adults. Strong evidence is accumulating that smokeless tobacco can cause cancer in humans. The evidence is strongest for cancer in the oral cavity. A high risk of cancer of the cheek and gum is approximately fifty-fold for chronic smokeless tobacco users. Potent carcinogens have been identified in smokeless tobacco and it can be addictive. Long term use of snuff can lead to the development of leukoplakia, particularly at the site of tobacco placement. The leukoplakia may develop dysplasia and, with further transformation, cancer. The purpose of the review of the literature was to generate additional information regarding food additives. The prospective longitudinal in vivo study would investigate the effects of smokeless tobacco and food intake on the oral mucosa of Air Force basic trainees. This

effort could generate a possible collaborative research endeavor between both the USAF/SGDG and my parent Institution.

Presently a collection of research papers are being reviewed in order to develop a protocol at both sites.

VIII. To collaborate in a research project related to the effects of bases and liners in dentistry as they affect bonding of the material to dentin and amount of fluorides released over a period of time.

Upon discussion with Col. John O. Burgess, D.D.S., Director of Dental Research for the Dental Directorate at Wilford Hall Medical Center, a research program was designed for Mr. Bobby Larry, a freshman dental student at Meharry Medical College and a Summer Graduate Student Fellow. This experience included participation in the above mentioned research projects and assignment to study **shear bond strength and fluoride release of bases and liners**. Mr. Larry has almost completed his independent research study and has been instrumental in the accomplishments of some of the above research activities.

IX. To review and update a questionnaire for diabetic patients.

Upon arrival of Mr. Antoinne Able (senior medical student at Meharry Medical College and a Summer Graduate Student Fellow), a conference with Col James Jacobson, M.D., Deputy Director of Hospital Services at Wilford Hall Medical Center, was scheduled. Dr. Jacobson is one of the

endocrinologist that I met during my pre-summer visit to Wilford Hall. Through his generous time and advice, we were able to develop the stress hormones protocol. Mr. Able was assigned to work with the Endocrinology staff and to be the liaison for the salivary study. Mr. Able was also assigned with me to update and develop an instrument to assess the nutrition knowledge of diabetic patients. This instrument was initially developed by Col. Jacobson and later revised and updated by myself. The questionnaire is pending revision from the Diabetic Dietitian in charge of patient education. Eventually this questionnaire will be given to diabetic patients before and after nutrition education. The purpose of the study is to assess the knowledge and application of knowledge of diabetic patients in their daily dietary management and performance.

X. To collaborate in a research project related to the effects of cold sterilization on the accuracy of dental casts.

Objective not fulfilled.

XI. To collaborate in a research project related to the evaluation of different etching techniques for the Maryland dental bridge.

Objective not fulfilled.

XII. <u>RECOMMENDATIONS</u>:

A. Upon completion of the research project related to the effects of resistance of various types of pins used in dentistry and based on the statistical analysis of the

generated data, recommendations will be generated for both manufacturers as well as for the Dental Profession. Participation in the development and review of the research manuscript will be encouraged.

B. Upon completion of the research project related to finishing techniques of composite resins and based on the statistical analysis of the generated data, recommendations will be generated for both manufacturers as well as for the Dental Profession. Participation in the development and review of the research manuscript will be encouraged.

C. Upon completion of the research project related to salivary endocrinology (an activity initiated by myself), I will analyze the data and prepare a manuscript for dissemination. The amount of time required to generate enough information for this research product to be significant requires follow-up research. I will in turn modify this proposal and submit it as a mini grant when I return to my parent Institution. This research has generated interest at the Dental Clinic as well as in the Endocrinology Section which in turn makes it possible for further collaboration within both Wilford Hall and my parent Institution.

D. Upon completion of the review of the literature related to smokeless tobacco, I will provide Dr. John O. Burgess with recommendations for possible additional variables to be included in his project with recruits and oral cancer.

E. The questionnaire related to the nutrition knowledge of diabetes patient will be reviewed by Dr. Jacobson and will be administered to the diabetic patients at Wilford Hall. Participation in the analysis of the results of the questionnaire will be encouraged and recommendations for improvement will be assessed.

F. In all the above mentioned research activities, participation through oral and/or poster presentations will be encouraged, after discussion with Dr. Burgess, at National, International and/or at our parent Institutions in order to disseminate the activities generated during the Summer Fellowship. This will encourage future participation of the Institution as well as of the Faculty in the on-going research efforts of Universal Energy Systems and the U.S. Air Force Office of Scientific Research.