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PART ONE

DETECTION OF Ureaplasma urealyticum
IN CLINICAL SAMPLES BY POLYMERASE
CHAIN REACTION DNA AMPLIFICATION

and

PART TWO

DETERMINATION OF THE SEQUENCE OF THE
AMPLICON OF Ureaplasma diversum GENERATED BY
POLYMERASE CHAIN REACTION WITH UNIVERSAL BACTERIAL 16s rRNA PRIMERS

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PART ONE

DETECTION OF Ureaplasma urealyticum IN CLINICAL SAMPLES BY POLYMERASE CHAIN REACTION DNA AMPLIFICATION

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Abstract

Ureaplasma urealyticum has been found to be associated with a variety of diseases in humans, adults, infants, and neonates. A DNA probe which is specific for a particular target DNA sequence unique to this bacteria has been developed (Brogan et al. 1991). This study details the use of this highly sensitive diagnostic technique in correctly determining the presence and absence of U. urealyticum in 75 clinical samples.

DETECTION OF Ureaplasma urealyticum
IN CLINICAL SAMPLES BY POLYMERASE
CHAIN REACTION DNA AMPLIFICATION

Ellen W. Doss

Introduction

There have been several species of the order Mycoplasmatales, commonly referred to as mycoplasma, isolated from the urogenital tract of humans. Of these species, Ureaplasma urealyticum and Mycoplasma hominis are the only two known to cause disease (Bondi 93). In the mid-1980s, Ureaplasma urealyticum emerged as a genital and urinary tract pathogen in adults, male and female, as well as in the fetus and the newborn. Physicians in many cases have failed to correctly diagnose a patient who has been infected with this bacteria. The difficulty with accepting this organism as a cause of disease in males, females, and neonates can be attributed to the fact that, in many cases, cultural samples cannot be obtained from the infected site, and to the fact that U. urealyticum has also been found in certain asymptomatic individuals. U. urealyticum can be found in the cervix or vagina of 40-80% of sexually mature and asymptomatic women. It has become necessary, therefore, to develop reproducible diagnostic tests that correctly identify the presence of Ureaplasma urealyticum in certain clinical specimens.

In women, colonization has been linked to younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity, and oral contraception. U. urealyticum has also been found to be transmitted to an average of 38% of babies born to infected mothers (Bondi 93). The colonization of U. urealyticum in neonates sharply declines after an age of three months. It has also been found that colonization is very low in older children and sexually inactive adults. After puberty, however, colonization increases with increased sexual activity.

U. urealyticum has been found to cause upper genital or urinary tract diseases in females and males. Some such diseases are nonchlamydial, nongonococcal urethritis in males; prostatitis; epididymitis; chorioamnionitis in females; congenital pneumonia; and, extragenital diseases, such as septic arthritis, in adults (Brogan et al. 1992). The presence of U. urealyticum has also been associated with female urethral syndrome, pelvic inflammatory disease, infertility, spontaneous abortion, prematurity, intrauterine growth retardation, postpartum fever, and low birth weight and respiratory distress in neonates born of infected mothers. Evidence has suggested that in order for the fetus or neonate to become infected, U. urealyticum from the cervix or vagina must invade the amniotic fluid at the beginning of labor and prior to membrane rupture. Risk of infection of the placenta with mycoplasmas increases with the rupture of the membranes, increased duration of labor, and the number of vaginal exams (Bondi 97).

Methodology:

One way that has been used to test for the presence of U. urealyticum is a color change test in 10B media (Remel, Lenexa, KS). This media is a product used to transport and identify U. urealyticum. U. urealyticum possess an enzyme, urease, capable of hydrolyzing urea with the resulting production of ammonia. The production of ammonia causes an increase in the pH of the media which, in turn, causes a change in the color of the test fluid from a salmon to a dark cherry red.

A8 agar (Remel, Lenexa, KS) is another diagnostic test that can be used to culture, isolate, and define U. urealyticum and other genital mycoplasmas. The medium contains peptones which supply nitrogenous substances and amino acids needed for bacterial growth. U. urealyticum produces intense dark, golden brown colonies on this media, while other mycoplasmas appear in a "fried egg" form, only slightly yellow or amber in color.

Currently, these diagnostic tests cannot be considered without

limitations. For example, 10B media is not totally selective for U. urealyticum. The presence of U. urealyticum in liquid and on solid media may be masked by the growth of other larger bacteria which are faster-growing. Also, the slow growth rate and the small size of U. urealyticum often result in long incubation periods in 10B media. These difficulties have prevented laboratories from offering tests for U. urealyticum that accurately identify the presence of this organism in clinical samples. Brogan et al. (1991) have developed a method of diagnosis which requires less time. This method employs the method of Polymerase Chain Reaction (PCR) developed by Kary B. Mullis and colleagues at the Cetus Corporation (1983). Brogan et al. (1991) have developed a set of primers specific to U. urealyticum that can be used with the GeneAmp PCR Core Reagent Kit (Perkin Elmer Cetus, Norwalk, CT).

The Polymerase Chain Reaction provides multiple copies of a particular DNA target template. It is a technique for the in vitro amplification of specific nucleotide sequences by the simultaneous primer extension of complementary strands of DNA. In 1955, Arthur Kornberg and his colleagues at Stanford University discovered an enzyme called DNA polymerase. In the cell, this enzyme repairs and replicates DNA molecules. DNA polymerase can extend a short oligonucleotide primer by attaching a nucleotide to the 3' end when the primer is bound to the complementary strand, or target template. The surrounding solution in the cell must also include dNTPs, nucleotide triphosphate molecules as building blocks of the segment of DNA strand to be extended. DNA polymerase carries out the synthesis of a complementary strand of DNA in the 5' to 3' direction using a single-stranded template.

The Polymerase Chain Reaction uses a similar technique in vitro. However, two primers, rather than one, each complementary to the opposite, heat-denatured strands of a particular region of DNA, are used in this reaction. Primers are used so that each primer extension allows the synthesis of each strand of DNA towards the other (Taylor 1992). Each primer directs the

synthesis of a strand of DNA which can then be used as the template for the other primer not used in the previous extension.

The ingredients of the reaction are deoxynucleotides (dNTPs), which provide energy and nucleosides for the synthesis of the new DNA strand; a DNA polymerase; two primers; a template; a buffer, with a specific pH; and, a magnesium chloride solution. The dNTPs should be present in the reaction mixture in excess as the synthesis step is repeated by heating the newly formed DNA strands to denature them, and then by cooling the reaction mixture to allow the chosen primers to completely anneal to their complementary strand sequences (Taylor 1992).

With each heating and cooling, the amount of DNA complementary to each primer will increase exponentially until one of the reactants is exhausted or the DNA polymerase is unable to quickly synthesize new DNA. Amplification then stops or produces nonspecific DNA strands after a certain number of cycles.

The reactants of PCR are very stable at high temperatures. Thermus aquaticus (Taq) is a heat stable DNA polymerase with two advantages. The replenishment of this enzyme after each denaturing step is not required. Previously used was E. coli DNA polymerase, I-Klenow fragment (Saiki et al. 1988), which required replenishment after each denaturing step. There are other heat stable enzymes now becoming available, such as Thermus thermophilus DNA polymerase, Thermococcus litoralis DNA polymerase, and Bacillus sterochermophilus DNA polymerase. The activity of Taq doubles from 65 C to 72 C, making the temperature of the reaction extremely important. Its optimal activity is over a pH of 8.2 to 9.0 in 10 mM Tris at 25 C. Although Taq DNA polymerase is limited in its ability to synthesize new strands of DNA above 90 C, it is extremely stable to high temperatures and is not irreversibly denatured by exposure to high temperatures. This provides for the more specific annealing of oligonucleotide primers. Taq polymerase is still 50% active after 40 minutes of activity at 95 C, the usual temperature at which denaturation takes place in the polymerase chain reaction.

Deoxynucleotide triphosphates, dATPs, dCTPs, dGTPs, dTTPs are relatively stable at -20 C. Relatively low concentrations of dNTPs have been observed to give satisfactory yields of PCR product. They have also shown to improve specificity and to increase the fidelity of Taq DNA polymerase. Very low concentrations of dNTPs may adversely affect the synthesizing ability of Taq DNA polymerase.

Taq DNA polymerase is very sensitive to varying concentrations of magnesium ion. Very high magnesium ion concentrations are inhibitory. Also, since dNTPs can bind magnesium ions, the exact magnesium ion concentration required to activate Taq DNA polymerase is dependent upon the dNTP concentration. Higher concentrations of dNTPs bind to magnesium ions and reduce the available magnesium ion concentration. The optimal magnesium ion concentration depends ultimately also on the primers and templates used, and therefore, must be determined empirically. Most often, a final concentration of 1.0 to 4.0 mM magnesium chloride in the reaction mixture works well (Perkin Elmer Cetus, Norwalk, CT).

PCR buffer provides a particular pH and ionic strength for amplification. Taq DNA polymerase activity is sensitive to the nature and concentration of monovalent as well as divalent ions (Elrich, Gibbs, Kazazian 12). Concentrations of KCl stimulate the synthesis rate of Taq polymerase by 50% to 60% with an optimum at 50 mM. Higher concentrations of KCl may inhibit enzyme activity.

Oligonucleotide primers are usually 18 to 30 bases in length. They should have similar G and C content (at least 50% G and C, minimal secondary structure, and low possibility of complementary 3' regions.

DNA can be obtained from many different sources as a starting template for successful PCR amplification. One main requirement for amplification is that the DNA template be completely intact. Full denaturation of the template must also occur before the PCR reaction can begin.

The selection of PCR times, temperatures, and number of cycles for each program depends on the DNA template to be amplified,

as well as the primers chosen. Reaction mixtures may vary from 25 ul to 100 ul.

Materials and Methods

Clinical Samples. Clinical specimens were obtained from respiratory tracts of neonates suffering from infection in the form of tracheal aspirants and throat cultures, and from adult patients in the form of vaginal, cervical, or urethral swabs. The isolates from these liquid samples were obtained using standard procedures (Walsh et al. 1991). These clinical samples were transported in Mycotrans transport medium (Irvine Scientific, Santa Ana, CA) from a variety of different Air Force Medical Centers. 10B broth (Remel, Lenexa, KS) was used for the testing of these clinical samples for U. urealyticum. The species of bacteria was determined by the growth and the microscopic observation on Mycotrim GU medium (Irvine Scientific, Santa Ana, CA).

Preparation of samples for PCR Analysis. A 1 ml aliquot of the liquid portion of Mycotrans media, 10B broth, or Mycotrim GU media of the clinical samples received was centrifuged at top speed, 14000 x g, for 15 minutes in a microcentrifuge in order to collect the cells. The supernatant was discarded and the pellet was resuspended in 100 ul of sterile distilled water and then boiled for 10 minutes to break up the cells. After boiling, the sample was then centrifuged again at top speed for 8 minutes to eliminate cellular debris. The supernatant was then saved and stored in the -20 C freezer until PCR analysis.

Oligonucleotides. During PCR analysis, a 186 base pair region of U. urealyticum DNA was amplified by using the 20 deoxynucleotide primers U2-1 (5'-ACTAAATTTATTGTTGTTAA-3') and U2-2 (5'-CAATGTAAATTCGTTTATCA-3') developed by Brogan et al. (1992) which were synthesized first by Midland Certified Reagent Company (Midland, TX).

DNA amplification by PCR. The clinical samples that had been isolated and stored in the -20 C freezer were thawed on

ice. Amplification was performed using the Techne PHC-3 Dri-Block Cyclor (Techne Corp., Princeton, NJ), the GeneAmp Kit with AmpliTag DNA Polymerase (Perkin Elmer Cetus Corp., Norwalk, CT.), and the PCR Optimizer Kit (Invitrogen Corp., San Diego, CA). After some slight problems with the magnesium concentration of the 10X PCR Buffer II from the GeneAmp Kit, the proper buffer, 5X Buffer D, producing optimal results with the particular template and primers was chosen from the PCR Optimizer Kit. The following is the reaction mixture used with reagents from the PCR Optimizer Kit and AmpliTag DNA Polymerase from Perkin Elmer Cetus:

25 ul Reaction Mixture

5X Buffer		
300 mM Tris-HCl		
75 mM (NH)SO		
17.5 mM MgCl	5	ul
PCR Water	10.125	ul
dNTP mix		
2.5 mM of each	2.5	ul
AmpliTag 5U/ul	0.125	ul
Template	6.25	ul
Primers 20uM		
U2-1	0.5	ul
U2-2	0.5	ul

The reaction mixture was overlaid with about 50 ul of mineral oil to prevent evaporation of reaction mixture (Sigma Chemical Company, St. Louis, MO). After an initial denaturing step at 94 C for 3 minutes, amplification was accomplished using 32 cycles, consisting of a denaturing step at 94 C for 1 minute, a primer annealing step at 58 C for 1 minute, and a primer extension at 72 C for 1 minute. A final extension to complete the extension of the primers at 72 C for 5 minutes after the 32 cycles had been completed was also carried out.

Analysis of PCR Products. Twenty-five ul of the reaction mixture was taken out of the PCR reaction tube and placed on

Parafilm "M" Laboratory Film (American National Can). The reaction mixture was then rolled down the Parafilm "M" to eliminate any excess mineral oil from the reaction mixture. About 10 ul of this was then used for analysis. The remaining 15 ul was stored at 4 C for future use. To the 10 ul of PCR product, about 2-3 ul of loading dye containing bromophenol blue was added. This product was electrophoresed for 30 minutes in a 2% Sea Kem GTG agarose gel (FMC, Rockland, ME) containing 1 ug/ml of ethidium bromide. A 1 kb DNA Ladder (Bethesda Research Laboratories Life Technologies, Inc., MD), BioMarker Low Ladder (BioVentures, Murfreesboro, TN), and BioMarker EXT Plus Ladder (AT Biochem) were used for size standards for the amplicons. PCR amplicons were visualized on a ultraviolet transilluminator.

Results:

PCR Analysis. The temperature and duration of each of the steps in the amplification cycles was determined by the melting temperature of the primers. Contamination was reduced by switching pipet tips after each different addition to the PCR reaction mixture. The following 3 pages indicate the results of the PCR and other diagnostic tests performed on 75 clinical samples.

U2-1 & U2-2 Primers

	Prime	#	Date	Media	PCR	ICP
①	1 Hudson, M.	2696	10/2/92	M	-	-
②	2 Jones, G.	2643	10/8/92	M	+	+
③	4 G. A. , M. L.	2017	8/18/92	M	+	+-
④	5 Melanson, M.		7/29/92	M	-	-
⑤	7 Rindsema, M.	2594	10/5/92	M	-	-
⑥	8 Younce, S.	2521	9/29/92	M	-	-
⑦	10 Evans, B. A.	2016	8/19/92	M	+	+
⑧	11 Evans, B. D.	2019	8/19/92	M	-	-
⑨	17 Anoa, C.	1239	4/18/91	arg	+	+
⑩	18 Davis	1286	5/7/93	arg	+	+
⑪	22 Belmonte, J.	2513	10/13/92	arg	-	-
⑫	23 Otis, B.	1592	6/10/93	M	-	-
⑬	38 Jones, B.	1604	6/11/93	M	+	+
⑭	41 Guerrero, B.	1264	5/4/93	M	-	-
⑮	43 Serenci, B.	3300	12/4/92	M	-	-
⑯	45 Krizdick, B.	3092	11/16/92	M	-	-
⑰	47 Evans, B. C.	2018	8/19/92	M	-	-
⑱	50 Lillian, S.	2115	8/27/92	M	-	-
⑲	51 Jones, S.	2116	8/27/92	M	-	-
⑳	53 Richardson, A.	788	3/19/93	Mtm	-	-
㉑	54 Diaz, B. F.	1744	5/9/92	M	-	-
㉒	57 Sherman, J.	2450	9/29/92	M	+	+
㉓	58 Barker, D.	2849	DOB 12/22/66	M	+	+
㉔	72 Davis, B.	1293	5/10/93	M	+	+
㉕	73 Flanagan, B.	1333	4/21/93	M	+	+

Ellen's #1's				5/6/93			
(26)	74	Davis, Inf	1292	~~5/6/93~~	m	+	+
(27)	75	Alvarado	1565	6/8/93	m	-	-
(28)	76	King, Ella	1610	6/15/93	u. media	+	+
(29)	77	Fleming, Inf	1438	5/25/93	m	+	+
(30)	80	Flanagan	1074	4/14/93	m	+	+
(31)	81	Dunham	1513	6/2/93	m	+	+
(32)	-	Lamney	610	3/21/93	u. biad ~~002~~	+	+
(33)	-	Guero, Inf	1263	5/4/93	m	+	+
(34)	-	Layman, A.	~~323~~	2/4/93	m	+	+
(35)	-	Wuhringer, K.	1112	4/21/93	m	+	+
(36)	-	Phillip, S.	1048	4/13/93	m	-	-
(37)	-	Dunham, D.	1513	6/2/93	m	-	-
(38)	-						
(39)	134	Crist, Inf.	1672	6/21/93	m	-	-
(40)	135	Chapman	1666	6/21/93	m	-	-
(41)	166	Rhesus A152	1755	6/29/93	m	-	-
(42)	165	Rhesus 9592	1758	6/29/93	m	-	-
(43)	167	Hall, Inf	1772	6/30/93	u. media	-	-
(44)	168	Hall, Inf	1776	6/30/93	m	+	+
(45)	169	Hall, A.	1779	6/30/93	m	+	+
(46)	171	Davis	1679	6/23/93	m	+	+
(47)	172	Chapman, Inf	1794	7/1/93	m	+	+
(48)	173	Chapman, Inf	1794	7/1/93	10B	+	+
(49)	174	McMichael	1838	7/7/93	m	Faint +	Contaminated
(50)	176	Brown	1824	7/7/93	m	+	+

⑤①	94	Wengert, P.	1777	5/27/92	1 media	-	-
⑤②	96	Spiess, C.	2047	8/20/92	u. media	-	-
⑤③	97	Soures, P.	1883	8/13/92	u. media	-	-
⑤④	99	Bonca, inf B	95	11/2/93	M. Transport	+	+
⑤⑤	101	Oris, inf B	1512	6/10/93	arg.	-	-
⑤⑥	102	Oris, inf B	1510	6/10/93	10B	-	-
⑤⑦	105	Eastling, inf	655	3/5/93	M. Transport	+	+
⑤⑧	106	Flanagan, inf	1074	4/10/93	10B	+	+
⑤⑨	107	Ramirez, F.	2002	10/6/92	arg.	+	+
⑥⑩	108	—	2285	7/23/92	10B	+	+
⑥①	110	Young, Joy	710	3/12/93	M. Transport	+	+
⑥②	111	Stell, L.	877	3/26/93	10B	+	+
⑥③	112	Buckley, L.	375	11/20/92	10B	+	+
⑥④	113	Byrston, P.	—	6/4/92	M	-	-
⑥⑤	178b	Clark	1892	7/19/93	M	-	-
⑥⑥	129	Johnson, E.	1916	7/12/93	M. Transport	+	+
⑥⑦	188	Fannery, inf	2006	7/19/93	M	+	+
⑥⑧	186	Hall, A.	1965	7/15/93	M	+	+
⑥⑨	192	Sandow, A.	2021	7/21/93	M	-	-
⑦⑩	191	Thuse, D.	2028	7/21/93	M	-	-
⑦①	187	Amos, E.	2019	7/21/93	M	-	-
⑦②	182	Witt, L.	1939	7/14/93	M	-	-
⑦③	181	Cudick	1948	7/14/93	M	-	-
⑦④	183	Bayless-Cudick	1971	7/15/93	M	-	-
⑦⑤	170	Strehl, K.	1782-84	6/30/93	M	+	+

Discussion

Currently, the diagnosis of U. urealyticum infections requires long incubation periods and concentrated microscopic examination of growth on certain supportive medium (Mycotrim GU, Irvine Scientific). Furthermore, there is always a possibility of contamination with other microorganisms. In this study, the Polymerase Chain Reaction has demonstrated that diagnosis of U. urealyticum can be accomplished in a matter of about five to six hours.

Some problems may be encountered when clinical specimens are not properly transported from the clinical setting to the laboratory setting. When supported in Mycotrans media, the proper temperature of transport to ensure the support of U. urealyticum is between 4 C and 8 C. This study has found that when proper transportation has not occurred, amplification by PCR does not occur and is visualized on agarose gels in particular lanes as streaks. Certain improvements and further investigations concerning these problems will be needed in the future. Regardless of these problems, this U. urealyticum specific amplification by Polymerase Chain Reaction removes many of the problems found in present clinical diagnostic tests. When certain refinements have been made, this method of analysis will certainly be a benefit in the testing for the presence or absence of this pathogen in cultural samples.

PART TWO

DETERMINATION OF THE SEQUENCE OF THE AMPLICON OF Ureaplasma diversum GENERATED BY POLYMERASE CHAIN REACTION WITH UNIVERSAL BACTERIAL 16S rRNA PRIMERS

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Abstract

The sequence of the amplicon generated by Polymerase Chain Reaction amplification of a sample of Ureaplasma diversum using universal bacterial 16S rRNA primers was determined employing the method of non-isotopic DNA sequencing.

DETERMINATION OF THE SEQUENCE OF THE
AMPLICON OF Ureaplasma diversum GENERATED BY
POLYMERASE CHAIN REACTION WITH UNIVERSAL BACTERIAL 16S rRNA PRIMERS

Ellen W. Doss

Introduction

The bacterium, Ureaplasma diversum belongs to the class, Mollicutes, which consists of procaryotic organisms lacking cell walls. They are among the smallest of all free-living organisms, ranging from 0.2 to 0.8 um in diameter. The genus, Ureaplasma is composed of organisms which possess urease. This enzyme allows for the hydrolyzation of urea.

Ureaplasma were initially isolated from humans. From humans, isolates show a few distinct serotypes within a single species, namely, Ureaplasma urealyticum (Shepard et al. 1974).

The second animal species from which ureaplasmas have been isolated was cattle (Howard, C.J. et al. 1983). The isolates were serologically heterogeneous and existed as three similar, but not identical strains. They were also distinct from the Ureaplasma urealyticum species already isolated from humans (Howard et al. 1983). The basis of this analysis was the comparison of the G and C (guanine and cytosine) content of the DNA of U. urealyticum and U. diversum and by the polyacrylamide gel electrophoresis (PAGE) comparison of the percentages of their respective polypeptides.

The traditional method for the identification of such pathogenic organisms has been their isolation and growth in the laboratory. These procedures, propagation and isolation in the laboratory, have proven to be time-staking and significantly limited in many situations.

An alternative method involves the use of 16S rRNA sequences for the diagnostic studies of these particular pathogenic bacteria, as well as many other bacterial strains. These 16S rRNA genes are found in all bacteria and do not succumb to a great amount of mutations over a short amount of time. It has been noted that highly variable portions of these sequences provide unique,

sensitive identification for any bacterium and, on the other hand, highly conserved regions provide quick identification for all bacteria.

Therefore, certain PCR primers can be used to recognize these highly conserved 16S rRNA gene sequences and then to amplify variable or similar regions. Sequence determination and analysis of PCR products can then be carried out, with subsequent comparison to other sequences of other bacteria determined in the same manner.

Methodology

The sequence of the 1500 base pair amplicon generated by Polymerase Chain Reaction with 16S rRNA universal primers was determined using a non-isotopic DNA sequencing system (United States Biochemical Corp., Cleveland, Ohio). This system is based on the detection of biotinylated DNA fragments on a nylon membrane with chemiluminescence. Sequencing reactions are done using Sequenase Version 2.0 T7 DNA Polymerase. Biotin is incorporated by sequencing with a 5'-biotinylated primer. After the electrophoretic separation of the biotinylated fragments, the membrane is allowed to air dry and is placed in a hybridization bag where a blocking step, a streptavidin-alkaline phosphatase conjugation step, and a series of washes is performed. Streptavidin binds to biotin and thus indirectly links alkaline phosphatase to each DNA fragment. After excess SAAP is washed away, Lumi-Phos 530 is applied to the membrane, which is then sealed in a plastic bag. Lumi-Phos 530 is a chemiluminescent substrate for alkaline phosphatase and when triggered, emits light at a maximum at 530 nm (USB Corp., Cleveland, Ohio). Decomposition of Lumi-Phos 530 is catalyzed by alkaline phosphatase and produces light emissions which can be detected with X-ray film. Exposures of about one to two hours are necessary to detect sequences of 300 bases. The chemiluminescence remains strong for several days so different exposure times can be tried over this time period.

Materials and Methods

DNA amplification by PCR. The U. diversum amplicon was obtained by PCR performed with 1 ul of U. diversum amplicon (1992), the Techne PHC-3 Dri-Block Cycler (Techne Corp., Princeton, NJ), PCR Optimizer Kit (Invitrogen Corp., San Diego, CA), AmpliTag (Perkin Elmer Cetus Corp., Norwalk, CT), and the following primers: GP1 (5'-AGAGTTTGATCCTGGCTCAGGA-3') and GP2 (5'-GGTAGGGATACCTTGTTACGACT-3'). Ten microliters of this PCR product and 2 ul loading dye containing bromophenol blue were electrophoresed in a 2% Sea Kem GTG agarose gel (FMC, Rockland, ME) containing 1 ug/ml of ethidium bromide. A 1 kb DNA Ladder (Bethesda Research Laboratories Life Technologies, Inc., MD) was used as the size standard. The PCR amplicon was visualized at 1500 bp on a ultraviolet transilluminator (Fotodyne, New Berlin, WI).

Direct Cloning of PCR Amplicon. The TA Cloning Kit (Invitrogen) was used for the ligation and transformation of the PCR amplicon of U. diversum. No purification of the amplicon was necessary. Twelve and a half microliters of the amplicon were diluted in 12.5 ul dH O. From this mixture, 5 different tubes were prepared with 0.125, 0.25, 0.50, 0.75, and 1.00 ul of the amplicon mixture, and after ligation into the EcoRI site of the PCR II vector and transformation into E. coli competent cells, were spread onto Luria-Bertani agar plates containing ampicillin (5 ul/5 ml) and X-gal (50 mg/ml). The plate with the ligation and transformation of the 1.00 ul of amplicon mixture had three clones and the plate with 0.125 ul had one clone. A plasmid miniprep was performed on these clones and the minipreps were then digested with EcoRI to see the size of the insert. It was found to be the correct size at 1500 bp.

Restriction Enzyme Mapping. Miniprep #2 was used in the mapping. Single digests and double digestions with EcoRI were performed (Promega, Madison, WI). The digestion found the EcoRI site not in the amplicon and the SalI site not in the plasmid. The results of the restriction endonuclease double digestion showed BamHI and KpnI suitable for nested deletions since two

enzymes that did not cut the amplicon were needed. A 5' cutter and a 3' cutter were necessary.

Nested Deletions. The plasmid miniprep of clone #2 was cut with BamHI and KpnI. After extraction with 1 vol TE saturated phenol/chloroform and ethanol, the pellet was dried and resuspended in 1x Exonuclease buffer (Promega) and then subjected to nested deletions using the Erase-a-Base System (Promega) which is based upon the method of Henikoff (1984). This procedure was necessary in order to obtain DNA fragments small enough to sequence since the 1500 bp amplicon was too large. Transformations of these nested deletions (a series of 8 time points) into DH5 competent cells and plasmid minipreps (4 clones from each of 8 plates) cut with EcoRI and HindIII showed the following subclones as suitable for sequencing: 1A, 2D, 3C, 4A, and 5A. These subclones were then subject to the Geneclean procedure (Bio 101, La Jolla, CA) and then were alkaline denatured.

Sequencing of Sub-Clones. The nucleotide sequences of the resulting smaller inserts were determined by Sequenase Images (USB Corp.). After exposure, the sequences were read two times with further analysis by Dr. Vito DelVecchio.

Results

The sequences are in the process of being read and analyzed. Overlaps in the sequences of the sub-clones will be looked for in order to obtain the complete sequence.

Discussion

After further analysis, the DNA sequences of 5 sub-clones will be placed into the gene sequence analysis program of the Genetics Computer Group (GCG, University of Wisconsin, Madison, WI) and compared to the sequences of other bacteria in order to see similarities and differences among them. In the future, the 1500 bp amplicon obtained by the PCR (using the same universal bacterial primers of this study) of a clinical sample from Rhesus monkey 959Z (Brooks AFB, TX) will also be sequenced and compared to the sequence of the U. diversum amplicon determined in this study.

PART ONE
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DEVELOPMENT OF A POLYMERASE CHAIN REACTION ASSAY
FOR *UREAPLASMA DIVERSUM*

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Abstract

A polymerase chain reaction (PCR) assay has been developed for the detection of *Ureaplasma diversum* DNA in bovine clinical samples. The assay uses oligonucleotide primers, derived from a DNA sequence unique to *Ureaplasma diversum* to generate a discrete product or amplicon. Hybridization studies enabled the definition of the unique fragment and partial sequencing of the segment led to the selection of primers. The PCR based assay is highly specific and sensitive for *U. diversum*. This technique lends itself well to a clinical laboratory setting since it can be performed on crude lysates of samples within six hours. *Ureaplasma diversum* pathogenesis is not well understood and once this assay is established and used regularly, it could help define this microorganism's exact role in the disease state.

Ureaplasma urealyticum, a human pathogen closely related to *U. diversum*, was also studied this summer. It is another poorly understood organism and various experiments were done in an attempt to characterize its etiologic and pathogenic roles in neonatal disease.

DEVELOPMENT OF A POLYMERASE CHAIN REACTION ASSAY FOR *UREAPLASMA DIVERSUM*

Heather R. Panek

Introduction

Ureaplasma diversum is of the class Mollicutes, which includes prokaryotic organisms that lack cell walls and is included in the genus *Ureaplasma* because of its ability to metabolize urea. (1) Ureaplasmas are among the smallest of organisms, 0.2um to 0.8um in diameter, and are thus difficult to define microscopically. Culturing of the ureaplasmas is also challenging due to their exacting nutritional requirements and slow generation time. *Ureaplasma diversum* is differentiated from other ureaplasma species by its guanine and cytosine content. For example, *U. diversum* has a G and C range between 28.7 to 30.2 mol. percent, while *Ureaplasma urealyticum*'s G and C content is 26.9 to 28.0 mol. percent and they do not overlap (2). *Ureaplasma diversum*, which is isolated from cattle, has three cross-reacting but not identical strains (3). This organism can be isolated from asymptomatic animals as well as from diseased urogenital tracts in both sexes of cattle, from eyes of bovine with keratoconjunctivitis, and from pneumonia infected calf lung. The pathogenicity reported includes; mastitis in cows, pneumonia in gnotobiotic calves, vulvitis and conjunctivitis. Virulent and avirulent strains have been described. (4)

Specific nucleic acid probes offer an alternative to culturing methods for the identification of *U. diversum*. A polymerase chain reaction assay, in particular, offers a specific, rapid and sensitive method for the diagnosis of this organism that would be of benefit for the care of domesticated animals and industries dependent on their welfare. Polymerase chain reaction involves two oligonucleotide primers that flank the DNA sequence to be amplified. The primers hybridize on opposite strands of the target sequence and are oriented so the polymerase synthesizes DNA across the target region. This initial synthesis doubles the amount of target. The

extension products are also complimentary to the primers. The cycle is repeated after a heat denaturation step. Rapid, exponential accumulation of the target sequence is accomplished with many (30-35) cycles of denaturation, annealing of primers and primer extension. Since the reaction must cycle between moderate annealing temperatures and high denaturation temperatures, the DNA polymerase from the thermophile *Thermus aquaticus* (Taq) is utilized as it is not inactivated at high temperatures. (5)

Ureaplasma urealyticum, even though it is recovered in a large percentage of asymptomatic adults, is also considered a human pathogen (6). It has been reported to be the most common microorganism isolated from the central nervous system (7) and lower respiratory tract of newborns, especially those born prematurely (8). It has been isolated as the cause of pneumonia and acute respiratory distress in infants in the absence of other microorganisms. (9) Like *U. diversum*, *U. urealyticum* is difficult to diagnose by culturing methods and previously this laboratory developed a PCR assay (10) to quickly identify this organism. On occasion, a positive clinical sample exhibits phenotypes unusual for *Ureaplasma urealyticum*. For example, the clinical isolate from the "Wells" infant grew extremely quickly under normal culturing conditions. Molecular Biology techniques were employed to try and determine what made this sample more virulent versus typical *U. urealyticum*.

Methodology

The mycoplasma and ureaplasma strains were purchased from the American Type Culture Collection (Rockville, MD). They were *M. hominus* ATCC 2314, *M. orale* ATCC 23714, *M. genitalium* ATCC 33530, *M. pneumoniae* ATCC 15531, *M. salivarium* ATCC 33130, *M. hyorhinitis*, ATCC 23839, *U. urealyticum* ATCC 27618, and *U. diversum*

ATCC 43321. Growth media and culture conditions are detailed in Velleca *et al* (11). The cloning vector was the plasmid pUC18 (Gibco BRL) and the competent host cells for the transformation experiments were the *Escherichia coli* strain DH5 α (Gibco BRL).

A fellow student isolated genomic DNA from *U. diversum* by the method of Razin (12). The genomic DNA and pUC18 were digested with the restriction endonuclease, EcoRI to generate fragments of both with "sticky" ends that will allow reassociation later by hydrogen bond interactions. The cut fragments were purified by Bio 101's GeneClean system and ligated to each other with T4 DNA ligase at 16°C. The enzyme seals single stranded nicks between adjacent nucleotides in a duplex DNA strand. Ligase splits ATP to form a enzyme-ATP complex which binds to a nick, exposing a 5' phosphate and a 3' hydroxyl group, ultimately forming a covalent bond between them. (13) The ligated vector and *U. diversum* fragment were then transformed into DH5 α competent cells via a short 42°C heat shock which facilitates DNA uptake (14). Using the host machinery, pUC18 begins to reproduce and express its own products. The plasmid contains an ampicillin resistance gene which it imparts on the host cell, allowing selection for *E. coli* colonies that contain plasmid on Luria Bertani agar/ampicillin (50ug/ml) plates. To select for colonies containing chimeric plasmids versus self-ligated pUC18, a colorimetric technique was used. The *U. diversum* fragment was inserted into pUC18's lac operon, inactivating the β -galactosidase gene. When transformants are grown on media containing X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactosidase), a colorimetric substrate for β -galactosidase, and IPTG (isopropyl- β -D-thiogalactopyranoside), an inducer of β -galactosidase, the *E. coli* containing chimeric vectors with the destroyed β -gal gene are white, while self-ligated pUC18 produces blue bacterial colonies. (15) White

colonies were selected for, grown up overnight in terrific broth and plasmid DNA was obtained by the boiling miniprep method of Holmes (16). The plasmid DNA was cleaved with EcoR1 and run on a one percent agarose gel to check fragment size.

Since *U. diversum* is only isolated from cattle and is fairly distinct from other bovine pathogens, several fragments between the sizes of one and two kilobases were selected to test their uniqueness for *U. diversum* against other species. The fragments were removed from the plasmid with EcoR1, run on a agarose gel to separate them and purified by the GeneClean system. They were then labeled using Boehringer Mannheim's Genius One system. This system incorporates digoxigenin (DIG), a steroid hapten into the *U. diversum* fragments by the random primed labeling method (17). The reaction is performed by Klenow enzyme (DNA polymerase I) which synthesizes complimentary DNA strands from single stranded DNA templates starting at a 3' OH group. A mixture of random sequence hexanucleotide primers, dNTP's, DIG-11-dUTP, Klenow enzyme and denatured (single-stranded) template DNA produces a uniformly DIG labeled copy of any sequence. (18) Several strains of mycoplasma as well as *U. urealyticum* and *diversum* were alkali-denatured, dot blotted onto positively charged nylon membrane (Boehringer Mannheim) and cross-linked to the membrane by a three minute exposure to ultraviolet light. The blots were incubated in hybridization solution containing approximately 5 ng/ml labeled probe (all hybridizations were done per Boehringer Mannheim protocol). After thirty-six hours of incubation at 65°C, the blots were washed successively with 2x and 0.1x SSC solutions containing 0.1% SDS, blocked with supplied blocking reagent to prevent non-specific antibody-enzyme conjugate binding and incubated with anti-digoxigenin-alkaline phosphatase conjugate. The conjugate complexes with digoxigenin adhering to the membrane through complimentary sequence interactions. The membrane was washed again and incubated with Lumi Phos 530 for

detection. Lumi Phos 530 contains a chemiluminescent alkaline phosphatase substrate, Lumigen PPD. Alkaline phosphatase dephosphorylates Lumigen PPD, forming an unstable intermediate which emits light proportional to the amount of alkaline phosphatase present. (18) Emitted light is detected with standard X-ray film placed against the membrane. Alternatively, colorimetric detection was sometimes used. X-phosphate (5-bromo-4-chloro-indolyl phosphate) and NBT (nitroblue tetrazolium salt) produce an insoluble blue precipitate, allowing hybridization detection. This reaction is also catalyzed by alkaline phosphatase. (18)

One fragment, after several verifying trials, was determined to be unique for *U. diversum*. The clone that contained this fragment was designated pUD#36. After the specificity was confirmed, a restriction map of this fragment was constructed using various restriction endonucleases. A restriction map enables the ordering of restriction sites on the DNA segment. It was determined that pUD#36 only contained one endonuclease site, HindIII. This information was obtained to help prepare the fragment for sequencing. Since the average sequencing reaction runs about 250-300 base pairs and pUD#36 is 1500 base pairs in length, it is necessary to create a set of overlapping deletions of the fragment to sequence it in its entirety. Using Promega's Erase-a-Base kit, pUD#36 was cut into nested deletions, each deletion about 250-300 nucleotides shorter than the previous. The deletions were made by Exonuclease III digestion, which cleaves bases from a 5' end in a regular manner (19). To insure unidirectional deletions, it was necessary to cut pUD#36 with two restriction endonucleases, one leaving a 3' overhang to protect that side and another generating the necessary 5' end for ExoIII. The restriction map indicated that Pst I and BamH I would be appropriate for this task since they do not cut inside the fragment and leave the correct overhangs.

Sequencing of the 1500 base pair fragment was done by the chain termination

method of Sanger *et al* (20). This method involves the synthesis of a DNA strand by T7 DNA polymerase from a single stranded template. Synthesis is initiated by an oligonucleotide primer which anneals to the template. Synthesis is terminated by the incorporation of 2'3'-dideoxynucleoside 5' triphosphates (ddNTPs); nucleotide analogs that cannot support continued synthesis. With appropriate concentrations of dNTPs and ddNTPs, synthesis is terminated at each site the analog can be incorporated. Four separate reactions, each with a different ddNTP, generates the complete sequence after running the reactions on a 6% acrylamide gel. (20)

To sequence pUD#36, Sequenase Images (United States Biochemical) was utilized. This is a non-radioactive system that incorporates a 5'-biotinylated primer into the DNA strands and can be enzymatically detected later. The double-stranded pUD#36 was alkali denatured and incubated with the biotinylated primer supplied, which corresponded a sequence on the pUC18. The annealed DNA was then incubated with the Sequenase polymerase and the dNTP (80uM each), ddNTP (8uM) mixture. The reactions were stopped and stored at -20°C until needed.

The sequencing run itself was done with the Auto Trans 350 Direct Transfer Electrophoresis System (Betagen Corporation). A vertical acrylamide gel (22x22cm) separates the DNA according to size. The DNA then moves out of the bottom of the gel onto a positively charged nylon membrane which is continually moved forward (1.8mm/min) on a webbing system attached to a motor. Because the DNA is directly deposited onto the membrane it is immediately ready for non-isotopic detection. The sequencing gel was pre-run at 1100 volts for thirty minutes. The sequencing reactions were heated to 85°C and 2ul of each reaction was loaded onto the gel. After the gel was run for approximately four hours at 1100 volts, the membrane was removed from the Auto Trans apparatus, air dried, blocked with Sequenase Images reagents and incubated with streptavidin alkaline phosphatase conjugate. The

streptavidin binds to the biotin label, indirectly linking alkaline phosphatase to the DNA segments. The membrane was then washed to remove excess conjugate and, as with the previous non-radioactive system, detected on standard X-ray film after incubation with Lumi Phos 530 for several hours. (21) The sequence was then read off the X-ray film and three oligonucleotide sequences were selected for the PCR assay primers. Several criteria were kept in mind for primer selection. The sequences should be between 15 and 30 bases long with approximately equal numbers of A or T's and G or C's. The melting temperature (T_m) should be similar for the two primers to avoid asymmetric priming and should ideally be between 55° and 65°C. The approximate T_m can be calculated by:

$$T_m = 4(\#G \text{ and } C \text{ bases}) + 2(\#A \text{ and } T \text{ bases}).$$

The primers were ordered from Midland Certified Reagents (Midland TX) as follows:

primer 1 (HRP1) 5' GTGATCAACTGCATACCAGC 3'

primer 2 (HRP2) 5' GCACTCTAGCACCTCAGTAG 3'

primer 3 (HRP3) 5' CTGTATGCTGAGTGCGATCG 3'

The PCR primers were first tested with the pUD#36 clone they come from. Amplification was performed using a Techne PHC-3 Dri Block Thermo- cycler and the GeneAmp kit with AmpliTaq DNA polymerase (Perkin Elmer Cetus). One microliter plasmid miniprep (0.05-0.1 ug) was added to 24 ul PCR mix containing final concentrations of 50mM Tris (pH 8.3), 1.5mM MgCl₂, 200uM each dNTP, 0.25U Taq and .2uM each primer. The reaction was overlaid with 50 ul sterile mineral oil. After initial denaturation at 95°C for three minutes, thirty-five cycles of denaturation at 94°C, primer annealing at 58°C and extension at 72°C (each for one minute) were carried out. Five minutes at 72°C was included at the end of amplification to insure complete primer extension. The amplified samples were analyzed on either a 2% agarose gel containing ethidium bromide (50ug/ml) or on an acrylamide minigel and later

stained with ethidium bromide. Once it was determined that the primers generated an amplicon, the assay was optimized with Invitrogen's PCR Optimizer Kit. Polymerase chain reactions were set up first with varying magnesium concentration (1.5mM to 3.5mM) at constant pH (8.5). Then the pH was varied at constant Mg concentration (2.0mM). Other reaction conditions were unchanged. Once the best reaction parameters were established, the PCR assay for both primer sets (HRP1 with HRP2 and HRP1 with HRP3) was tested against *U. diversum* genomic DNA as well as various other mycoplasmas and *U. urealyticum*. The primer sets must next be tested with bovine clinical samples. To obtain an isolate for the PCR assay, clinical samples are spun down to pellet the cells, resuspended in sterile water, boiled for ten minutes, then spun to remove cell debris. The PCR reaction is done with 12.5 ul of the supernatant (per 50 ul total reaction volume). The reaction is cycled 30-35 times as described previously and positive samples identified by a particular sized fragment on a 2% agarose gel. The PCR assay from clinical sample to amplified product determination takes about six hours.

The *Ureaplasma urealyticum* experiments were started before arriving at Brooks AFB. Large amounts of the "Wells" clinical sample were grown up in standard *Ureaplasma* media and genomic DNA was isolated by the method of Razin (12). The genomic DNA was cleaved with the endonuclease EcoR1 for three hours, as was the plasmid vector, pUC18. The digested genomic DNA and the vector were ligated with T4 DNA ligase and transformed into DH5 α *E. coli* competent cells. The transformation yielded approximately 150 chimeric clones which were streaked out on LB agar/ampicillin master library plates. The transformants were grown in 5ml LB/amp broth and plasmid DNA was obtained by the boiling miniprep method (16). The plasmid DNA was then cut with EcoR1 again to separate the plasmid from the "Wells"

DNA on a 1% agarose gel. The gels were vacu-blotted (variation of Southern (22)) onto positively charged nylon and cross-linked to the membrane with three minutes exposure to ultraviolet light. The membranes were then pre-hybridized according to the Boehringer Mannheim Genius protocol. They were probed with digoxigenin-11-dUTP labeled genomic *U. ureaplasma* DNA for thirty-six hours. The blots were washed, blocked for at least six hours and incubated with anti-DIG alkaline phosphatase conjugate for thirty minutes. The membranes were incubated with Lumi Phos 530 for detection. The film was exposed to the blots approximately two hours. *Ureaplasma urealyticum* hybridized with all of the "Wells" fragments except for two, designated pUW #62 and #63. To make sure that these fragments were unique to the clinical sample, the insert DNA was isolated from the plasmid on a 1% agarose gel, excised and purified by the GeneClean method. They were then labeled with DIG-11-dUTP and used to probe a dot blot with several mycoplasmas, *U. urealyticum*, *U. diversum*, pUW#62 and pUW#63 DNA cross-linked on it. If these sequences were specific, they were, as a first step in analysis, to be sequenced and entered into the GCG gene bank program to see if they matched any previously entered sequences.

Results and Conclusions

The *Ureaplasma diversum* project was successful in that a polymerase chain reaction assay was developed. The primers for this assay were selected from partial sequence information of pUD#36. The entire sequence was not determined for several reasons. The new Auto Trans 350 system was very user-friendly and will be beneficial for future non-radioactive sequencing. The problems with the sequencing came from the sequencing reactions, not that they were difficult to set up, but it takes several trials of a new system before optimal reaction parameters can

be determined. Time did not allow these experiments to take place. One change in the basic protocol was that the provided extender mix, which increases the dNTP concentration, was used since the Auto Trans system allows readable sequences in excess of the standard 250-300 base pairs. Also, modifications must be made to the sequencing reactions for adenine and thymine because ureaplasmas have a very high percentage of these bases. Since it was possible to choose oligonucleotide primers from the partial sequence, time was not spent on making many necessary protocol changes. It is recommended that optimal conditions be determined since Sequenase Images seems like a good system. Hopefully, full sequencing of pUD#36 will be done in the future.

Polymerase chain reactions of primer HRP1 with HRP2 were disappointing since they always generated a 200 base pair amplicon. This result usually indicates contamination in the assay. However, even with repeated efforts of eliminating any contamination, it was not possible to alter this result. Primers HRP1 and HRP3 were much more successful. They generate a 500 base pair amplicon for *U. diversum* and not for any other closely related species tested. Primer HRP1 has a T_m of 60°C and HRP3 has a T_m of 62°C. They will generate amplicons at annealing temperatures of 58, 59 and 60°C. The PCR optimization kit helped determine that a Mg^{2+} concentration of 3.5mM at pH 8.5 (buffer "D") is best for these primers, generating an easily detected product with 30-35 cycles. Several tests, that time did not allow this summer, must still be done on this assay. The highest annealing temperature that will still generate an amplicon must be found, as well as the lowest template concentration necessary for detection. Most critical is that this assay be used with a large number of clinical samples. This will, of course, determine the validity of the assay.

The *Ureaplasma urealyticum* project was not successful. There were many setbacks that delayed the progress of these experiments. First of which was a lack of

pUW#62 and #63 DNA. The master plates died and it took several attempts at subcloning to transform the DNA into competent cells. During these attempts #63 was lost and work on #62 continued to move very slowly. When there finally was enough pUW#62 to label and double check its specificity for the "Wells" clinical isolate, there was a problem with the colorimetric detection system that could not be resolved despite many modifications to the protocol. The colorimetric system was abandoned for chemiluminescent detection, which ultimately revealed that pUW#62 was not totally specific for the clinical isolate. However, that result was not as definite as it could have been, since the signal generated by the interactions of pUW#62 fragment with *U. urealyticum* and another mycoplasma were much weaker than the positive control. Even though this particular study did not result in any specific differences being characterized between the "Wells" strain and standard *U. urealyticum*, more investigation should be done on this strain.

In conclusion, the polymerase chain reaction assay developed will be a powerful tool in the diagnosis of *U. diversum*. With rapid and specific means of diagnosis, it will be easier to define *U. diversum* pathogenesis in the bovine population.

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**PCR Detection of Mycoplasma hominis
in Clinical Samples**

**Study of a DNA Probe for Escherichia
coli Strain 0157:H7**

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PCR Detection of Mycoplasma hominis:

Abstract: Mycoplasma hominis was detected in clinical samples by means of a Polymerase Chain Reaction based assay. The detection resulted from the generation of a 152 base pair PCR product which represented a fraction of a 471 base pair Mycoplasma hominis genomic DNA segment. The PCR based assay was developed during last summer's AFOSR program by a colleague who is currently enrolled in a M.D./Ph.D. program at Thomas Jefferson University. My task was to test the specificity of the PCR assay by compiling data using clinical samples supplied in various types of transport media.

Introduction: Mycoplasma hominis is one of the most common organisms associated with neonatal infections. It has been associated with acute urethritis of males as well as vaginitis and cervicitis of females¹. Infections of the female genital tract provide the organism with the opportunity to impact upon pregnancy by infecting the fetus and neonate².

The detection of Mycoplasma hominis is most often done with the use of various types of media. These methods are too slow and are easily contaminated with other organisms. The PCR based assay is a quick and inexpensive way of detecting Mycoplasma hominis in clinical samples.

PCR Detection of *Mycoplasma hominis*:

Experimentation: Clinical samples used included: throat swabs, blood samples, tracheal aspirants, lung biopsies, urethral and vaginal swabs, and placental tissue. The specimens were shipped in various types of transport media, including: Mycotrans transport media (Irvine Scientific, Santa Ana, CA.); Remel Arginine broth (Remel, Lenexa, KS.); and Remel 10B media (Remel). *Mycoplasma hominis* identification was done by microscopic observation of colony characteristics on Remel A8 media (Remel) and Mycotrim GU media (Irvine Scientific). The media based results were compared with the PCR based results (Table #1).

Clinical samples were prepared for PCR analysis by aliquoting 1 ml of transport media into a microcentrifuge tube. This was centrifuged at 14,000 x g for 15 minutes in a microcentrifuge. The supernatant was discarded and the remaining pellet was resuspended in 100 ul of sterile distilled water, boiled for 10 minutes, and stored at -20 C until use.

Clinical samples containing cotton swabs were placed in 1 ml of sterile distilled water and vortexed gently for 15 seconds. The swab was then discarded and the liquid portion was centrifuged at a low speed for a short period of time. The supernatant was transferred to a microcentrifuge tube, boiled for 10 minutes, and stored at -20 C until use.

PCR amplification was done using the Gene Amp Kit with AmpliTaq DNA Polymerase (Perkin Elmer Cetus, Norwalk, CT.) in conjunction with the Techne PHC-3 Dri Block Cyclor (Techne Corporation, Princeton, NJ.). Twenty-five ul of isolated clinical sample was added to 75 ul of PCR reaction mix containing: 50 mM Tris (pH 8.3); 1.5 mM MgCl₂; 200 uM dNTP's; 0.25 ul of Taq Polymerase; and 0.15 mM 20-mer primers Mh-2 (5'-GGTGATTACGTTGTATGC-3') and

PCR Detection of *Mycoplasma hominis*:

Mh-3 (5'-GGTCCTAGACAACTTATAAG-3') (Midland Certified Reagents, Midland TX.). The PCR reaction mix was overlaid with 50 ul of sterile mineral oil (Sigma Chemical Company, St. Louis, MO.). PCR times and temperatures were as follows: initial denaturation at 95 C for 3 min.; amplification using 35 cycles of: denaturation at 94 C for 1 min, annealing at 60 C for 1 min., and extension at 72 C for 1 min. An additional 5 min. at 72 C was added at the end of the 35 cycles to allow for complete extension of the primers.

Twenty-five ul of amplified PCR product was electrophoresed for 60 min. using a 2% Nu Sieve agarose gel (FMC Bioproducts, Rockland, MD.). The gel was prepared with Tris-Acetate-EDTA (40 mM Tris, 20 mM Acetic Acid, 1 mM EDTA, pH 8.3), containing 0.625 ug/ml ethidium bromide (Amresco). The PCR amplicons were visualized on an ultraviolet transilluminator (Fotodyne, New Berlin, WI.) and sized by comparison with the standard 100 bp DNA ladder (Gibco BRL, Bethesda, MD.). Those amplicons which tested positive for *M. hominis* displayed the 152 bp band. A total of 82 clinical samples were tested for *M. hominis* and the PCR based results were in 100% accordance with the media based results recorded in the Epidemiology Lab/Bacteriology Section at Brooks Air Force Base, San Antonio, TX. (Table #1).

PCR Detection of Mycoplasma hominis:

Results:Table #1

	Clinical Sample #	Media	PCR	Media
1)	#924	10B	-	-
2)	#2672	"	-	-
3)	#925	Arginine	+	+
4)	#1592	"	-	-
5)	#1231	"	-	-
6)	#2057	"	-	-
7)	#1239	"	-	-
8)	#2513	"	+	+
9)	#2463	"	+	+
10)	#2044	"	+	+
11)	#1641	"	-	-
12)	#2016	"	-	-
13)	#2017	"	-	-
14)	#2018	"	-	-
15)	#2019	"	-	-
16)	#1293	"	+	+
17)	#1498	"	+	+
18)	#2153	"	-	-
19)	#2116	"	+	+
20)	#523	"	-	-
21)	#2838	"	-	-
22)	#1048	"	+	+
23)	#2645	"	+	+
24)	#2447	"	-	-
25)	#788	"	+	+
26)	#2451	"	-	-
27)	#2603	"	+	+
28)	#2696	Mycotrans	-	-
29)	#2960	"	-	-
30)	#2448	"	-	-
31)	#1286	"	+	+
32)	#3562	"	-	-
33)	#3563	"	-	-
34)	#2115	"	+	+
35)	#3299	"	-	-
36)	#1292	"	-	-
37)	#1438	"	-	-
38)	#2450	"	-	-
39)	#1569	"	-	-
40)	#1309	"	-	-
41)	#3453	"	-	-
42)	#1074	"	-	-
43)	#1913	"	-	-
44)	#1565	"	-	-
45)	#1513	"	-	-

PCR Detection of *Mycoplasma hominis*:

Results: Table #1

Clinical Sample #		Media	PCR	Media
46)	#710	Mycotrans	-	-
47)	#1672	"	-	-
48)	#329	"	-	-
49)	#2242	"	-	-
50)	#1768	"	-	-
51)	#1034	"	-	-
52)	#787	"	-	-
53)	#1838	"	-	-
54)	#1713	"	-	-
55)	#1839	"	-	-
56)	#1847	"	-	-
57)	#1916	"	+	+
58)	#1782	"	-	-
59)	#2802	"	-	-
60)	#1751	"	-	-
61)	#1794	"	-	-
62)	#2807	"	-	-
63)	#95	"	-	-
64)	#1666	"	-	-
65)	#2270	"	-	-
66)	#2292	"	-	-
67)	#2425	"	-	-
68)	#2849	"	-	-
69)	#655	"	-	-
70)	#2700	"	-	-
71)	#1679	"	-	-
72)	#1882	"	-	-
73)	#3444	"	-	-
74)	#2695	"	-	-
75)	#1844	"	+	+
76)	#1945	"	+	+
77)	#1948	"	-	-
78)	#1939	"	-	-
79)	#1968	"	-	-
80)	#1965	"	-	-
81)	#2003	"	-	-
82)	#2006	"	-	-

PCR Detection of Mycoplasma hominis:

Discussion:My goal for the M. hominis project was to confirm the specificity of the DNA Probe which was developed in our lab last summer. According to my results, 82 clinical samples were in 100% accordance with the media based results recorded at Brooks Air Force Base, San Antonio, TX. This data fully supports the accuracy of this PCR based assay for the detection of M. hominis. In the very near future, our lab hopes to begin distribution of a diagnostic test kit for the detection of M. hominis which would be used in clinical labs nationwide.

DNA Probe for EHEC Strain 0157:H7:

Abstract:Enterohemorrhagic Escherichia coli (EHEC) 0157:H7, often referred to as the "Jack in the Box Strain", was first recognized in 1982 during an outbreak of severe bloody diarrhea caused by contaminated hamburger meat. Infection is commonly associated with the consumption of undercooked ground beef, but can also occur from drinking raw milk or sewage contaminated water. The illness often causes bloody diarrhea and abdominal cramps. In more severe cases, involving children under five years of age and the elderly, the illness could cause Hemolytic Uremic Syndrome (HUS). This syndrome leads to the destruction of red blood cells and kidney failure. Currently, there are several clinical tests available for EHEC but they are expensive, time consuming, and problematic with respect to accuracy. The lack of standardization of these assays from one laboratory to the next represents another obstacle in worldwide diagnosis of EHEC. A DNA probe for EHEC would circumvent these problems and also result in rapid therapy for patients.

Introduction:In this study, the development of a DNA probe for EHEC Strain 0157:H7 is outlined. The development of such a system would include in situ hybridization and nucleic acid amplification of EHEC 0157:H7. DNA probes are economical and highly specific ways of diagnosing illness. After the probe specificity has been tested, a PCR based assay can be developed for use in clinical laboratory testing.

DNA Probe for EHEC Strain 0157:H7:

Experimentation: Plasmid DNA from E. coli strain 0157:H7 was isolated by the Magic Miniprep Method (Promega). The 60 megadalton plasmid was separated from genomic DNA by agarose gel electrophoresis using a 1% Agarose II Gel (Amresco). The resulting band of plasmid DNA was excised from the gel and filtered through a 0.45 um Ultrafree-MC Filter Unit (Millipore). The filtrate was then precipitated using 7.5M ammonium acetate and 95% ethanol. The purified DNA was then cut into restriction endonuclease fragments with the enzyme Pst I (Promega). The resulting fragments were gene-cleaned (Bio 101, La Jolla, CA.) and ligated into pUC 18 (Boehringer Mannheim Biochemicals, Indianapolis, IN.), and recombinant DNA molecules were cloned into E. coli DH5 competent cells. These cells were transformed by the method of Hanahan³ and were grown on Luria-Bertani agar containing 60 ug/ml ampicillin in the presence of 50 ul - 2% X-gal (5-bromo-4-chloro-3-indoyl-B-D-galactoside) and 10 ul - IPTG (isopropyl-B-D-thiogalactopyranoside). Colonies containing plasmids with inserts appeared yellow in color and non-recombinants were blue in color. The recombinant plasmids were isolated from each clone by the Miniprep Boiling Lysis Method of Holmes and Quigley⁴, cut with the restriction enzyme Pst I (Promega), and electrophoresed on a 1% Sea Kem GTG agarose gel (FMC Bioproducts, Rockland, ME.) containing 0.625 ug/ml ethidium bromide (Amresco). DNA was visualized using an ultraviolet trans illuminator (Fotodyne, New Berlin, WI.) and sized by comparison with the standard 1 Kb DNA ladder (Gibco BRL, Bethesda, MD.).

DNA Probe for EHEC Strain 0157:H7:

The resulting library will be examined for EHEC 0157:H7 specific genes. Recombinant plasmids containing inserts will be blotted onto positively charged nylon membrane (Boehringer Mannheim) by the method of Southern⁵. Probing will be done with other diarrheagenic strains which I have isolated (88, 189, 194, 221) and clones containing inserts unique for 0157:H7 will be further evaluated.

The EHEC specific clones will be highlighted using a non-radioactive signaling system. The Southern blot will be probed with digoxigenin labeled DNA probes. The hybrids will be detected by complexing digoxigenin DNA with anti-digoxigenin antibodies complexed with alkaline phosphatase. The presence of hybridized probes will be signaled by the action of alkaline phosphatase on Lumi-Phos 530 (Lumigen). The enzyme causes the hydrolysis of Lumi-Phos 530 which results in the emission of photons which are analyzed on Fuji Medical X-Ray Film (Fuji Photo Film Company, Japan). Clones of interest will be sequenced to determine the nucleotide content of the insert DNA.

Sequencing will be done using a non-isotopic DNA Sequenase Imaging System (United States Biochemical Corporation, Cleveland, OH.) in conjunction with the Auto Trans 530 direct transfer electrophoresis system (Betagen). After the sequence has been read, PCR primers will be synthesized and amplification studies will be done. At this point in the study, PCR detection of EHEC strain 0157:H7 can be accomplished.

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ANALOG TO DIGITAL CONVERSION METHODOLOGY AND DOCUMENTATION,
DATA ACQUISITION / CALIBRATION SOFTWARE

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ANALOG TO DIGITAL CONVERSION METHODOLOGY AND DOCUMENTATION, DATA ACQUISITION / CALIBRATION SOFTWARE

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Abstract

A methodology for analog to digital (A/D) conversion and subsequent data analysis of cardiovascular hemodynamic data was developed. Specifically, documentation and computer software were written to support the conversion process.

Two forms of documentation were developed. First, a "how to" manual describing in detail the operation of the hardware and software used in the data conversion process was written. Second, a series of A/D records were created to support, document and simplify the conversion process. These records describe all hardware and software settings that effect the final digital output, the location of the digital output, calibration information, dates and study names, signal names and numbers, and signal quality comments.

Computer software was written to: 1) import raw binary data, 2) aid in calibrating the data, 3) display the data, and 4) automatically pick individual heart beats. These routines are accessible through a user-friendly user interface.

ANALOG TO DIGITAL CONVERSION METHODOLOGY AND DOCUMENTATION, DATA ACQUISITION / CALIBRATION SOFTWARE

Craig Reister

Two main areas of research will be discussed: 1) Development of an analog to digital (A/D) conversion methodology supported by an instructional manual and documentation, and 2) Development of software that will import the raw digital data, calibrate the data, and plot the calibrated data using an object oriented user interface.

A/D METHODOLOGY AND DOCUMENTATION

Introduction

With the increasing demand for digital data at the Laboratory for Aerospace Cardiovascular Research (LACR), the existing methodology and documentation for A/D conversion of data was determined to be inadequate. It was also decided that the data acquisition procedure would be re-evaluated.

Data were recorded on a multi-channel recorder and stored on VHS analog tapes. The A/D process involves using four pieces of hardware; 1) the tape playback system, 2) a low-pass filter, 3) an A/D board, and 4) a computer system. There are software and/or hardware settings involved with each piece of equipment that need to be set properly to produce reliable digital data. Research was performed to determine these optimal settings (see following two paragraphs). The settings used during actual A/D conversion should be documented properly to facilitate future research efforts. Four documents were created for this purpose.

When A/D converting, a main concern is *aliasing* of signals. Basically, aliasing occurs when frequency components of the signal to be sampled exceed $1/2$ of the sampling rate (the Nyquist frequency). These frequencies "fold over" the Nyquist frequency and when A/D converted,

appear to be at a different frequency than they actually are. To prevent aliasing problems, a low-pass filter with a carefully selected cutoff frequency is used (see Carlson, 1986).

Another concern is the selection of the sampling frequency. For signal frequency reproduction, the sampling rate need only be twice the highest peak signal frequency component for 100% accuracy. For signal magnitude reproduction, it is a slightly more complicated issue. To get good accuracy, the generally accepted sampling frequency is ten times the highest peak signal frequency component. However, at 5 times the highest signal component, fair reproduction can be obtained.

Method

From discussions with researchers at LACR, it was determined that the maximum signal frequency content that would be measured is typically 25 Hz, except for ECG which would be at 40 Hz. All of the signals except ECG need to be very accurately reproduced. Therefore it was decided that the sampling frequency would be 250 Hz, and the low-pass filter cutoff frequency would be set at 100 Hz (this is slightly below the Nyquist frequency of 125 Hz due to filter characteristics, see Precision Filters specifications sheet for filter characteristics). This would give 100 % frequency reproduction of all signals, good magnitude reproduction of signals below 25 Hz, and fair magnitude reproduction from 25 to 40 Hz.

The A/D conversion hardware, shown in Figure 1, consists of: an analog tape playback system, low-pass filters, an A/D board, and a host computer.

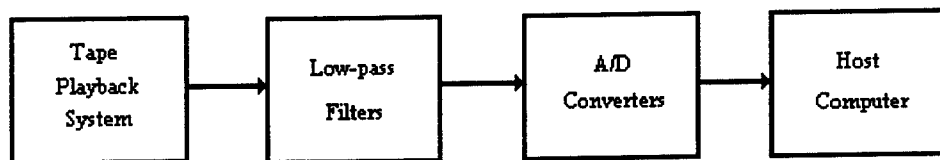


Figure 1

The tape playback system that is in use by LACR is a RACAL V-Store VHS, 24-channel analog recording unit. The settings that can be adjusted on playback are: filter, unipolar, offset level, I/P offset, bandwidth, cal signal, record enable, and I/P atten (see Appendix A, A/D RECORD #1 for optimal settings). No amplification, attenuation, or offset is applied at this stage. A signal of ± 1 V is output by the RACAL playback system.

The second block is a set of low-pass filters from Precision Filters (System 6000, model 6614 low-pass filters). The programmable settings for the filters are: cutoff frequency (FC), pre-gain, bypass, post-gain, zero, and filter type (see Appendix A, A/D RECORD #1 for optimal settings). A post gain of 5 is used to amplify the ± 1 V from the RACAL to match the input range on the A/D board which is ± 5 V.

The A/D conversion board (AD12V26 from Concurrent Corporation) resides in the host computer and is controlled with software that resides on the computer. The software controls the timing of the A/D converters and the user supplies a sampling rate for the converters to use.

The final block is the host computer. The one in use at LACR is a Concurrent 7500 running the UNIX operating system. The software that runs the A/D board and that stores the digital data is located on this machine. There are two pieces of software involved in this process. The software that drives the A/D converters is a commercial package called Lab Work Bench (LWB). The second piece of software is a program, written in-house, that archives the data to an appropriate storage location. These data are also archived on this computer.

The documentation for the entire process described above consists of three A/D records (see Appendix B). A/D record #1 documents all of the analog playback settings as well as the low-pass filter settings. A/D record #2 is used to document the interconnections of the tape playback system, the low-pass filters, and the computer as well as physical signal name and signal quality comments for each signal. A/D RECORD #3 describes the location of all converted data, the sampling frequency used, the analog tape name, and the tape counts from the analog tape that were converted.

The next piece of documentation is the calibration sheet (see Appendix A). This sheet is used to document the calibration gains and offsets of every signal. The gains and offsets are applied to the raw digital data using the formula: $\text{calibrated data} = \text{gain} \times \text{raw data} + \text{offset}$. The result gives a calibrated set of data that represents some physical quantity (eg. pressure in mmHg).

The last document is a "how to" instructional manual for the entire A/D process (see Appendix B)¹. The manual is a complete guide to the A/D conversion process at LACR. It covers the setup of all necessary hardware throughout the entire conversion process. It is a very detailed, self-explanatory document that allows a user to A/D convert data without any prior exposure to the equipment or minimal instruction on the conversion process, other than the manual itself.

Results and Discussion

The research into the methodology of A/D conversion of data produced a set of optimal A/D hardware and software parameters, a series of four documentation sheets, and a "how to" manual describing the A/D process.

Given that the maximum frequency that needs to be accurately reproduced is 25 Hz, the set of optimal parameters are as follows; 1) sampling frequency = 250 Hz, 2) low-pass filter cutoff frequency = 100 Hz, and 3) a gain of 5 is used at the filter stage.

The documentation sheets are to be filled out during the A/D conversion process. The first sheet documents the settings used for the playback system and the low-pass filters. The second sheet shows the signal channel path from the playback system, through the low-pass filters, and to A/D converters. It also matches the physical signal name to the channel numbers. Signal quality comments are also included. The third sheet documents the tape name, UNIX directory where the files reside, the file name(s), the dataset name, the sampling frequency used, and the tape counts from the analog tape that were converted. The final sheet is the calibration sheet. This documents the UNIX file used for the calibrations, the A/D channel, the physical signal name, and the calibration gain and offset.

The "how to" manual is a very detailed, self-explanatory document that allows a user to A/D convert data with no prior exposure to the equipment and little or no instruction other than the document itself.

DATA ACQUISITION / CALIBRATION SOFTWARE

Introduction

After the data have been A/D converted, calibrated and imported into a suitable data analysis software package, one must be able to view the data. We chose MATLAB 4.0 as a tool in developing software for these purposes.

MATLAB 4.0 is a WINDOWS based software package that utilizes an object-oriented, event-driven user interface. Object-oriented simply means that everything that is presented to the user is presented as an object. For example, if a user is to be asked a yes or no question, a 'yes' box and a 'no' box will appear on the screen. To answer yes, the user simply moves a cursor over the 'yes' box and pushes a button (this is done using a mouse). Event-driven software works on the principle that things happen only when events (actions) occur. For example, using the yes or no scenario above, the program would remain idle until the 'yes' button was pushed, then it would execute the appropriate pre-determined software routine. With such an interface, one can build programs that are very powerful, yet easy to operate.

Method

While the software was being developed, one of the things that was kept in mind was ease of use. The reason for this is that the resulting software package is to be operated by many users with various disciplinary and academic backgrounds. To that end, the program is written to be entirely menu driven with a main menu that has six options: *files*, *calibrate*, *pick beats*, *plot*, *analysis* (future development), and *exit* program.

The *files* menu allows the user to import the raw data into MATLAB. The user selects the file they want using a WINDOWS style file selection window and the software imports the raw data into MATLAB.

The *calibrate* menu will contain programs that will aid in the calibration process and will save the calibration data. This part of the program was not fully completed. One of the main calibration routines was written and is in use, but integration into the other programs was not completed.

Pick beats automatically picks heart beats and saves them to disk (this is for the analysis portion of the program and is for future use).

The next menu is the plotting routines. It has a secondary menu that asks if the user wants to display any of five types of plots. They are: single variable, overlaid plots, "movie" which will display the data in motion, beat-by-beat display, and finally a plot that one can select the view and move ones view right or left.

The last menu item is the *analysis* section. This section has not been written and is for future study. Some of the possible routines that will be included here are: analysis of total peripheral resistance and arterial compliance per beat, ventricular dP/dt, arterial elastance ratios, and Pmax.

The final item is used to *exit* the program.

Results and Discussion

MATLAB 4.0 was used to produce a set of programs to aide in importing raw digital data and plotting the calibrated data. The programs use a uniform, object-oriented, graphical interface to allow ease of use.

Acknowledgments: The author wishes to thank Steven C. Koenig for working with me on the documentation, and all the help he has given me. I would also like to thank Dr. Dan Ewert for giving me the opportunity to do my research, for believing in me and for giving me the

opportunity to show what I can do. And last but not least, I would like to thank everyone else at LACR, especially Dr. Swope, Dr. Crisman, and Dr. Latham, for giving me the opportunity to work at LACR and the support once I arrived.

REFERENCES

Carlson, A. Bruce, Communication Systems, Chapter 10, pp. 352-355, McGraw Hill, 1986.

¹ The "how to" manual was a joint effort between this author and Capt. Stephen C. Koenig, USAF, Biomedical Engineer, Laboratory for Aerospace Cardiovascular Research, Brooks Air Force Base, Texas.

Appendix A

A/D RECORD #1

Subject: _____

Study Date(s): _____

A/D Conversion Date: _____

Initials: _____

PRECISION FILTER SETTINGS (All Channels)

Parameter	Suggested Setting	Setting Used	Comments
FC	100 Hz		
PRE GAIN	1		
BYPASS	OFF		
POST GAIN	5		
ZERO	0		
FILTER TYPE	TD6B		

RACAL SETTINGS (All Channels)

Parameter	Suggested Setting	Setting Used	Comments
FILTER	LIN - Ø		
UNIPOLAR	OFF		
OFFSET LEVEL	+000		
VP OFFSET	OFF		
BANDWIDTH	WB2		
CAL SIGNAL	OFF		
RECORD ENABLE	OFF		
VP ATTEN	1		

NOTE: A check mark can be used to signify the suggested setting was used.

A/D RECORD #2

Subject: _____

Study Date(s): _____

A/D Conversion Date: _____

Initials: _____

Filter Channel	A/D Channel	RACAL Channel	Physical Signal	Signal Quality
0	1			
1	2			
2	3			
3	4			
4	5			
5	6			
6	7			
7	8			
8	9			
9	10			
10	11			
11	12			
12	13			
13	14			
14	15			
15	16			

SIGNAL QUALITY RATING: Excellent, Good, Fair, Poor, Non-existent

NOTES:

A/D RECORD #3

Subject: _____

Study Date(s): _____

A/D Conversion Date: _____

Tape Name: _____

UNIX Directory(s): _____

Initials: _____

File Number on UNIX	Dataset	Sampling Frequency	Tape Counts Converted

NOTES:

CALIBRATION SHEET

Subject: _____

Study Date(s): _____

Initials: _____

Protocol Type: _____

Cal Type (pre/post): _____

UNIX File: _____

A/D Channel	Physical Signal	Calibration Gain (units)	Calibration Offset (units)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			

NOTES:

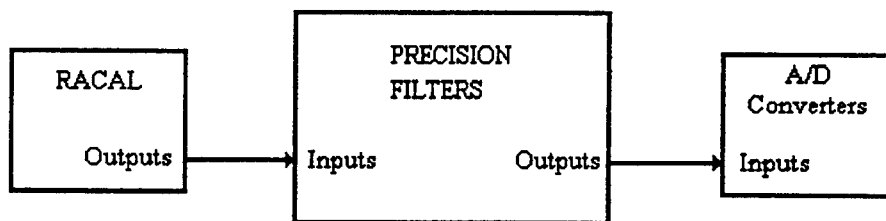
Appendix B

PROCEDURE FOR A/D CONVERTING DATA

Last update: 7/13/93

- 1) Determine the *animal number*, *experiment type*, and *experiment date* that you want to study. This can be accomplished by using the "**LACR Data Inventory**" database. The database can be accessed --- to be finished after the database is completed ---.
- 2) The **LACR Data Inventory** will identify the availability of the following items for each study; strip chart recordings, video recordings, analog tapes, and A/D data tapes.
- 3) Sign out the Data Record Book (DRB) and the Data Record File (DRF) from the LACR data storage custodian (SSGT Joe Weston).
- 4) Review DRB and DRF for all pertinent information for the study(s).
- 5) Sign out strip chart recordings if available (see data storage custodian). If strip charts are unavailable, one may ask engineering to print out desired signals. This will require giving engineering a listing of all *necessary signals*, desired *physiological ranges*, appropriate *chart settings* (speed and layout), and *tape counts* to be printed.
- 6) After reviewing the DRB, DRF, and strip chart recordings, determine epochs, approximate tape counts and viable channel selection (up to 12) for A/D conversion.
- 7) Sign out appropriate tape(s) (see data storage custodian), RACAL (see engineering), and PRECISION FILTERS (see engineering).
- 8) RACAL / PRECISION FILTER Settings:
 - a) Create an A/D record to go into the DRF. The A/D record should contain the following information; *channel assignments*, *channel settings* (gains, offsets, cutoff frequency, description, sampling rate, tape speed), *calibration factors* (gain, offset), *signal quality* comments, and *data file naming convention*.
 - b) Connect the outputs from the RACAL (lower row of BNC connectors) to the FILTER inputs (BNC connectors located on back panel) with appropriately labeled BNC connector cables. Connect the outputs from the FILTER to the A/D converter inputs using the appropriately labeled BNC connector cables. **NOTE:** FILTER output channels should be connected to similarly numbered A/D input channels (these cables should already be connected). RACAL output

channels should be connected to FILTER input channels using the *channel assignments* determined in 8a).



c) "PROGRAM THE FILTER"

Definitions

DISPLAY WINDOW = the LCD display window

SEL(system) = select (SEL) button in the system block below **DISPLAY WINDOW**

ARROW(system) = arrow buttons in the system block below **DISPLAY WINDOW**

ENT(system) = enter (ENT) button in the system block below **DISPLAY WINDOW**

NEXT(system) = next (NEXT) button in the system block below **DISPLAY WINDOW**

SYS(system) = system (SYS) button in the system block below **DISPLAY WINDOW**

DISPLAY(group) = red 7-segment LED display in the group block

ARROW(group) = arrow buttons in the group block on the upper left

DISPLAY(channel) = red 7-segment LED display in the channel block

ARROW(channel) = arrow buttons in the channel block on the upper left

CURSOR = the underscore bar that is movable inside **DISPLAY WINDOW**

"**ARROW TO**" = moving the cursor with the **ARROW(system)** buttons

NUM KEY = group of buttons in the system block to the right of **DISPLAY WINDOW**

*The channels on the filter are labeled 0 - 11.

Procedure

- Turn the power to the precision filter **ON** (lower right corner).
- Push **SYS(system)**
- **ARROW TO** "modify group" using **ARROW(system)**
- Push **SEL(system)**
- Adjust **ARROW(group)** until 6d appears in **DISPLAY(group)**
- **ARROW TO** "add channel" using **ARROW(system)**

- Push **SEL(system)** => green LED to left of **SEL(system)** will light.
- Push "0" button in **NUM KEY**
- Push **ENT(system)** => green LED to left of **SEL(system)** will turn off.
- Push "A" button in **NUM KEY** (this selects all channels) => green LED to left of **SEL(system)** will light.
- Push **ENT(system)** => green LED to left of **SEL(system)** will turn off.
- Push **SYS(system)**
- Push **NEXT(system)** if "FC" is not displayed in **DISPLAY WINDOW** on left below "FILTER". Check to see that "FC" is set to 100 Hz, "PRE GAIN" is set to 1, "BYPASS" is set to OFF, "POST GAIN" is set to 5, and "ZERO" is set to 0 for all channels. This is done by *scrolling* through **DISPLAY(channel)** using **ARROW(channel)** while verifying parameter values in the **DISPLAY WINDOW**.
- If any of these settings are incorrect, **ARROW TO** incorrect parameter and change to appropriate value by typing in correct parameter value using **NUM KEY** and then pushing **ENT(system)**.
- Once these parameter values have been verified, push **NEXT(system)**, which will display another set of parameters in the **DISPLAY WINDOW**.
- Check to see that "FILTER TYPE" is set to TD6B for all channels as previously done.
- If this setting is incorrect, **ARROW TO** "FILTER TYPE" and change by pushing **SEL(system)**, pushing "up" **ARROW(system)**, and pressing **ENT(system)**.

d) Setup the RACAL

- Turn the power to the RACAL on (upper right corner). NOTE: Switch on back panel above power cable must be in the down position, and the front panel "power" on button must be pressed.
- Hook up speaker / microphone to connector just below "volume" button on lower right side of front panel. The audio track will play through the speaker and the volume level can be controlled by pushing + or - on the "volume" button.
- Set the tape speed on the RACAL, located on panel to left of **DISPLAY WINDOW**, to the same value as data was recorded (see DRB or DRF ... usually 15/32 IPS).

- Load analog data tape and push "f.fwd" button for 2-3 seconds, then push "stop" button.
- Push "f.rew" button, to rewind analog data tape to it's beginning.
- Push "tape reset" button to reset tape count to 0000 (this re-matches tape counts on DRB and strip chart recordings).
- Push "search" button, located to right of **DISPLAY WINDOW**.
- Push "adjust" + or - , located below the display screen, until the tape number selected in 6) (start of data set to be A/D converted) appears. NOTE: Watch "TAPE POSITION" in center of **DISPLAY WINDOW** while pressing "adjust" + or -.
- Push "go" button, located below the display screen (this advances analog data tape to desired tape count ==> analog data tape now ready for A/D conversion).

9) Set up the Laboratory Workbench (LWB) header file

- Log in to the A/D workstation, located in Data Analysis room, by entering your account name and password. If you do not know it or have one see System Administrator (???). NOTE: The login name is all in lower case letters. The password will not be displayed, and is case-sensitive (ie. a lower case letter must be typed in as lower case, and an upper case letter must be typed in as upper case).
- Place the cursor inside the new window that appears and push "Return" key, located on the computer keyboard.
- Double-click (push the left mouse button twice) the "A/D Convert" icon, located in the lower left corner of the screen.
- Place the cursor inside the new window that appears and leave it there (this activates the window).

NOTE: The information displayed in [brackets] are default settings for the following sequence of selections, ... they may be selected by pushing *return* only.

- Select *l* (LACR)
- Select *d* (Data Acquisition)
- Select *b* (baboon) or appropriate animal
- Enter the *animal / test number* (eg. a_166)

- Enter the *protocol type number* (eg. 6 for kc_135)
- Enter the procedure date, not the current *date* in the form: YYMMDD where YY is the year number, MM is the month number, and DD is the day number.
- Select 3 (new_12_ch)
- Select n (NEW CHANNEL ASSIGNMENT), if you wish to create a new channel assignment scheme. If an appropriate channel assignment scheme already exists, it may be selected by entering the filename identification number (eg. (1) demo ... ==> enter 1 to select "demo" settings). Else, repeat the following 5 steps *n* number of times where *n* is the number of channels to be A/D converted (*n* should be 12 ==> 12 channels).
 1. A/D channel *assignment* - enter n -- where n is the next channel number (start with 0)
 2. A/D channel *gain* - enter 1
 3. A/D channel *label* - enter the channel label (eg. RA Pressure)
 4. A/D channel *units* - enter the units for this channel (eg. mmHg)
 5. select o (ok to continue)

NOTE: See DRF for channel labels and channel units. Ao Flow is in cc/s. Use NU for ECG and other unitless signals.
- Select r (review / end)
- Select s (save to file)
- Enter the *file name* (eg. "hydration")
- Select c (correct - continue)

At this point, LWB will be run automatically with the header file you just created (it will take a few seconds to activate). Place the cursor inside the LWB window.

10) A/D convert the data wanted using LWB

General Information

Scope #1 contains data channels 0-3

Scope #2 contains data channels 4-7

Scope #3 contains data channels 8-11

Scope #4 contains data channels 0-2 with a wider time base

The signals displayed on each scope are color coded, where;

signal 0,4,8 = white

signal 1,5,9 = red

signal 2,6,10 = yellow

signal 3,7,11 = blue

The headings for each scope are the labels for the first signal (ie. labels for signals 0,4,8)
Signal displays for each window may be adjusted by;

V = vertical setting (amplitude), adjust by clicking mouse on arrow keys

H = horizontal setting (time base), adjust by clicking mouse on arrow keys

red bar (-) = adjust signal offset within scope range, adjust using mouse button

==> click onto red bar, hold down mouse button, and drag bar up or down

Procedure

- Use mouse to click on "Run" command in "Setup" box in top right corner of LWB window. The A/D converters will now be operating, but no data will be stored.
- Push "fwd" on the RACAL. The data signals will now be displayed in two second blocks on the scopes. Visually verify that the data is being converted. NOTE: H, V, and/or red bar (-) may need to be adjusted to see the proper signals (see General Information section above).
- Once satisfied with A/D signal quality, rewind data tape back to desired starting tape count using "f.rew" on RACAL (==> ready to write data to data file).
- Push the "fwd" button on the RACAL.
- Use mouse to click the "On" command in "Switch" box in top center of LWB window. The number in the "Record Data" box, just to the right of the "Switch" box, will start incrementing (be sure to note start and end block #'s). The incoming analog signals are now being A/D converted and stored in a data file.
- Monitor all signals on the scopes to insure data is being A/D converted properly.
- Once all desired data has been A/D converted and you are ready to stop writing it to a data file, use the mouse to click the "Off" command in the "Switch" box in top center of LW window (Note and annotate the end block #).
- Push the "stop" button on the RACAL to stop tape playback.
- If this is all of the desired data to be A/D converted and stored in a data file (==> ready to exit LWB), use the mouse to click the "Exit" command in "Setup" box in top right of LWB window (this allows one to exit LWB). NOTE: the A/D converted data is automatically stored into a data file (tmp.1).

11) Store the file.

- Use mouse to move cursor inside the "A/D Convert" window.
- Select *k* (Keep)

- Select *I* (1:1 ratio)
- Select *c* (Continue) if another batch of data is to be A/D converted, otherwise select *q* (Quit) and the A/D conversion is complete.
- Select *c* (Continue), to create a new data file.
- Go to step 10).

12) Verifying Data File

- Use mouse to place mouse in remaining window.
- Enter directory where data file is stored, for example;
`cd /u/data/LACR/LWB/baboon/a_166/centrifuge`
- Type "ls" to view all data files stored in this directory
 ==> should see appropriate file name (ie. 930126.1.D and 930126.1.H), there should be a data file (D) and a header file (H). If the file does not show up, consult engineering.

13) Logout of A/D Computer Station

- Use mouse to move cursor into "blue area" of computer screen.
- Push and hold far right mouse button which will display "Root Menu".
- Using mouse, drag cursor to "Exit" command, and release mouse button.
- "QUIT" verification menu will appear. Use mouse to move cursor to "OK", push and release the left mouse button. The screen will blank and the "Welcome to the X Window System" screen will appear.

ELASTANCE RATIOS FOR OPTIMAL VENTRICULO-ARTERIAL COUPLING

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Final Report for:
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August, 1993

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Abstract

Computer programs were developed to utilize and verify equations from Ewert, et. al. (1992), which were used to estimate total peripheral resistance and arterial compliance under transient gravitational conditions. These equations along with an equation to estimate the optimal ratio of arterial elastance to left ventricular elastance for maximal external work transfer were then used to analyze high +Gz and micro-gravity data. Preliminary results indicate that the heart and arterial system are at times maximally coupled; however, under most cases, it seems that the two are not. In fact, the trend of the experimental elastance ratio seems to be opposite the trend of the calculated optimal elastance ratio.

ELASTANCE RATIOS FOR OPTIMAL VENTRICULO-ARTERIAL COUPLING

Mark J. Schroeder

Introduction

Two popular theories on how the heart is coupled to the arterial system have been proposed. One theory states that the heart and arterial system are coupled to provide maximum external work, while the other states that maximum efficiency is the desired result (Elzinga and Westerhof, 1980, Sunagawa et. al., 1983). To date, subjects have been studied under both normal conditions and during exercise in an attempt to test these theories. The analysis of data under steady conditions is relatively straightforward. However, since jet pilots and astronauts are not always under normal gravitational conditions, aortic pressure can become very unsteady. Therefore, a set of equations was developed by Ewert, et. al. (1992) in order to study how the cardiovascular system reacts under these conditions. These equations not only provide a better estimate of TPR but attempt to upgrade the standard "effective" arterial elastance index (Sunagawa et. al., 1983) to a more physiological arterial elastance. They also developed an equation to determine the physiological arterial elastance necessary for maximum external work transfer from the left ventricle to the arterial system. This is expressed as an elastance ratio (E_a/E_{es}) which, when compared to the actual elastance ratio, will give researchers insight into how the left ventricle is coupled to the arterial system. This might then provide new ideas on how to better equip our pilots and astronauts when under transient gravitational conditions.

The purpose of this research was to write computer programs to integrate these equations with real, transient +Gz data in order to evaluate the efficacy of the equations and also to process physiological data. Once the data was processed, conclusions were to be drawn on how the cardiovascular system responds to transient gravitational conditions, especially the elastance ratio of the left ventricle and the arterial system during ejection.

Methods

The following two equations (with two unknowns) were used to calculate lumped arterial resistance and lumped arterial capacitance:

$$\int_{t_0}^{t_1} i_{ao} dt = C_A [(P_{ao}(t_1) - P_p(t_1)) - (P_{ao}(t_0) - P_p(t_0))] + \frac{1}{R_A} \int_{t_0}^{t_1} (P_{ao} - P_v) dt$$

$$\int_{t_1}^{t_2} i_{ao} dt = C_A [(P_{ao}(t_2) - P_p(t_2)) - (P_{ao}(t_1) - P_p(t_1))] + \frac{1}{R_A} \int_{t_1}^{t_2} (P_{ao} - P_v) dt$$

i_{ao} = aortic flow

C_A = lumped arterial capacitance

R_A = lumped arterial resistance

P_{ao} = aortic pressure

P_v = venous pressure

P_p = pleural pressure

P_v was estimated as the right atrial pressure and P_p as a low-pass filtered right atrial pressure. If right atrial pressure was unavailable, pleural pressure and venous pressure were omitted from the above equations causing minimal error. Thus, given only blood flow and aortic pressure one can estimate C_A and R_A .

Below are diagrams which illustrate the two elastances $E_a (= 1 / C_A)$ and E_{es} .

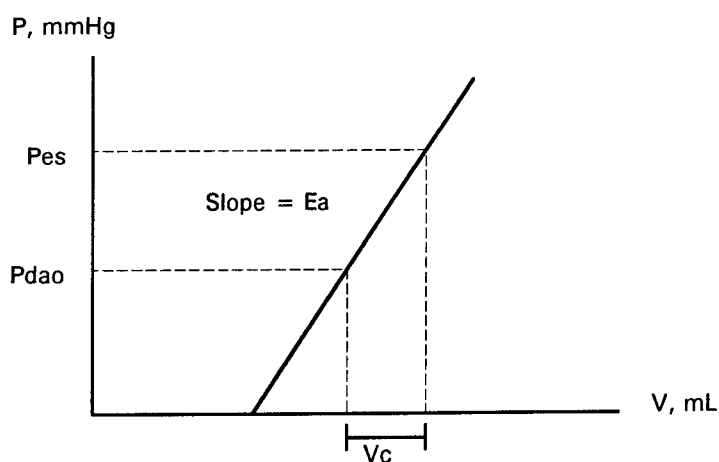


Figure 1: Arterial Elastance

where:

P_{es} = end systolic pressure

P_{dao} = aortic pressure at end diastole

V_c = blood volume in the arterial capacitance

E_a = lumped arterial elastance (mmHg/mL).

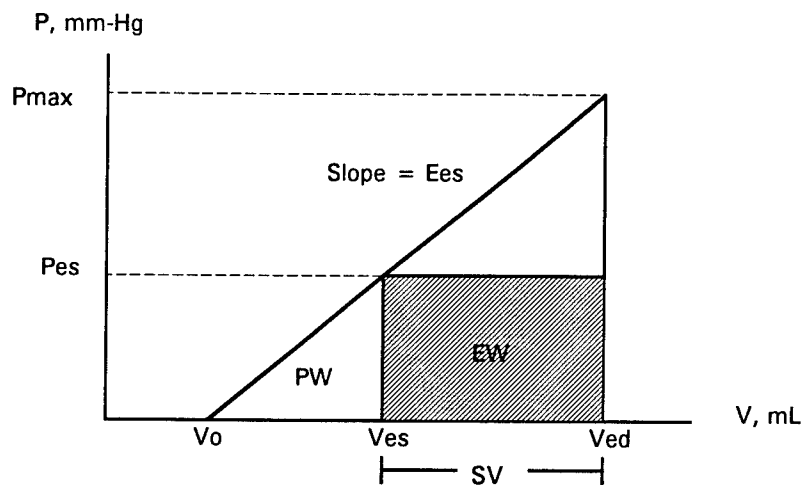


Figure 2: Left Ventricular Elastance

where:

P_{max} = isovolumic maximum pressure*

V_0 = volume in left ventricle when pressure is zero

V_{es} = end systolic volume

V_{ed} = end diastolic volume

SV = stroke volume

E_{es} = end systolic elastance (mmHg/mL)

EW = external work (mmHg*mL = .133 mJ)

PW = potential work (mmHg*mL = .133 mJ).

* estimated using an optimization technique described by Sunagawa, et al. (1980)

From the above figures, the elastances are as follows:

$$E_a = \frac{P_{es} - P_{dao}}{V_c}$$

$$E_{es} = \frac{P_{es}}{V_{ed} - SV - V_o}$$

Note: E_a is assumed to be constant throughout ejection

The blood ejected from the left ventricle must be stored in either the arterial capacitance (C_A) or passed on through the arterial resistance (R_A) to the venous system. This can be written as:

$$SV = V_c + V_R$$

where during ejection:

$$V_c = C_A(P_{es} - P_{dao}) = \frac{1}{E_a}(P_{es} - P_{dao})$$

$$V_R = \frac{1}{R_A} \int_{T_{ej}} (P_{ao} - P_v) dt$$

The above equations were then used to maximize external work (EW) of the left ventricle with respect to E_a by differentiating $EW = P_{es} \cdot SV$ and setting it equal to zero. The final equation that Ewert et al. used to calculate the optimal elastance ratio for maximum external work transfer is:

$$\frac{E_a}{E_{es}} = \frac{\left(\frac{V_R}{V_{max}} - 1 \right) + \frac{P_{dao}}{P_{max}} \left[3 - 2 \left(\frac{V_R}{V_{max}} + \frac{P_{dao}}{P_{max}} \right) \right]}{\left(1 - 2 \frac{V_R}{V_{max}} \right) \left(\frac{V_R}{V_{max}} + \frac{P_{dao}}{P_{max}} - 1 \right)}$$

where $V_{max} = V_{ed} - V_o$.

Results and Discussion

As already shown, E_a is calculated simply as the inverse of C_A . E_{es} , however, could not be calculated by means of the above equation due to the fact that the volumes of the left ventricle were unattainable. This was easily remedied since E_{es} can also be calculated as $(P_{max} - P_{es})/SV$, where SV is simply the time integral of the flow.

The optimal elastance ratio possessed a similar problem in the use of V_{max} . This too was easily remedied by letting $V_{max} = P_{max}/E_{es}$.

External work was approximated to be $P_{es} * SV$. Realizing that an average of left ventricular pressure during ejection would be a better estimate of EW than just P_{es} , the equations were adapted accordingly. By the same token, an average of left ventricular pressure can also be taken during the diastolic phase and adapted into the EW equation to give $EW = (P_{esavg} - P_{davg}) * SV$.

One of the more puzzling problems in estimating R_A and C_A lies in choosing the location in time (t_1) for which the two equations are separated.

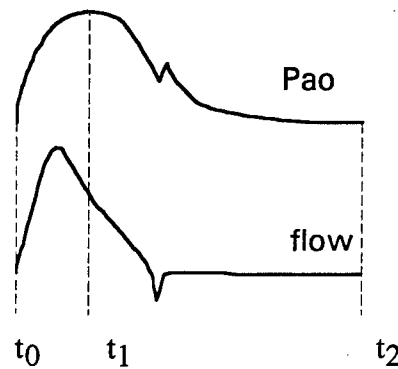


Figure 3: Aortic pressure and flow

Choosing t_1 at various locations produces different results in R_A and C_A . Currently, it seems fit to choose t_1 near maximum aortic pressure but not too near t_0 . This is accomplished by forcing t_1 to be chosen at a distance from t_0 of at least 25% of the total time of the beat. Use of this method has produced consistent results; however, more study is necessary.

During some experiments the flow baseline can be seen to drift. This is particularly noticeable in centrifuge runs due to the high +Gz forces obtained. As harmless as this might seem at first, it is of utmost concern to the laboratory because it raises the question of whether the shift is due to real, physiological affects or instrumentation limitations. More work is being done in this area, but for now, the best estimate is that the drift should not be present. Therefore, before stroke volume was calculated, the blood flow signal was shifted such that the diastolic portion of the flow signal was zero for each beat. A comparison of calculated stroke volumes with and without this offset can be seen in the figure below.

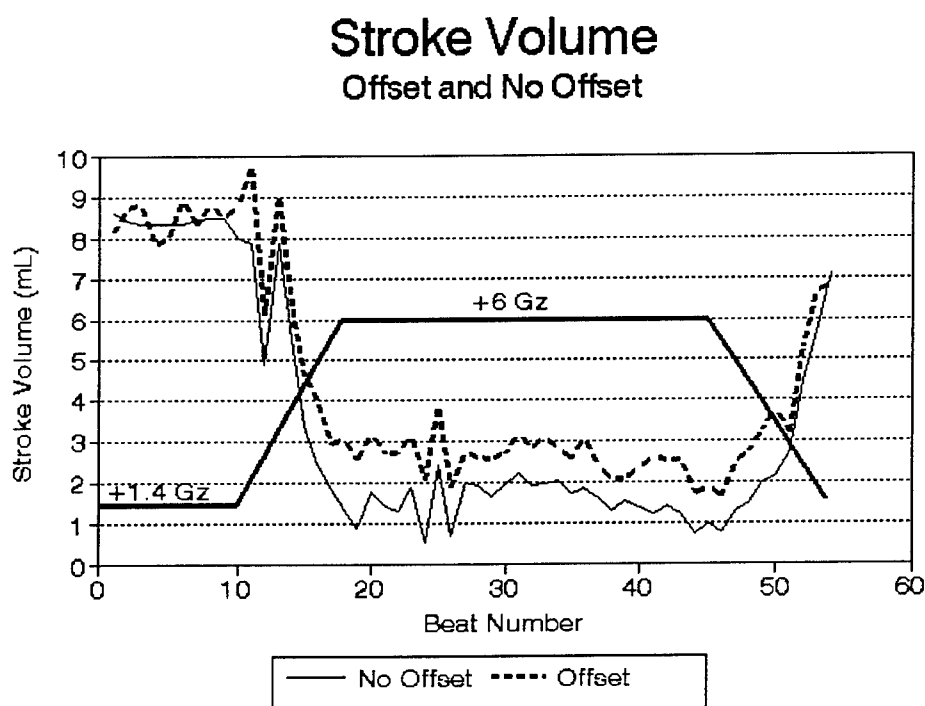


Figure 4: Comparison of offsetting blood flow

The last main concern is the fact that once R_A and C_A are calculated and inserted into their respective volume equations (V_C and V_R), the sum of the two volumes does not add up to the total stroke volume (SV) as can be seen in Figure 5. This discrepancy might indicate an error in the calculation of R_A and C_A . The cause of this problem is still being looked into.

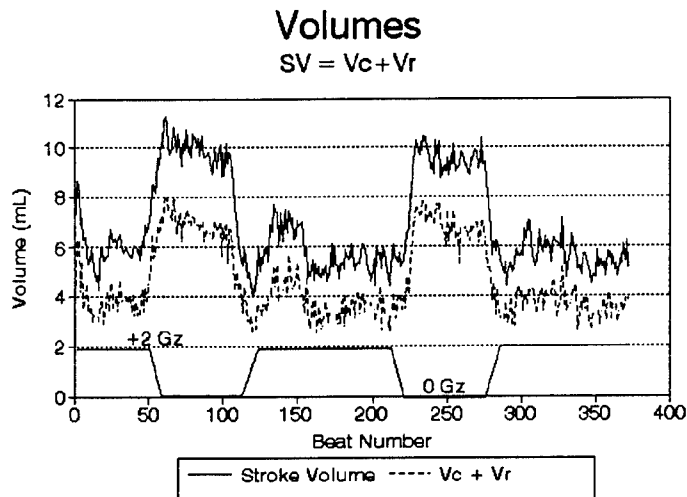


Figure 5: Sum of the volumes

Preliminary results of R_A , C_A and the elastance ratios for two different situations have been calculated. Figures 6, 7 and 8 show the parameters for the case of a parabolic flight in which conditions of both 0 Gz and +2 Gz are achieved intermittently for 30 second periods. Figures 9, 10 and 11 show the parameters for a centrifuge run that begins at approximately +1.4 Gz and momentarily achieves +2 Gz and +3 Gz.

The results for the parabolic flight indicate that arterial capacitance increases and TPR decreases when the body experiences less gravitational force. The converse occurs when experiencing greater gravitational forces. This would agree with conventional thinking in that the systemic arterial system acts to keep the brain supplied with blood during increasing gravitational conditions, i.e. increase resistance and decrease capacitance. The elastance ratios during the parabolic flight indicate that the arterial elastance is not acting in a manner to optimize external work; perhaps a different mechanism is being optimized at this time.

Capacitance and TPR react in a similar manner during the centrifuge run; however, the elastance ratios tend to follow each other in this case. Due to previously mentioned discrepancies, these results are not yet absolute and precaution must be taken before drawing too many conclusions on these studies.

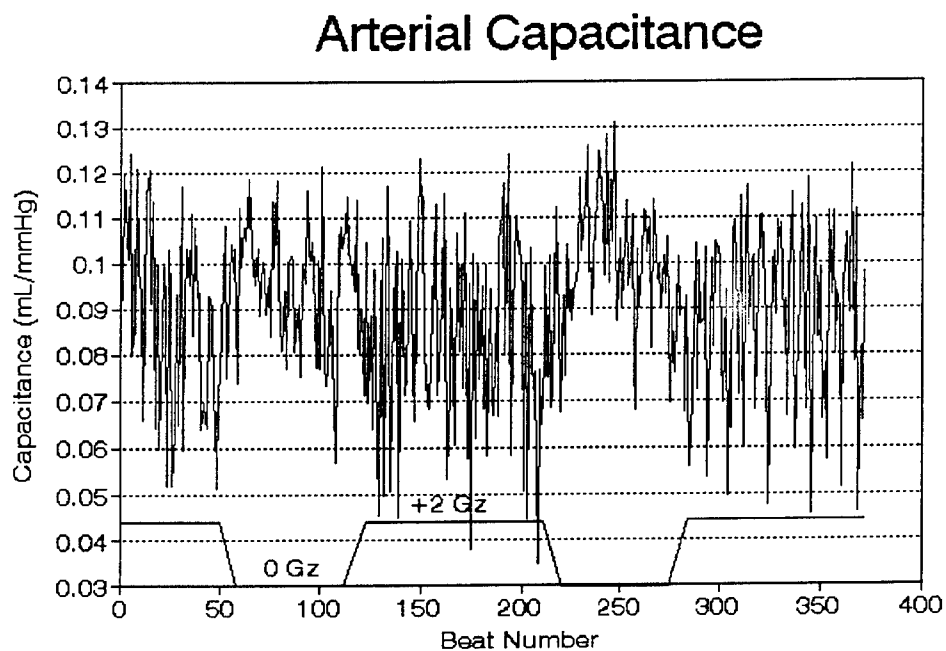


Figure 6: Arterial Capacitance during Parabolic Flight

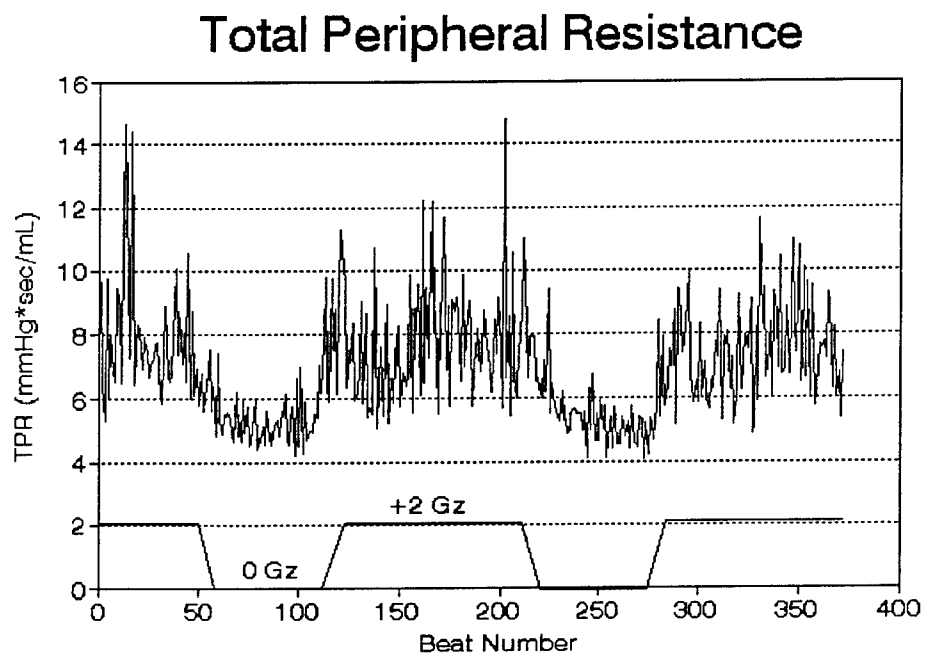


Figure 7: TPR during Parabolic Flight

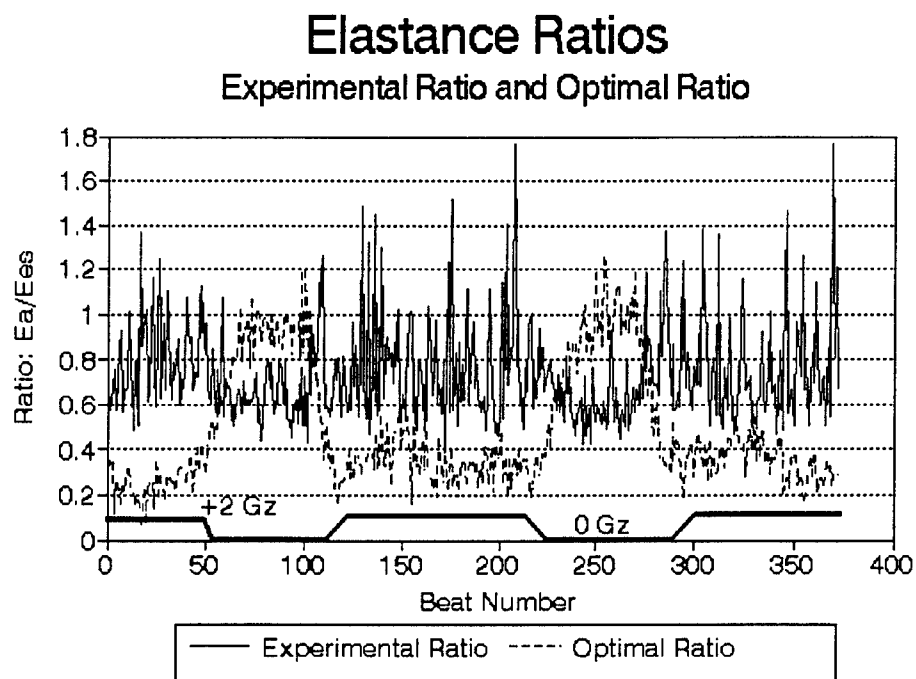


Figure 8: Elastance Ratios during Parabolic Flight

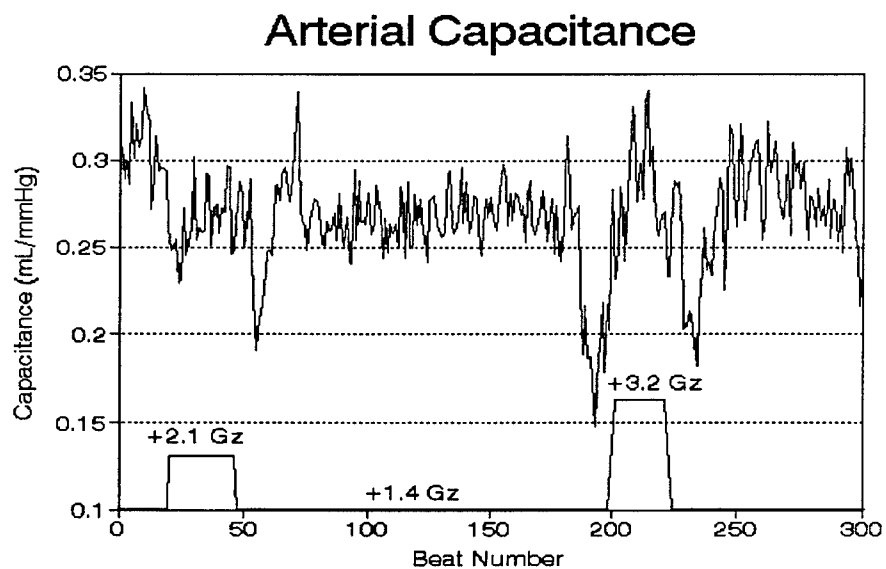


Figure 9: Arterial Capacitance during Centrifuge Run

Total Peripheral Resistance

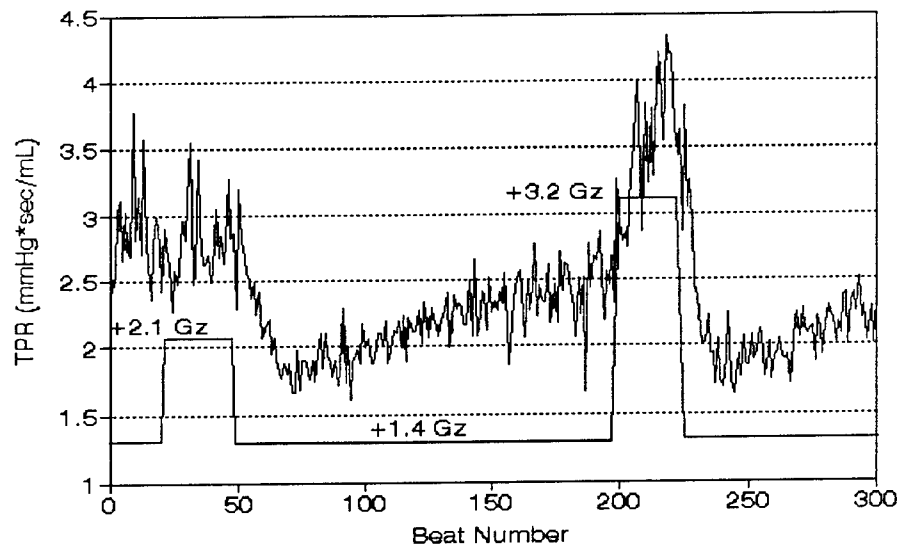


Figure 10: TPR during Centrifuge Run

Elastance Ratios

Experimental Ratio and Optimal Ratio

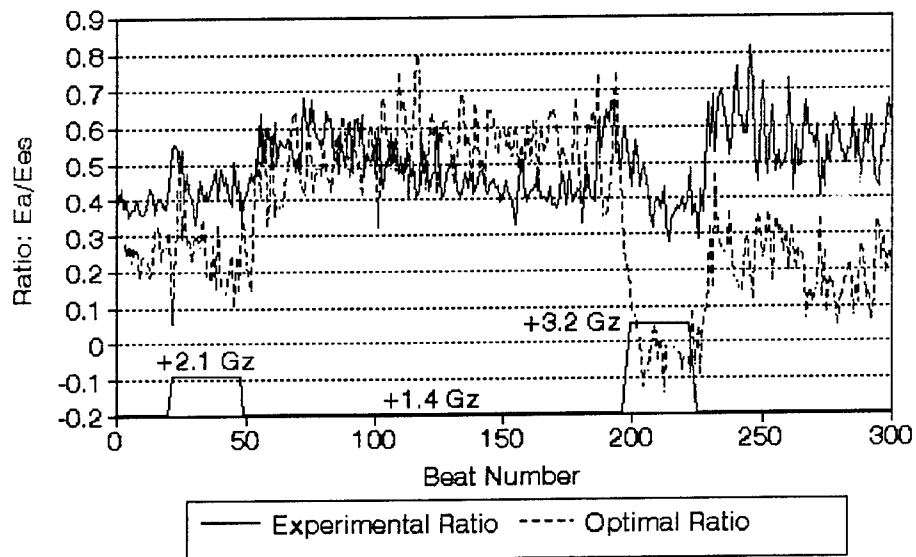


Figure 11: Elastance Ratios during Centrifuge Run

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**PROPOSED STUDY OF HYPERBARIC OXYGENATION INFLUENCE ON HEPATIC CYTOCHROME
P-450 MICROSOMAL ENZYME SYSTEM IN THE MALE SPRAGUE-DAWLEY RAT**

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**Final Report for:
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P-450 MICROSOMAL ENZYME SYSTEM IN THE MALE SPRAGUE-DAWLEY RAT

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ABSTRACT

Hyperbaric oxygenation is currently used by many in the medical community as a standard treatment for various disorders, but there is little information on the subject of hyperbaric oxygenation and drug metabolism. This proposed study will link basic scientific data and suggest new experiments that will further define and validate the role of hyperbaric oxygenation and provide information so other areas of applications, such as novel drug delivery and xenobiotic detoxification, can be investigated.

The main goal of this proposed study is to systematically study the relationship between hyperbaric oxygenation and cytochrome p-450 hepatic microsomal enzyme system in the male Sprague-Dawley rat. Three model drugs antipyrine, quinidine, and acetaminophen would be used to identify changes in the hepatic monooxygenase enzyme system. Data could be gathered from this study that would provide a mechanistic understanding of drug metabolism in a hyperoxic environment.

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INTRODUCTION

Drug handling in the body is dependent on a number of variables. These variables range from drug formulation, absorption, plasma binding affinity, biomembrane barriers, and relative extraction by the specific organ of the body concerned with metabolism of a particular substance to the biotransformation and excretion of the metabolites.¹

The majority of drugs undergo metabolic change. Metabolism usually results in increased polarity, and this reduces the degree of tissue penetration and increases the rate of urinary excretion.² Alteration of drug metabolism may either increase or decrease the biological half-life, depending on the mechanism.³ The most frequent outcome of metabolism is the deactivation of a drug and thus termination of its pharmacologic effect. The converse may happen, resulting in the activation of a prodrug or a change in the nature of the pharmacological activity of a drug.¹ The enzyme system responsible for biotransformation of many drugs are primarily located in the hepatic smooth endoplasmic reticulum. Fragments of this network are isolated by centrifugation of liver homogenates in the microsomal fraction. This study will utilize the two principal methods to assess drug metabolizing enzyme activity in man.

1. Direct measurement of enzyme activity to parallel *in vivo* studies.
2. Measurement of the pharmacokinetics of a model compound. Such as antipyrine, which is used to study factors, which modulate enzyme activity through induction or inhibition.

Cytochrome p-450 monooxygenases, in hepatic microsomes, mediate metabolism by inserting a single atom of oxygen, derived from a diatomic oxygen molecule, into a substrate. Depending on the specific reaction and the nature of various unstable intermediates, different reactions can occur.⁴

Drug metabolism reactions observed in man consist of phase I and II (see fig 1). Phase I reactions include oxidases and oxygenases(i.e. enzymes that require oxygen as a substrate). Over 100 mammalian oxidases and oxygenases are known; including xanthine oxidases, aldehyde oxidases and amine oxidases.⁵ However, most of these are not involved in xenobiotic metabolism. Also included in phase I metabolism are oxidative and reductive dehalogenation; N-hydroxylation and N-oxidation; oxidative deamination; S-, N-, and O-dealkylation; and aliphatic and aromatic hydroxylation. Phase I reactions often lead to introduction of a substituent, which can then combine with the conjugating group.² The major groups involved in the conjugation of substrates are sulfates, glucoronides, glycine, methyl and acetyl groups.⁴ The increased hydrophilicity of the compounds following conjugation results in increased elimination from the body via the urine or bile.

MICROSOMAL ENZYME ACTIVITY

The activity of the microsomal enzymes can be increased by administration of certain drugs and by exposure to various chemicals in the environment.⁶ The classic drugs used to study the induction of the hepatic microsomal enzyme system are barbiturates such as phenobarbital. Stimulation of the microsomal system by phenobarbital results in altered biotransformation of a wide variety of substrates.³ The increase in enzyme activity is attributed to induced synthesis of cytochrome p-450 , cytochrome b₅, cytochrome p-450 reductase, and other monooxygenase enzymes.⁶ In fact, phenobarbital has the ability to cause an increase in hepatic blood flow, bile flow, and other hepatic proteins, including those thought to be important in the uptake of organic anions into hepatocyte.⁶

Since chronic administration of a drug may stimulate its own metabolism and that of other drugs, interactions may occur between either drugs that are simultaneously administered or a drug may "interact" with itself. The concomitant administration of phenobarbital and warfarin results in lower plasma levels of warfarin. Thus the therapeutic concentration of warfarin is not reached resulting in a less than desirable anticoagulant effect.⁶ The desired therapeutic effect can be attained if the dosage of anticoagulant is increased. However if the administration of phenobarbital is discontinued, after

the dosage of warfarin has been increased, severe bleeding may occur. Also repetitive administration of phenobarbital results in a gradual decreased response to the same dose. From the previously described clinical situation, it is apparent that any changes in the drug metabolism enzyme system can result in altered pharmacokinetics and/or pharmacodynamics.

Interestingly, it has been noted following the administration of some known drug to be a hepatic metabolic enzyme inducer (i.e. phenobarbital) that an increase in acute phase protein alpha-1-acid glycoprotein (AGP) occurs.^{7,8} AGP is a plasma protein which binds to basic lipophilic drugs including propranolol, meperidine, quinidine, and chlorpromazine. If a variation in the plasma level of AGP occur, then the free plasma level of the drug in question can vary considerably, whereas the total drug concentration of the drug in plasma may only be affected slightly.^{9,10} Therefore an increase in AGP correlates to a decrease in active (free) drug that will be able to bind to the receptor and elicit a response. Therefore the dose response curve for these basic lipophilic drugs shift to the right when AGP increases. It can be established that changes in protein binding directly effects both drug disposition and pharmacological response. The biochemical mechanism responsible for increased AGP following the administration of hepatic enzyme inducers has not been discovered.

Acetaminophen, a widely used over-the-counter analgesic, is known to cause hepatotoxicity when ingested in large quantities in both animals and man. Hepatotoxicity stems from acetaminophen activation by microsomal p-450 monooxygenases to a reactive intermediate (N-acetyl-p-benzoquinoneimine, NAPQI) that binds to tissue macromolecules, thereby initiating centrilobular hepatic necrosis.¹¹ Studies show that hepatic metabolic enzyme inducers causes an increase in susceptibility to the hepatotoxicity of acetaminophen.¹²

A minimum amount of work has been done with regards to the effects of hyperbaric oxygenation on drug metabolism *in vitro* and even less has been carried out *in vivo*. However it has been demonstrated that hyperbaric oxygenation can increase the cytochrome P-450 content and catalytic activity of rat.¹³ Another study showed that exposure of a dark or light adapted mice to 1-3 ATA oxygen for times ranging from 15 min to 4 hours caused a progressive increase in hepatic cytochrome p-

450, *in vitro* and *in vivo*.¹⁴ It was also demonstrated that induction was a specific effect of increased oxygen tensions and was not due to pressure per se. Neither equivalent pressures of compressed air in the *in vivo* experiments nor equivalent pressures of nitrogen in the *in vitro* experiments induced cytochrome p-450¹⁵. Moreover, when isolated liver cells from mice were made anoxic there was a 30% loss of cytochrome; at 30-50 torr there was a 30% increase; and at 700 torr or higher, 30-60% increase. This means that many enzymes are oxygen dependent under normal physiological conditions.⁴ The reviewed literature also suggest that hyperbaric oxygenation is a profound inducer of lung cytochrome P-450^(11,12,13,14,15,16).

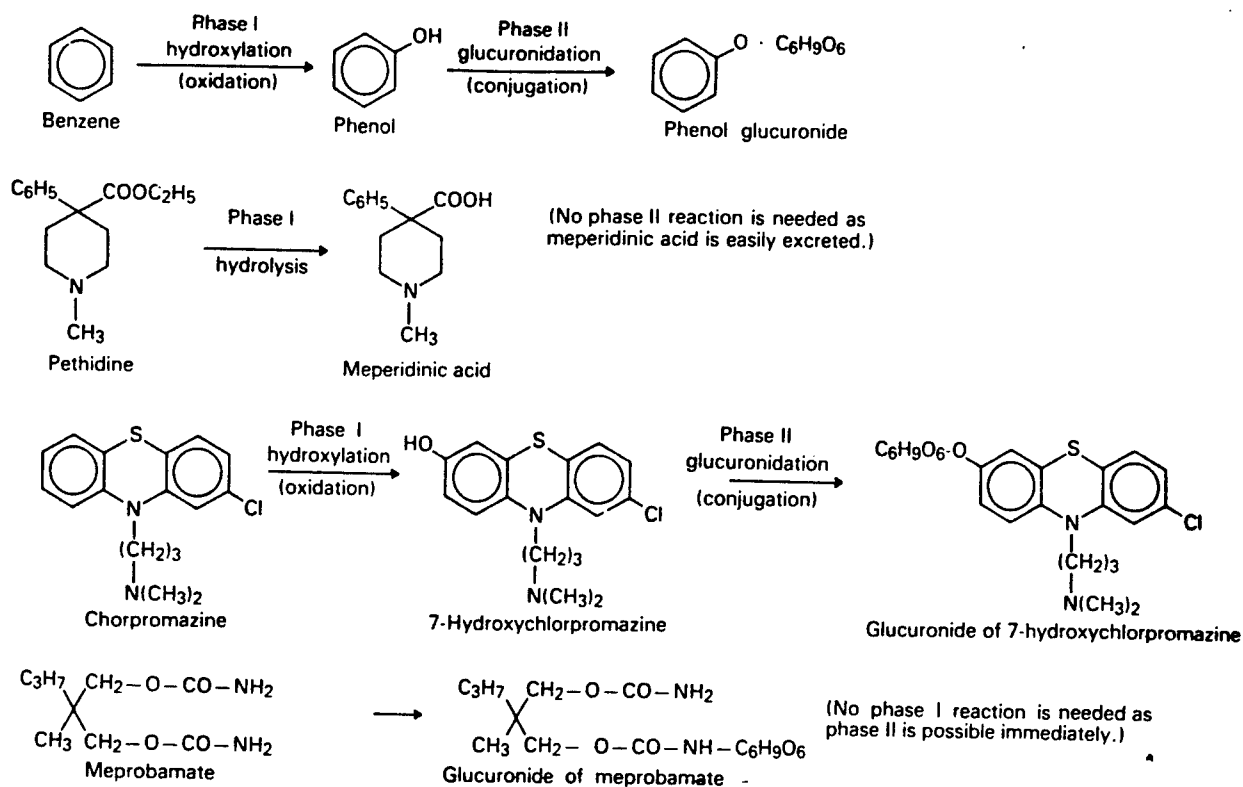


Fig 1.1 A selection of phase I and phase II drug metabolism reactions

Methodology

A minimal amount of basic research has been done investigating the role of HBO in drug metabolism. This study proposes to examine the relationship between HBO and hepatic microsomal drug-oxidizing enzyme activities in male Sprague-Dawley rats by using cytochrome p-450 content and seven cytochrome p-450 mediated hepatic microsomal monooxygenase enzyme activities; UDP-glucuronyl transferase, aminopyrine-n-demethylase, aniline hydroxylase, aldrin epoxidase, 7-ethoxycoumarin-O-deethylase, 7-ethoxyresorufin-O-deethylase, and serum glutamate pyruvate transaminase.

Next the relationship between HBO and alpha-1- glycoprotein concentration, will be accomplished by two methods. The first method is to measure the alpha-1-glycoprotein concentration. The second method to assess the changes in alpha-1-glycoprotein concentration will be through the binding of quinidine, a basic lipophilic drug that binds primarily to alpha-1- glycoprotein. Any changes in alpha-1- glycoprotein concentration will be manifested in an alteration of the bound/unbound ratio of quinidine. Thirdly, the area of oxidative metabolism will be assessed *in vivo* in a subject undergoing hyperbaric oxygenation using the drug antipyrine. Antipyrine will be used because it exhibits the following properties: exponential decay in plasma; negligible protein binding (<10 per cent); one compartment pharmacokinetics; and a high rate of metabolism by microsomal enzymes.

As outlined previously in the proposal, it has been documented that hepatic enzyme inducers are known to accelerate the formation of metabolites which cause cellular damage to the liver. In the final phase of this proposal, the effect of hyperbaric oxygenation on acetaminophen pharmacokinetics and hepatocellular damage will be investigated by using high performance liquid chromatography to assay the acetaminophen concentration and gross histopathological examination to evaluate the insult of acetaminophen metabolite on the liver.

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Target Discrimination in a
Geodesic Sphere

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Geodesic Sphere

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Abstract

Auditorily aided visual localization tasks were compared with non-aided visual localization tasks in a geodesic sphere at 260 points. Reaction times were at their best when the sound was correlated (aided) with the visual target than when the sound and target were uncorrelated (unaided). Performance declined as the targets moved out into the periphery for both conditions, at an accelerated rate for the uncorrelated condition. When in the rear hemifield, performance for the correlated fell to almost 2 1/2 times what it was in the frontal field, while it was as much as 8 times for the uncorrelated condition. Preliminary tests using synthesized sound over headphones have proven to be about as accurate as the free-field correlated condition. This may have strong implications for the use of 3-D headphones in applications for cockpit designs and entertainment.

Target Discrimination in a Geodesic Sphere

John Cisneros

Introduction

Experiments involving visual tasks have given evidence toward the superiority of discrimination at the frontal field of view, at a location of 0 deg. azimuth, 0 deg. elevation (subject facing directly forward) compared to other fields of view (Perrott et al., 1990; Perrott et al., 1991; Perrott and Saberi, 1989). Reaction times are at their lowest at this location. As the target being discriminated moves out into the periphery, reaction times tend to increase. The addition of a sound source, located at the same point as the visual target, has proven to improve performance (Perrott et al., 1991).

As of yet, studies of the rear hemifield have been quite scarce. This is most likely due to the fact that the rear hemifield is not part of the visual field. For purposes of free field testing, it would seem one of the last areas to explore. The consequences of such experimentation include target-acquisition for military use, i.e. pilot awareness of incoming enemy fire, wing man location, target location etc. Also, the perfection of such studies as virtual reality, which is not only relevant for military purposes, but pseudo-reality training and endless opportunities in entertainment.

Apparatus

The Biological Acoustics Branch of the Armstrong Aerospace Medical Research Laboratory maintains an auditory localization facility which measures auditory localization performance in three dimensions. The apparatus used is a fourteen foot diameter, geodesic sphere containing two-hundred and seventy-two loudspeakers spaced about fifteen degrees apart. Each speaker housed an array of four L.E.D.'s (forming a diamond shape) directly in front of it. The sphere was covered with foam to reduce auditory reflections, although the speaker-L.E.D. configurations were left uncovered. The sphere itself was in an acoustically sound-proof room. All speaker-L.E.D. configurations were controlled with a micro-processor located outside of the anechoic chamber.

Procedure

A two-alternative, forced-choice paradigm was used. Subjects were instructed to sit in the dome, so that their head was positioned at the center of the sphere. The subjects sat naturally and were not restrained in any way, but were told to look forward after completion of each trial. They were then instructed to press one of two buttons if the L.E.D. array formed a vertical line or to press the second of the two buttons if the array formed a horizontal line. The room was completely dark if no L.E.D.'s were on. A light reading of a typical L.E.D. display, immediately in front of the array, registered a 16 foot-candle

measurement.

In the first experiment, the speaker correlated with the L.E.D. array and turned on at the exact same time as the array. This was to cue the subject, who was facing forward, where the target appeared. In the second experiment, an uncorrelated sound, positioned under the subject, turned on also at the same time as the visual target. This uncorrelated sound only signaled to the subject when the visual target turned on, but unlike the correlated condition, did not cue the subject to where the target was located.

Two-hundred and sixty targets around the subject were used. The only locations not being used were ten configurations located under the subject, forming an unequal hexagon. Two additional speakers located at -60 deg. elevation, -90 deg. azimuth and -75 deg. elevation, -90 deg. azimuth were also unused due to technical reasons.

Presentations of the targets were randomly displayed one at a time. The intertrial interval was three seconds. The end of a trial occurred when the subject responded to the target orientation with one of the two possible button responses. Five paid, college-aged subjects (ages 20-25) were tested across both positions. A minimum percent correct of 80% was used to give validity to any given session and reaction times were recorded and compared.

Results

As previously tested (Perrott et al., 1991) performance at 0 deg. azimuth and 0 deg. elevation is very accurate and reaction times at their fastest for the correlated condition. As the target moved out into the periphery, reaction times rose from about 720 ms. to about 1050 ms. As the targets moved behind the subject, out of the field of view, reaction time went up an additional 500 ms. This constitutes nearly a two-fold increase in time needed to locate and discriminate a target in the rear hemifield when compared to the initial line of gaze.

In the uncorrelated condition, the reaction time in the initial field of view (0,0) rose from the previous condition by about 60 ms. Peripheral performance jumped close to 2 full seconds, while localization of targets in the rear-hemifield took between 3 and 6 seconds.

Discussion

Clearly, correlated sound cues facilitate target localization not only in the rear-hemifield, but also assist in the acquisition at and around the frontal field of view. This experiment gives credence to a phenomena that may already seem obvious. However it is interesting to note that even in the initial line of gaze, performance is better when an auditory cue is correlated as opposed to uncorrelated with the target.

Reaction times around the median (0 deg. and -180 deg. azimuth), even right next to it were curiously faster than times recorded directly on the median in both conditions. The uncorrelated conditioned results showed slower times in elevations under the line of gaze compared to above the line of gaze.

A very important continuation of this report has already started and preliminary results show that synthesized sound may be almost as accurate as the free field testing. The Auditory Localization Cue Synthesizer (ALCS) developed in the Armstrong labs have shown, in two of the previously tested subjects, to be almost as accurate when using KEMAR Head-Related Transfer Functions (HRTF) over headphones. This follow-up may be crucial in the development and implementation of Synthesized, three dimensional sound.

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**AUGMENTING ADAPTATION AND NATURAL
SELECTION IN MILITARY AVIATORS**

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Abstract

The tendency towards the restriction of biological diversity in aviators is an attempt by the United States military to create a rational process for selecting individuals with the highest potential for battlefield success. A major objective is to use Darwinian selection advantageously, by first understanding the human and machine performance characteristics that lead to battlefield success. Two areas have emerged as areas where improvements may enhance aviator survivability and performance; enhanced prescreening of aviator cardiovascular performance and the development of "smart systems" for biological countermeasures.

Cannon's homeostasis suggests a constancy of the internal environment. Yet, homeostasis is a matter of perspective. It is obvious that maintaining oxygen delivery to the brain is important. Yet to maintain this many other systems are altered along the production-delivery-consumption complex. For instance, the lungs may increase frequency and depth of breathing and the heart may beat faster. Thus, the constant environments of these two are altered.

Thus, the understanding of physiological response to +Gz can not be discussed in terms of homeostasis alone. Therefore LeChateliers principle is important. LeChateliers states that a system in equilibrium will adjust to minimize the effect of an applied force. This gives a unique perspective when considering the response to +Gz. We can view the systemic physiological responses in an n- dimensional state space. A certain trajectory will be traced in this space over time while the system is in equilibrium. When perfusion is altered, there needs to be a compensation. Thus, the organism searches for the best new solution to maintain a new equilibrium with the altered variables. If it is assumed that the system is ordered and not random, then it can tentatively be concluded that some individuals with high +Gz tolerance employ a better search algorithm or that the search can be artificially manipulated.

The purpose of this paper is to explore the analysis of biological signals in aviators with the goal of eventually predicting +Gz tolerance and modeling the physiological response to elevated +Gz.

I. INTRODUCTION

Evolutionists long have held the belief that biological diversity is essential for the propagation and advancement of a species. Yet, military leaders hold an opposing view that biological diversity is incompatible with the efficient and effective resolution of military engagements. The examples of such military belief structures include vision screening for aviators, height and weight requirements, and psychological screening. Additionally, an aviator must also have an education equivalent to a Bachelor's degree. Sociological factors have also influenced biological diversity in the selection and retention of aviators, as females were previously restricted from combat flying positions.

The tendency towards the restriction of biological diversity in aviators is an attempt by the United States military to create a rational process for selecting individuals with the highest potential for battlefield success. The modern battlefield is an environment where Darwinian natural selection determines survival in aviators. A major objective is to use Darwinian selection advantageously, by first understanding the human and machine performance characteristics that lead to battlefield success. With the objective assessment of performance criteria, it is then possible to institute a more encompassing pilot prescreening and training program to enhance those characteristics essential for battlefield success. This approach coupled with the development of advanced aircraft and life support systems provides an opportunity for achieving both a biological and technological advantage over adversaries. With continual improvements in aircraft, life support, and physiological assessment tools, performance criteria can be continually refined along with enhancing prescreening programs and countermeasures. Thus, the more effort placed on understanding and enhancing aviator performance the greater our ability will be to truly understand and combat aviator stressors.

Two areas have emerged as areas where improvements may enhance aviator survivability and performance; enhanced prescreening of aviator cardiovascular performance and the development of "smart systems" for biological countermeasures. These systems may include the deployment of biological sensing devices along with advanced life support systems such as Combat EDGE and ATAGS. The design of life support systems is beyond the scope of this paper as its focus will be on the assessment and prediction of cardiovascular factors essential for success as an aviator.

The modern aviator must evade or seek out and engage his adversary while at the same time striving to maintain cognitive and visual awareness. However, the task of enemy engagement or evasion decreases the ability to maintain cognitive and visual awareness. An aircraft may fly at very high speeds through six degrees of freedom (3 linear and 3 rotational), while to a more limited degree the aviator can also move around in six degrees of freedom. Gravity, flight, and aviator movements results in acceleration being placed on the aviator. A common nomenclature is necessary when discussing the magnitude and direction of the acceleration (Table 1). The measure of G as a unitless multiplier of gravitational acceleration (9.81 m/sec^2) is used to describe the relative intensity of the acceleration. Of major concern to aviators is +Gz acceleration.

Elevated +Gz acceleration leads to pooling of blood in the vasculature of the legs and reduction of blood flow to the cerebral and visual regions. Maximum levels of +Gz of selected worldwide aircraft are presented in Table 2. Paradoxically, perfusion to the

cerebral region is minimized during enemy engagement and evasion where high +Gz is encountered. Thus, a perfusion paradox can be stated:

Perfusion Paradox: During times of elevated cognitive and physiological stress cerebral and ocular perfusion are minimized.

Such perfusion minimization has led to a series of crashes and fatalities in high performance aircraft. Ten crashes of high performance aircraft occurred in one four year period due to G induced loss of consciousness (GLOC). There is a Gaussian distribution of tolerance to elevated +Gz, suggesting that some individuals are better equipped to handle the elevated +Gz. However, there is a subset of the aviator population that will have below normal tolerance to +Gz. The common feature among the high tolerance and low tolerance groups is their effort to resolve the reduction in cerebral and ocular perfusion due to elevated +Gz. Therefore, it would be appropriate to attempt to predict an individuals +Gz tolerance and determine the temporal organization of physiological responses in high +Gz tolerance and low +Gz tolerance groups.

The successful resolution of +Gz tolerance prediction can only be achieved after a more complete understanding of the organized physiological response to high +Gz is developed. The physiological response to high +Gz encompasses many systems including renal, cardiovascular, and respiratory systems. It is their integrated response over time which determines the tolerance to +Gz. Two points of view may be interpreted when considering the response to +Gz; homeostasis and LeChateliers principle.

Cannon's homeostasis suggests a constancy of the internal environment. Yet, homeostasis is a matter of perspective. It is obvious that maintaining oxygen delivery to the brain is important. Yet to maintain this many other systems are altered along the production-delivery-consumption complex. For instance, the lungs may increase frequency and depth of breathing and the heart may beat faster. Thus, the constant environments of these two are altered.

Thus, the understanding of physiological response to +Gz can not be discussed in terms of homeostasis alone. Therefore LeChateliers principle is important. LeChateliers states that a system in equilibrium will adjust to minimize the effect of an applied force. This gives a unique perspective when considering the response to +Gz. We can view the systemic physiological responses in an n- dimensional state space. A certain trajectory will be traced in this space over time while the system is in equilibrium. When perfusion is altered, there needs to be a compensation. Thus, the organism searches for the best new solution to maintain a new equilibrium with the altered variables. If it is assumed that the system is ordered and not random, then it can tentatively be concluded that some individuals with high +Gz tolerance employ a better search algorithm or that the search can be artificially manipulated.

Therefore, it is evident that two types of modeling are necessary. In one case, we are trying to rapidly ascertain and predict the physiological responses to a G challenge. On the other hand we are trying to prescreen for quality pilots. Although the objectives appear on the surface to be different, commonalities exist including the involvement of the autonomic nervous system. In assessing the cardiovascular control loops, it is possible to use preexisting noninvasive clinical tests of autonomic function. These tests in many

laboratories have been computerized to aid in the efficient gathering of results, but provide little in the way of garnering new information.

The autonomic tests are often used to evaluate physiological responses to transient interventions. Yet, clinically often only maximum and minimum responses are recorded with little emphasis on the time response or the relationship of the physiological variables following the transient interventions. The following background objectives are necessary if the responses to elevated +Gz are to be adequately assessed in flight and predicted using ground based ANS tests. These objectives are being pursued at our institution in collaboration with researchers at the Armstrong laboratory.

Objective 1: To develop an efficient and accurate approach to the detection of biological signals and analysis in the time domain.

Objective 2: To develop an efficient algorithm for frequency domain analysis of biological signals.

Objective 3: To apply the approaches in objectives 1 and 2 to the conventional ANS tests along with techniques to model the transient response.

II. BACKGROUND

II. A. High Performance Aircraft and Earth Bound Simulations

Modern jet aircraft have the ability to transverse rapidly the three dimensional space of flight. The plane also carries an aviator which may also move in 6 d.f. However, the aviators movement may be presented as a deviation from the movement of the center of mass of the aircraft. The aircraft has the ability to generate elevate G stress on the aviator. A certain terminology for this G stress is presented in table 1. What should be noted is that G represents a unitless number which is a multiple of gravitational acceleration although the direction may vary. The capabilities of modern aircraft extend up to a wide range (Table 2).

Physiological monitoring of aviators in flight presents some thorny technological and sociological issues. There is a resistance to monitoring aircrew in flight as it may detect abnormalities resulting in grounding of the aviator. The technological obstacles exist but can be overcome readily through solid design. Therefore a ground based approach is necessary for studying aviators. Although a centrifuge does not allow all 6 degrees of freedom of movement, it has the advantage of being a rather well controlled environment. This provides the opportunity to develop countermeasures under a controlled environment. It also allows for extensive physiologic monitoring, including the use of catheters. Thus, the centrifuge is a valuable tool to develop countermeasures and to assess models of the cardiovascular system.

II. B. Biological Signal Processing

II. B. 1. Analog Prefilters

The purpose of prefiltering the analog signals in biological variability analysis is to avoid the introduction of errors due to aliasing. The ideal prefilter for variability studies should have minimal attenuation in the frequencies of interest (passband, 0 to 125 Hz) (1), and create a large signal attenuation at higher frequencies. The cutoff and stopband are two critical frequencies in the design of a prefilter. The cutoff frequency (F_c) is the point

where signal attenuation is initiated (an attenuation of -3 dB). The stopband frequency (F_s) is where attenuation is typically greater than -70 dB. The ratio of stopband frequency to cutoff frequency is often calculated to give a measurement of the rolloff from passband to stopband. In a lowpass filter, the passband region is before the cutoff frequency. In the passband region, minimization of ripples is important.

There are currently four popular types of analog filters; Butterworth, Chebyshev, Elliptic, and Bessel. The Butterworth offers a maximally flat passband, while the Chebyshev has the steepest transition from passband to stopband. The Bessel filter has a maximally flat time delay. The Butterworth filter trades off everything else for maximal flatness. However, the Butterworth has less than ideal phase characteristics. If 1 dB ripples in the passband are acceptable, then a Chebyshev filter can be used. It allows greater sharpness at the knee (3 dB), but has less than ideal phase characteristics. However, no matter what design is employed, flatness of the passband is never really possible. Design criteria calls for very tight tolerances of the resistors and capacitors (1%), but some ripple still occurs. The elliptic or Cauer filter is a variation of the Chebyshev filter and is easy to implement with CAD. The elliptic filter is indicated where the shape of the waveform is important. When accuracy and signal quality are necessary and some delay in the resulting signal output is acceptable, filters with a linear phase response are desirable. If all channels sampled are filtered with the same type of linear phase filter, then all signals will be shifted equally in the time domain.

II. B. 2. Sampling Frequency

The sampling frequency is determined by multiplying the stopband of the prefilter by 2. This value is then multiplied by the number of channels to get the total sampling rate. Sampling at twice the stopband frequency allows for 1 bit resolution to be achieved if the stopband is at -72 dB. To simplify the calculation of storage space requirements, the sampling rate should also be a power of two.

Through the use of signal decimation techniques, it is possible to alter the sampling rate. For a signal sampled at 256 Hz and 1024 Hz, the errors for sampling a 100 BPM heart rate signal range from 2.34 to 0.59 msec, respectively. However, it is possible that both sampling rates may give the same information about the heart rate intervals. The only way to assess this is by doing a signal to noise analysis.

Pizzuti et al. did an analysis of sampling rate and ECG using a system with ± 9 bits accuracy (20). Sampling at 250 Hz rather than 500 Hz is acceptable for large interval measurements such as R-R interval (2). However, sampling at 125 Hz introduced significant errors in interval measurements (2). Merri et al. studied signal to noise ratios of R-R interval measurements at various sampling frequencies (3). The highest quality signal was at 256 Hz, followed by sampling at 128 Hz and 64 Hz (3). Again, supporting the contention of sampling at greater than 250 Hz. It was suggested that the more variable the R-R intervals are, the greater the noise and therefore the higher sampling frequency.

To insure the integrity of the data analysis, it is appropriate to conduct a signal to noise test on each set of data. This will insure that no errors are introduced in the analysis through improper sampling rate selection.

II. B. 3. ECG Noise Suppression

A byproduct of using the surface electrode for the collection of the ECG signal is noise and baseline wander. A new method for the suppression of noise in the ECG signal has recently appeared in the scientific literature and involves the concept of mathematical morphology (4). Mathematical morphology is a technique that has been used primarily in image processing and has been used by some Hollywood studios in the film making process. A example of mathematical morphology would be to take a digital representation of an apple and having it follow a transition path turn into an orange.

A detailed explanation of the mathematical morphology technique applied to the ECG signal is presented elsewhere, but will briefly be described here (4). The objective is to convert a signal containing random noise into a signal devoid of noise. To achieve this objective, mathematical operations referred to as opening and closing are performed on the original signal and a five point structuring element. Basically, the original ECG signal is parallel processed along two paths. One path consists of an opening function followed by a closing function. The other path is a closing function followed by an opening function. The two paths are then averaged, resulting in an ECG signal with reduced random noise content.

II. B. 4. ECG Baseline Wander Normalization

Once random noise is removed from the ECG signal, it would be appropriate to normalize the baseline wander in the signal. Baseline wander can have a negative influence on the QRS detection algorithms. Additionally, it may make interpretation of the ECG difficult. Two approaches will be considered for using software post-processing to remove baseline wander (4,5). The first method involves mathematical morphology which was described previously (4). However, in the removal of baseline wander a longer structuring element (41 elements) is used (4). Because of the recursive nature of the mathematical morphology algorithm, it may be possible to use the algorithm in real time processing (with a minor delay).

The other software based approach was to develop a digital high pass filter to remove baseline wander (5). using this filter design the data is first passed through the filter in the forward direction. The resulting array is the reversed and passed through the filter again, yielding the final array with a normalized baseline. Sample code for this procedure is presented in the Appendix. Each filtering procedure causes a nonlinear phase shift in the data. However, because the filtering of each stage occurs in opposite directions the resultant phase shift is minimized, and is in fact close to zero (5).

II. B. 5. Peak Detection Algorithms

Once an analog ECG signal has been pre-filtered and converted into digital form by sampling at an appropriate rate, the R peaks of each QRS complex must be detected. Over thirty seven different methodologies have been published concerning the detection of QRS peaks (6). One of the simplest approaches to use, if the ECG signal has a stable baseline, is a peak threshold detection of a negative derivative. When the derivative signal crosses the zero line (corresponding to a R peak) the peak detector records the time of occurrence. If the data is not free of noise or unstable this will not work accurately. For instance, the baseline wander may cross the zero or threshold line yielding a faulty detection. This method may also be corrupted by high T waves.

Friesen presents two approaches by others that appear to be very appropriate for the analysis of the ECG during centrifugation studies (6). The Menard method is a simple approach to QRS peak detection. The first derivative of the ECG voltage is computed by formula, and the maximum derivative of the array is used to set a threshold to compare other values against for possible detections (6). Sample code for this method is presented in the Appendix. The Okada method is mathematically more intensive as it requires numerous steps for a digital filtering approach. The Okada algorithm is also presented in the appendix.

Once the complexes are detected it is necessary to find the true peak. A simple approach would be to find the first point following the detection where the derivative is zero. However, with the noisy environment there may be many such points a short distance from the QRS detection. Thus, it is more appropriate to find the maximum value of the ECG signal within a short window following the QRS detection.

II. B. 6. Instantaneous Heart Rate Determination

Although the heart rate power spectrum can be computed using just the R-R intervals, it tends to create errors in the spectrum. To obtain a more precise power spectrum representation, the heart rate needs to be sampled at regularly spaced intervals; in other words an instantaneous heart rate. Five methodological approaches exist for the determination of instantaneous heart rate (7,8). Factors to be considered include correcting for the delay encountered in some of the methods and the errors induced by each methodological approach. De Boar et al. present three methods; spectrum of intervals, spectrum of counts, and spectrum of inverse integrals (8). Berger et al. present two methods (7). The first method involves taking the reciprocal of the R-R interval duration and passing it through a low pass filter window with a width of 2 times the sampling frequency.

An equivalent approach to determine instantaneous rate is to let:

$R_i = f_r * n_i / 2$ where R is the instantaneous rate, f_r is the sampling rate and n_i is the sum of all fractions of intervals covered by that window (7).

II. B. 7. Computing the Power Spectrum

The power spectrum can be computed after the biological signal is in digital form and preferably converted to an instantaneous sampling rate. Nine methods have been described for the generation of power spectrums from time domain signals (9). One of these approaches has three variations, bringing the total to eleven possible methods with their own distinct advantages and drawbacks (9). The power spectrum is an assessment of the relative contribution of each frequency to the signal. For instance, if a signal repeats at exactly a 2 Hz period, such as a sine wave a single sharp peak will appear. However, if a signal is composed of repeating components of various periods of duration, multiple peaks are possible.

The generation of a power spectrum is typically based on two approaches; the autoregressive approach and the fast Fourier transform (FFT) based approaches. Press et al provide C algorithms for both approaches (10). The AR approach has the advantage over the FFT in that it does not require a power of two for the number of samples (9). Additionally, less samples are required (9). On the other hand, more care must be

employed in the use of the AR approach. The proper order of the AR approach can be determined using the Akaike Information Criteria (AIC) (11). The easiest method is the FFT based, periodogram approach. Minimal computational and software design strain is necessary to generate the power spectrum. A variation on this theme is the Blackman-Tukey approach, employed by Cohen's group (7). A practical application of the AR approach is in Bartoli et al (12) and an additional algorithm is in Marple (11).

Recently, additional approaches to frequency domain analysis have been proposed including complex demodulation and the use of a forgetting factor with the autoregressive approach. Complex demodulation is based on harmonic analysis and was used by Shin to study autonomic responses in dogs (13). This approach appears to show promise for the frequency domain analysis of ECG signals in aviators, this is due to what Shin refers to as the time-local characteristics of the complex demodulation process (13). The AR approach with forgetting factor, uses a geometric window that allows the more recent data to be considered to a greater degree than previous data in the power spectrum calculations. This allows for a more rapid response in the power spectrum to alterations in the underlying time domain signal.

II. B. 8. Presentation of Power Spectrum Results

After the selection of a method for computing the power spectrum, the results must be presented in an understandable format. On a two dimensional graph of the power spectrum, the frequency is along the X axis. Conversely, the x-axis may also be in units of cycles/beat if the De Boar approach is followed. This may also then be converted to equivalent Hertz by multiplying the x-axis values by the mean R-R interval. The values along the y-axis are unitless for normalized data and in units of msec^2/Hz if not normalized. If the De Boar method is used the units are $\text{msec}^2/\text{cycles/beat}$. Normalization of the power spectrum occurs when the power spectrum values are divided by the mean of the interval squared. The next factor to consider is whether the amplitude or power spectrum is being plotted on the y axis. Amplitude is simply the square root of the power spectrum.

Once the various methodologies are sorted out, it is necessary to interpret the power spectrum density plots. Of considerable importance is if the subjects were under controlled breathing or free breathing. Controlled breathing at a regularly spaced interval will cause a large spectral peak at the frequency of breathing. Whereas free breathing causes a more diffuse peak. This points out the stunning influence of vagal innervation on heart rate.

Resolution of the spectral plot is very important. The maximum resolution is a function of T^{-1} , with T equaling the period. For 5 minutes the resolution would be $1/300$ Hz or .0033 Hz. A longer period is necessary to generate smaller interval values. Thus, resolution is enhanced significantly as the period is increased.

Two of the published approaches to the assessment of biological variability are presented below, before a more detailed discussion of the autonomic nervous system is presented.

De Boar Method of Power Spectrum Analysis (14)

1. For a period of 512 beats the R-R interval duration (I), Systolic Pressure (S), Diastolic Pressure (D), Mean Pressure (M), and Pulse Pressure (P) will be determined.
2. Determine the means for the above variables.
3. Subtract the d.c. component
4. do an FFT
5. Power Density Spectra will be FFT^2
6. Smooth with a 31 point triangle window. this is also known as a Bartlett window.
7. Convert to equivalent HZ by multiplying by the 1/mean R-R interval. If this is not done the units are cycles/beat
8. Letting $B = S, M, D, P$ then compute cross spectra for each blood pressure variable with heart rate.

$$C_{BT}(f) = X_B(f) * X_T(f) = L(f) - iQ(f)$$

$$L(f)$$
 is called the cospectrum and $Q(f)$ is the quadrature spectrum. Remember that these have been smoothed.
9. Compute the smoothed estimator of the phase spectrum for each variable:

$$\text{psi}(f) = \arctan(-Q(f)/L(f))$$

$$\text{psi}(f) \text{ over the range of } \pm 180 \text{ degrees}$$

The Berger et al. method (7)

1. Determine R-R intervals
2. Plot as $1/\ln$ for all n
3. Pass through a boxcar (rectangle window) with the following parameters:

$$W(f) = \{[\sin(2\pi f / fr)] / [2\pi f / fr]\}^2$$

where fr is the new sampling frequency and $2/fr$ is the width of the window in Hz. The value for fr is usually taken to be 4 Hz.

4. Compute mean and remove d.c. component
5. Compute $(\text{FFT})^2$
6. apply a $1/W(f)$ correction to the $(\text{FFT})^2$
7. Compute the square root of the values in number 6 to get amplitude
8. Plot

II. C. Autonomic Nervous System (ANS) Assessment

The autonomic nervous system (ANS) has partial responsibility for the maintenance of homeostasis in the human body. The ANS can be considered an "unconscious control system" that can sense stimuli impinging on the organism and then initiate a response through the parasympathetic and sympathetic nervous systems. Such responses to maintain homeostasis include the regulation of heart, vessels, lungs and kidneys. With the large number of disorders that may affect ANS function it is necessary to employ a variety of tests that may aid in the differential diagnosis of the patients illness or establish the degree of ANS ability. Such tests are necessary to classify an individual because descriptions such as "diabetic neuropathy" do not adequately define the severity of the impairment. The ANS impairment may be borderline neuropathic, or a severe impairment may be present. Once the disorder is known, the ANS tests can be viewed as tools to evaluate the progression of the disorder.

The neurologist has a wide range of ANS function test choices to pinpoint ANS deficiencies and the tests may be placed into four broad classifications: non-invasive, invasive, other tests of vasomotor control, and tests of pupillary innervation (15). A major constraint in the testing of patients in a clinical environment or of potentially assessing capabilities of an aviator is the desire for rapid non-invasive tests or tests that do not involve drug administrations which would adversely affect aviator performance. Because the pupillary innervation tests use drug administrations, they will not be considered for potential tests; nor were the infusion of pressor drugs for the baroreceptor sensitivity test. Plasma drug levels were also not considered as potential tests, as a rapid classification that does not involve lab tests is desirable.

II. C. 1. Sympathetic Nervous System (SNS)

Dividing the ANS into parts, the sympathetic reflex arc can be considered first. Four potential tests exist to study this reflex arc. The first test is to evaluate orthostatic hypotension. Various authors have defined orthostatic hypotension as a drop in blood pressure greater than 25-30 mmHg, while borderline hypotension is a drop in blood pressure of 15-30 mmHg (16). Two tests have been widely used in the measurement of orthostatic tolerance; response to standing or tilt. In the tilt test a subject typically is tilted from the horizontal to a 60 or 70 degree angle (17). Power spectrum methods similar to those described by Berger et al. have often been used in conjunction with the tilt table test. However, these methods do not adequately assess the transient nature of the biological signals. Therefore, methods such as the power spectrum with forgetting factor may be more appropriate.

Blocked blood pressure response to the Valsalva maneuver or Lower Body Negative pressure are additional measures of the total sympathetic reflex arc. There are 4 phases to the Valsalva maneuver (18). An increase in intrathoracic pressure due to straining causes an initial increase in blood pressure in phase 1. Due to elevated blood pressure and continued subject straining a reflex tachycardia is evident (phase 2). With removal of straining, and concomitant drop in intrathoracic pressure, there is a fall in BP while HR remains stable. Phase 4 is noted by an overshoot in BP and the presence of bradycardia. To quantify the Valsalva maneuver, the longest R-R interval in phase 4 is divided by the smallest R-R interval in phase 2. Abnormal values are less than 1.5 or less than 1.4 (16,18).

Originally the Valsalva maneuver was an invasive test. A catheter was necessary to obtain the appropriate blood pressure data. However, now a Finapres or similar device can be used to measure finger arterial pressure, which correlates with the aortic pressure (16). An interesting note is that subjects with sympathetic failure and orthostatic hypotension had a large increase in HR during phase 2 but no bradycardia during phase 4 (19). With the shifts in blood pressure, the Valsalva maneuver may provide valuable information about an aviators physiological response to fluid shifts.

In the Lower Body Negative Pressure test the subject is placed in an apparatus which causes a negative (suction) pressure to be applied to his lower limbs. This negative pressure causes a pooling in the legs, leading to a decreased venous return. Therefore, heart rate will become elevated in a normal response (16). The presence of supine hypertension would also be an indicator of sympathetic neuropathy. The LBNP test also

creates the opportunity to evaluate a rapid shift in blood volume. the negative pressure can be released rapidly, creating a situation analogous to the rapid reduction in +Gz.

II. C. 1. a. Afferent SNS

Due to their simplicity, the tilt table test and Valsalva maneuver have been chosen as the two tests to be used for the quantification of the total sympathetic arc. Additional tests may be employed to discriminate a more specific location of a sympathetic nervous system (SNS) deficiency. Only one test exists for the measurement of the afferent pathway of the SNS. If vasopressin levels are abnormal with an elevated renin level the afferent pathway is impaired (16). If the total SNS reflex arc tests indicate a neuropathy and the efferent pathway is ruled out by the tests presented below, the afferent pathway is likely impaired (16). Therefore, for a rapid ANS assessment testing of the afferent SNS is not warranted.

II. C. 1. b. Efferent SNS

The efferent limb of the SNS can be assessed using a variety of approaches including; loud noise, ice, mental concentration, LBNP, sweating during central temperature elevation, and postural alterations in blood catecholamines (16). From this list, the easiest test to administer and control is to place a subjects hand in ice water (4 degrees Celsius) and assess the changes in blood pressure (17)

Further segmentation of the efferent path may be made into preganglionic and post ganglionic divisions (16). However, these tests involve blood work and the administering of drugs. Therefore, they would not be feasible in a rapid assessment scheme. Only one test was provided to evaluate the preganglionic pathway (16). That is the presence of efferent SNS neuropathy along with normal axon reflex. To test for the normal axon reflex acetylcholine is injected intradermally. If piloerection (hairs stand up around injection site) or sweating occurs, then the reflex is normal. The receptors can be measure by evaluating receptor density in platelets for alpha receptors and receptors in lymphocytes for beta receptors (16).

A variety of methods exist for the assessment of the peripheral fibers in the post ganglionic pathway of the efferent SNS, but again involve tests that are not feasible for rapid deployment. Abnormal sympathetic nerve activity, depressed catecholamine and dopamine beta-hydroxylase concentrations, and no acetylcholine axon reflex are the examples of positive tests for peripheral fiber disorders (16).

II. C. 2. Parasympathetic Nervous System

With the completion of the assessment of the sympathetic nervous system, the attention may be shifted to assessing the other part of the ANS, the parasympathetic system. If no bradycardia is present during elevated carotid sinus pressure a deficit will be present in the parasympathetic nervous system (16). Impaired parasympathetic function leads to the absence of heart rate change during a variety of tests. These tests include LBNP, neck suction, drug infusion, Valsalva maneuver, and hypotension or hypertension induced by posture change (supine to standing for example) (16). Of these tests, posture change and the Valsalva maneuver can be employed most rapidly.

To test for baroreflex sensitivity various doses of Phenylphrine or Angiotensin can be injected into the patient. Following a dose response pattern, R-R intervals will be longer

in control subjects compared to individuals with impaired parasympathetic nervous systems. Additionally a neck suction device may be used to cause progressive drops in carotid pressure. This would lead to a reduction in PNS stimulation and an elevation in HR (16). PNS neuropathies would show a reduced elevation in HR when compared to controls (16).

II. C. 2. a. Efferent PNS

In the presence of an abnormal test for the PNS and with the exclusion of an efferent PNS neuropathy, the afferent PNS is likely affected. Yet, no direct tests exist to quantify afferent PNS neuropathy. The efferent PNS pathway is checked by evaluating the vagus nerve. The possible tests include elevated heart rate at rest, no increase in HR with atropine, reduced or absent sinus arrhythmia, no variation during deep breathing or diving reflex or isometric exercise, and after injection of insulin there is no elevation stomach acid (16). Isometric handgrip exercise is the most convenient test to apply.

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Appendix A. Algorithm for Baseline Wander Suppression by Pottala

Suppress Baseline Wander

by

Pottala & Bailey et al.

/* Name the function */

double filtered_ecg(*ecg_voltage, sample_interval, freq_cutoff);

/* Input to this procedure is the array of ECG points- ecg_voltage[n]*/

/* note that ecg_voltage[n] is an integer */

/* Input sampling interval- sample_interval */

/* Input damping coefficient- sigma Pottala recommends 0.6*/

/* Input cutoff frequency -freq_cutoff */

/* compute g coefficients */

g= tan(pi*sample_interval*freq_cutoff*0.001)

g1= 1 + 2*sigma*g + g*g

g2= (2*g*g) - 2

g3=1- (2*sigma*g) +(g*g)

/* compute a and b coefficients */

b1=b3=(g*g)/g1

b2=(2*g*g)/g1

a2= g2/g1

a3=g3/g1

/* compute initial values for lowpass_out[1] and lowpass_out[2] */

lowpass_out[1]= (b1+b2+b3-a2-a3)*ecg_voltage[1];

lowpass_out[2]=b1*ecg_voltage[2]+(b2+b3-a3)*ecg_voltage[1]-
a2*lowpass_out[1];

/* compute lowpass_out[n] for the remaining ecg_voltage[n] */

size=size (ecg_voltage[n]);

for (n=3;n<=size;n++)

lowpass_out[n]=b1*ecg_voltage[n]+b2*ecg_voltage[n-1]+b3*ecg_voltage[n-2]-
a2*lowpass_out[n-1]-a3*lowpass_out[n-2];

/* compute from lowpass_out[n] and ecg_voltage[n] */

size=size (ecg_voltage[n]);

for (n=3;n<=size;n++)

highpass_out[n]=ecg_voltage[n]-lowpass_out[n];

```

/* compute initial values for rev_lowpass_out[1] and rev_lowpass_out[2] */

rev_lowpass_out[1] = (b1+b2+b3-a2-a3)*highpass_out[1];
rev_lowpass_out[2] = b1*highpass_out[2] + (b2+b3-a3)*highpass_out[1] -
                    a2*rev_lowpass_out[1];

/* compute rev_lowpass_out[n] for the remaining highpass_out[n] */
size = size(highpass_out[n]);
for (n=3; n<=size; n++)
    rev_lowpass_out[n] = b1*highpass_out[n] + b2*highpass_out[n-1] +
                        b3*highpass_out[n-2] - a2*rev_lowpass_out[n-1] -
                        a3*rev_lowpass_out[n-2];
next n;

/* compute filtered_ecg[n] from rev_lowpass_out[n] and highpass_out[n] */
size = size(highpass_out[n]);
for n=3 to size;
    filtered_ecg[n] = highpass_out[n] - rev_lowpass_out[n];
next n;

/* The output of this array is a filtered ecg array which meets the AMA standards */
return filtered_ecg[n];

```

Appendix B. Algorithm for Okada QRS detection

```

/*****
                                Peak Detection
                                by
                                Okada
*****/

/*input to this procedure is the filtered_ecg[n] */
/*input m, default should be 6 see okada for explanation */

/* first use three point moving average filter */
for n=2 to size(filtered_ecg)-2;
    Y0[n] = (filtered_ecg[n-1] + 2*filtered_ecg[n] + filtered_ecg[n+1])/4;
next n;

/* low pass filter */
for n= m+1 to size-m-1;
    for k=n-m to n+m;
        sum = Y0(k) + sum;
    next k;
    Y1(n) = [1/(2*m+1)]*sum;
next n;

```

```
/* square the difference between filter input and output */
```

```
  for n=m+1 to size -m -1;
    Y2[n]=(Y0[n]-Y1[n])^2;
  next n;
```

```
/* filter the squared difference */
```

```
  for n= m+1 to size-m-1;
    for k=n-m to n+m;
      sum=Y2(k)+sum;
    next k;
  Y3[n]=Y2[n]*{sum}^2;
next n;
```

```
/* Build a fourth array */
```

```
  for n= m+1 to size-m-1;
    if [(Y0[n]-Y0[n-m])]*[Y0[n]-Y0[n-m]]>0 then
      Y4[n]=Y3[n];
    else
      Y4[n]=0;
  next n;
```

```
/* Determine threshold for the fourth array */
```

```
  threshold_okada_ecg=0.125*max(Y4[n]);
```

```
/* critique a fourth array */
```

```
  for n= m+1 to size-m+1
    if Y4[n]> threshold_okada_ecg then
      i=i+1;
      possible[i]=n;
  next n;
```

Appendix C. Algorithm for Menard QRS Detection

```
*****
```

Peak Detection

by

Menard, et al.

```
*****/
```

```
/* The input to this procedure is the filtered_ecg[n] */
```

```
/* input sample interval */
```

```
/* Compute the maximum derivative of the first 1.2 seconds of filtered_ecg[n] data */
```

```
/* compute # of samples in 1200 milliseconds */
```

```
samples_1200=1200/sample_interval;
```



```

for n =2 to samples_1200-2;
derivative_ecg[n]=-2*filtered_ecg[n-2]-filtered_ecg[n-1]+filtered_ecg[n+1]
+2*filtered_ecg[n+2];
next n;
slope_threshold=0.70*max(derivative_ecg[n]);

/* Compute derivative and detections for filtered_ecg[n] */
for n =2 to size(filtered_ecg[n])-2;
derivative_ecg[n]=-2*filtered_ecg[n-2]-filtered_ecg[n-1]+filtered_ecg[n+1]
+2*filtered_ecg[n+2];
if derivative_ecg[n]>slope_threshold then; /* check if derivative is > threshold */
i=i+1; /* if it is then compare index to the */
if n-possible_ecg[i-1]>200 then
possible_ecg[i]=n /* to value of possible_ecg */
next n;

/*find true peak of the ecg */
for (i=1;i<=size (possible_ecg);i++)
true_ecg_peak[i]=max(filtered_ecg(possible_ecg[i]) to +144)
next i;

```

Table 1. Physiological Nomenclature for G force

Inertial Resultant of Body Acceleration

Linear Acceleration Physiological Response

Forward +Gx	Transverse A-P G Supine G Chest to back
Backward -Gx	Transverse P-A G Prone G Back to chest G
Headward +Gz	Positive G Toward feet
Footward -Gz	Negative G Toward head
To Left +Gy	Right lateral G
To right -Gy	Left lateral G

From: Burton RR, Meeker LJ, Raddin JH. Centrifuges for studying the effects of sustained acceleration on human physiology. IEEE, 1991; 56-65

Table 2. + Gz Capabilities of Selected Fighter Aircraft

Country	Manufacturer	Aircraft	+/-Gz
France	Dassault	Mirage 2000	+9
Yugoslavia	Soko	G-4 Super Galeb	+8/-4.2
USA	Northrop	F-20 Tigershark	+9
USA	McDonnell-Douglas	F-15 Eagle	+9/-3
USA	Lockeed	F-22	+9
USA	Lockeed	F-117A	+6
USA	General Dynamics	F-16	+9
USA	Bell Helicopter	AH-1(4B)W Viper	+3.39
UK	British Air	BAe Hawk T-45A Goshawk	+8/-4
UK	British Air	Harrier	+7.8/-4.2
USSR	Mikoyan	Mig 29 Fulcrum	+9.5
USSR	Mikoyan	Mig 25 Foxbat	+5
USSR	Mikoyan	Mig 27 Floger	+7
Swiss	JAS	39 Gripen (Griffen)	+9
Poland	IL	Iridium	+8/-4
Israel	IAI	Lavi (Young Lion)	+7.2
Israel	IAI	Kfir (Lion Cub)	+7.5
Israel	IAI	Nammer (Tiger)	+9
International	Eurofighter	EFA	+9/-3
International	Dassault/Dornier	Alpha Jet	+12/-6.4
International	Panavia	Tomado IDS	+7.5
International	Rockwell/MBB	X-31 A EFM	+9/-4
Czechoslovakia	Acro	Albatross	+8/-4
China	CAC	Mikoyan J-7	+8.5

Data extracted from Jane's World Aircraft 1993.

MULTIMODAL MEASURES OF MENTAL WORKLOAD DURING
COMPLEX TASK PERFORMANCE

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Abstract

Central and autonomic nervous system measures of mental workload were examine concurrently during tasks that varied in their perceptual/central and physical demands. A cognitive arithmetic and continuous manual tracking task were performed singly and together. The perceptual/central demand of the cognitive arithmetic task was manipulated by varying the number of addition and subtraction operations required to solve a problem. The physical demand of a single-axis, second-order compensatory tracking task was manipulated by varying the amount of force operators had to apply to the joystick. Multiple psychophysiological responses were recorded during task performance including: electroencephalographic, cardiovascular, pulmonary, and eye blink measures. Data will be collected from twenty-four subjects, but only preliminary analyses on a subset of responses from selected subjects are available at this time.

MULTIMODAL MEASURES OF MENTAL WORKLOAD DURING COMPLEX TASK PERFORMANCE

Richard W. Backs and Arthur M. Ryan

Introduction

Mental workload (MWL) has been described as an intervening variable that reflects the extent to which the information processing abilities of an operator are actively engaged during task performance (Gopher & Donchin, 1986). MWL is a multidimensional construct that has been assessed using performance, subjective, and physiological measures that have often been observed to dissociate during task performance (see O'Donnell & Eggemeier (1986) for a review). Some investigators have emphasized that these between-class dissociations are important sources of information regarding the structure of cognitive resources underlying complex task performance (Wickens, 1990; Yeh & Wickens, 1988). Measure dissociation can also occur within each of the performance, subjective, and physiological classes that may elucidate cognitive resource structure. Of interest in the present study is the pattern of association and dissociation observed among physiological measures, specifically the relation between measures of central nervous system activity (i.e., the electroencephalogram and event-related potential) and measures of autonomic nervous system activity (i.e., the electrocardiogram, impedance cardiogram, and respiration).

In the present study, MWL is assumed to result from the consumption of limited capacity cognitive resources, where increased resource utilization leads to increased MWL. The structure of these resources is assumed to conform to Wickens's (1980; 1984) multiple resource model. According to this view, attentional capacity is considered to be limited within three independent resource dimensions: processing stages (perceptual/central, response); processing codes (verbal, spatial); and input/output modalities (auditory and visual input, manual and speech output). The present study focused on task demands that affect resources along the processing stage dimension.

Historically, event-related potentials (ERPs) have played a prominent role in demonstrating the veracity of the processing stage dimension (e.g., Isreal, Chesney, Wickens, & Donchin, 1980; Isreal, Wickens, Chesney, & Donchin, 1980; Sirevaag, Kramer, Coles, & Donchin, 1989). For example, manipulations of tracking system order increase the demand upon perceptual/central resources and reduce the amplitude of the P300 to a secondary task, while manipulations of disturbance bandwidth increase the demand upon response resources and do not affect the P300 to a secondary task. The effects of these manipulations on cardiovascular measures is unclear. On the one hand, many cardiovascular measures (e.g., heart rate: Jennings, 1986ab; heart rate variability: Mulder & Mulder, 1981; Grossman & Svebak, 1987) are thought to index central processing and would be expected to correlate positively with ERP measures. On the other hand, Backs, Ryan, and Wilson (in press) did not find that heart period or heart rate variability were sensitive to tracking system order.

A cognitive/energetic approach to information processing may be useful in understanding central and autonomic dissociations. According to Kahneman's (1973) energetic resource model, total information processing capacity can vary, with the capacity limit determined by the operator's state of arousal. Autonomic measures are considered to reflect the regulation of the capacity limit. As tasks increase in difficulty they consume more resources; however, if the resources available are sufficient to perform the task the capacity limit will not increase with increased task difficulty. In the case of perceptual/central resources, the ERP measures would be expected to vary with task difficulty, but not the autonomic measures. Only when perceptual/central resource availability is not sufficient to perform the task, and more resources are needed, will the limit increase. In this case, both the ERP and the autonomic measures would be expected to vary with task difficulty.

Alternatively, central and autonomic nervous system measures may dissociate as a result of the dual innervation of the autonomic nervous system. Effector responses such as heart period are determined by a combination of sympathetic and parasympathetic inputs that may cancel each other under some circumstances. Thus, when cardiovascular measures based upon heart period

dissociate from the ERP it may not be because the heart was unaffected by the task, but because of the autonomic mode of control operating during task performance (Berntson, Cacioppo, & Quigley, 1991). Only when the sympathetic and parasympathetic inputs are separated will task effects be apparent.

A difficulty with the use of autonomic responses, and with heart period in particular, is that MWL effects upon the response tend to be small in relation to the primary, metabolic, function of the physiological system. Effects of MWL may be masked by homeostatic adjustments of the autonomic response to variables such as the physical workload required to maintain task performance and the level of arousal or stress, which may or may not be associated with task performance. Thus, the signal-to-noise ratio of the MWL effects on the autonomic response may decrease as background activity due to variables such as physical workload increases. However, heart period responses to MWL may still be able to be separated from responses to physical workload by their autonomic mode of control.

In the present study, central and autonomic measures were examined concurrently while perceptual/central processing resource demands of a cognitive arithmetic task and the physical demand of a second-order compensatory manual tracking task were varied. Participants performed the cognitive arithmetic task and the tracking task singly and together. The central measures were expected to be sensitive to the perceptual/central demand of the arithmetic task, but not to the physical demand of the tracking task. The sensitivity of the cardiovascular measures was assessed separately for the individual effector responses and for the sympathetic and parasympathetic inputs as determined statistically across the measures (Bucks, in press). Specifically, perceptual/central demand was expected to elicit primarily sympathetic activity consistent with the central measures, while physical demand was expected to elicit primarily parasympathetic activity independent of the central measures.

Method

Subjects

Sixteen subjects (9 female) from the subject pool maintained by Armstrong Laboratory participated have participated in the experiment. A total of 24 subjects will be run.

Apparatus

The electrocardiogram (ECG), electro-oculogram (EOG), and electromyogram (EMG) data were amplified by Grass amplifiers (Model P511K) and sampled at 1000 Hz by the Psychophysiological Assessment Test System (Wilson & Oliver, 1991). The impedance cardiogram (ZCG) was collected using a Minnesota Impedance Cardiograph (Model 304B), and stored on magnetic tape for later analysis. Respiration was collected using a Resptrace (Model 10.9000). The electroencephalogram (EEG) was collected at 100 Hz with a Biologic Brain Atlas topographic mapping system.

The task was controlled by a microcomputer. The joystick was a Measurement Systems (Model 446) force joystick.

Tasks

The cognitive arithmetic task was the Math Processing task from the Criterion Task Set (Shingledecker, 1984). Perceptual/central processing was varied across two levels using the medium and high difficulty Math Processing tasks. These tasks consist of two (medium) or three (high) operation addition and subtraction problems, where the operator's task is to decide whether the answer is greater than or less than five. The Criterion Task Set version of the task was modified for use in the present study so that the proportion of addition and subtraction operations and the proportion of problems that required carry operations was balanced within each task. Thirty-six problems were visually presented at a fixed interstimulus interval of 5 s in each 3 min. trial. All operators responded with the first (greater than) and second (less than) fingers of their left hand. Problems were terminated after the button push or 4 s, whichever came first. Participants were instructed to respond as fast as possible while maintaining 100% accuracy, and

given performance feedback after each trial.

The physical demand of a single-axis, second-order compensatory manual tracking task was varied at two levels. The input to the tracking system was scaled so that either 4 or 8 lbs. of force was required for full range movement of the joystick. The tracking cursor changed color when operators applied too much force to the joystick. Operators performed the tracking task with their right hand. Performance feedback regarding tracking error and whether too much force was applied was given at the end of each trial.

Procedure

Operators participated for four sessions distributed over successive days. The first three sessions (1.5 hrs. each) were practice; participants received 20 3-min. trials on each day. A total of 20 single task trials were presented: 4 medium and 6 high difficulty cognitive arithmetic; 10 tracking, 5 each at 4 lbs. and 8 lbs. of force. A total of 40 dual task trials were presented: 9 medium and 11 high difficulty cognitive arithmetic with each tracking force condition. Participants wore the physiological transducers on Day 3 for adaptation purposes. On the test day, participants performed one trial of each of the eight task conditions, where condition presentation order was balanced across participants with a Latin square. The block of task conditions was preceded and followed by a 3 min. eyes-open resting baseline.

Data Reduction

Performance. Reaction time in ms for correct trials was measured for the cognitive arithmetic task. Probability correct was examined as a manipulation check to make sure that no speed-accuracy trade-off occurred. Root mean square (RMS) error was measured for the tracking task. Stick RMS was measured as a manipulation check on the force manipulation.

Subjective. The NASA Task Load Index (NASA-TLX, Hart & Staveland, 1988) was collected after each test trial. The NASA-TLX contains six subscales that are each given rated on a scale from 0 to 100: mental demand, physical demand, temporal demand, performance, effort, and frustration level. The mean of the six subscales was used for analysis, where higher scores mean

greater MWL. The physical demand scale was examined separately as another manipulation check on the tracking force level.

EEG. EEG was filtered to pass between 1 to 30 Hz with the 60 Hz notch filter in. The middle minute of the trial was submitted to power spectral analysis.

ERP. Artifact-free trials were converted to source derivations (Hjorth, 1975, 1980) and averaged to obtain the ERP.

ECG. ECG was conditioned with a gain of 2,000 and a half-amplitude bandpass of 10 - 100 Hz. Heart period was defined as the time between successive heart beats measured in ms from the R wave-to-R wave interval (i.e., the interbeat interval (IBI)). Mean IBI across the trial was analyzed. Two measures of heart rate variability were derived from the IBIs across the trial, the Traube-Hering-Mayer (THM) wave (0.06 - 0.14 Hz) and respiratory sinus arrhythmia (RSA, 0.15 - 0.4 Hz).

ZCG. Systolic time intervals were derived from ensemble-averaged ZCG (Kelsey & Guethlein, 1990). Pre-ejection period (PEP) was used as a measure of cardiovascular sympathetic activation.

Respiration. Respiration rate in breaths-per-minute and tidal volume in mL were measured across the 3-min. trial.

EMG. EMG was conditioned with a gain of 10,000 and a half-amplitude bandpass of 1-1000 Hz. Total power (μV^2) from 1-500 Hz was summed for analysis.

Results

Due to equipment failure, only a subset of data from selected subjects was currently available for analysis.

ERP

Twelve subjects were available for analysis, but not all are represented in the data for each experimental condition: seven subjects for the single task medium difficulty cognitive arithmetic

task; ten subjects for the high difficulty cognitive arithmetic task; eleven subjects for the 4 lbs. force-medium cognitive arithmetic dual task; eleven subjects for the 4 lbs. force-high cognitive arithmetic dual task; ten subjects for the 8 lbs. force-medium cognitive arithmetic dual task; and eleven subjects for the 8 lbs. force-high cognitive arithmetic dual task. Since we were interested in a central nervous system measure of perceptual/central processing, we focused our analyses on the P300 component. Topographic maps of the P300 are presented in Figure 1 for the single tasks and in Figure 2 for the dual tasks.

Because the P300 is most prominent over the parietal cortical area, we selected the P₃, P_z, and P₄ electrode sites for closer inspection. Group mean Hjorth-corrected amplitudes from the three sites in each of the six tasks are presented in Figure 3. Several trends are apparent in the figure. First, P300 amplitude is greater in high difficulty cognitive arithmetic (MH) than in medium difficulty cognitive arithmetic (MM), consistent with the interpretation that more perceptual/central resources are required to process more difficult arithmetic problems. The second trend is that P300 amplitude to the arithmetic problems is reduced during the dual tasks compared to the single tasks. This reduction in P300 amplitude was greatest for the high difficulty cognitive arithmetic dual tasks (4H and 8H for the 4 and 8 lbs. force conditions, respectively) compared to the cognitive arithmetic single task (MH); the reduction for the medium difficulty dual tasks (4M and 8M for the 4 and 8 lbs. force conditions, respectively) compared to the cognitive arithmetic single task (MM) appeared to be smaller than for the difficult cognitive arithmetic task. This pattern of results is also consistent with the interpretation that the tracking task also requires perceptual/central processing resources, and when tracking and cognitive arithmetic are performed currently they compete for these resources. Further, resource competition was greatest for the high difficulty cognitive arithmetic task. Even though the cognitive arithmetic task was treated as having the highest priority, there appears to be insufficient perceptual/central capacity to allocate the single task level of resources to the arithmetic problem in the dual task.

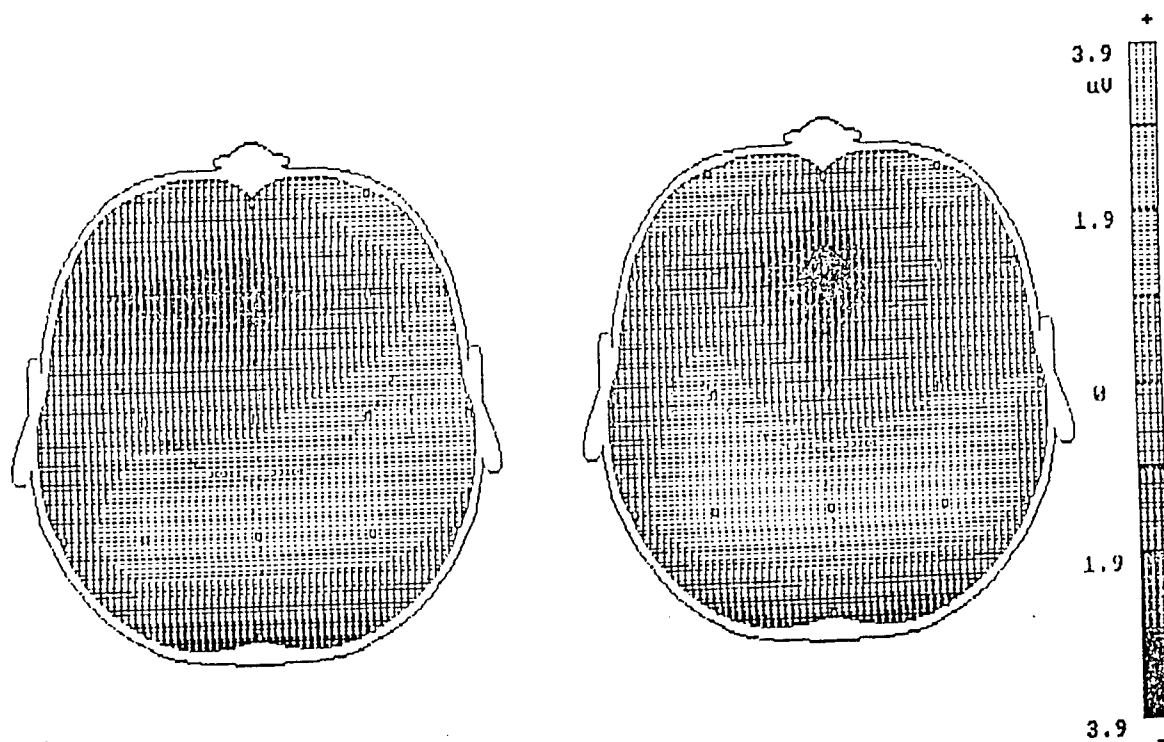


Figure 1. Topographic maps at 300 ms after the onset of the arithmetic problem for the medium (left) and high (right) difficulty cognitive arithmetic single tasks.

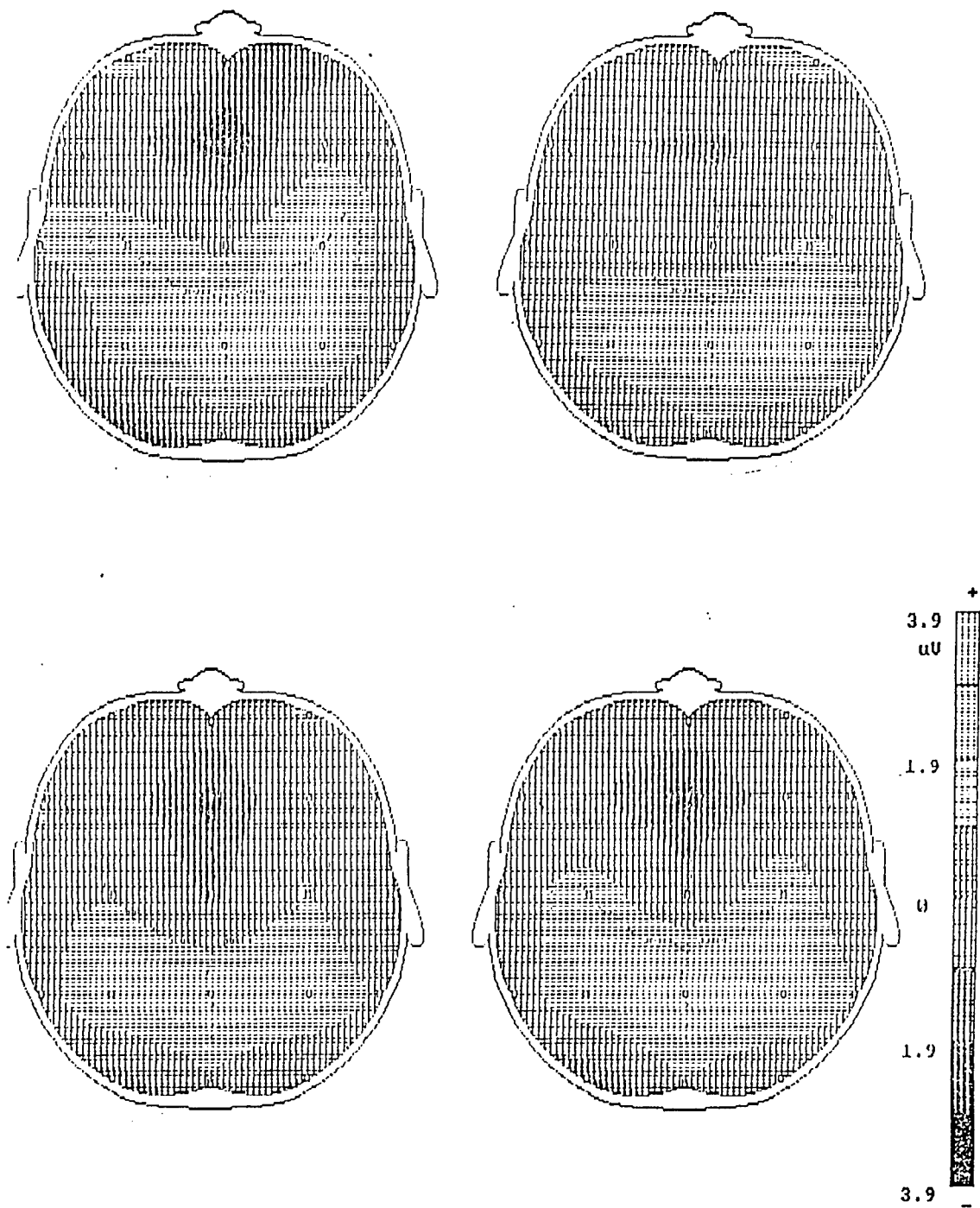


Figure 2. Topographic maps at 300 ms after the onset of the arithmetic problem for the cognitive arithmetic dual tasks. The top row is medium and the bottom row is high difficulty cognitive arithmetic. The left column is 4 lbs. force and the right column is 8 lbs. force tracking.

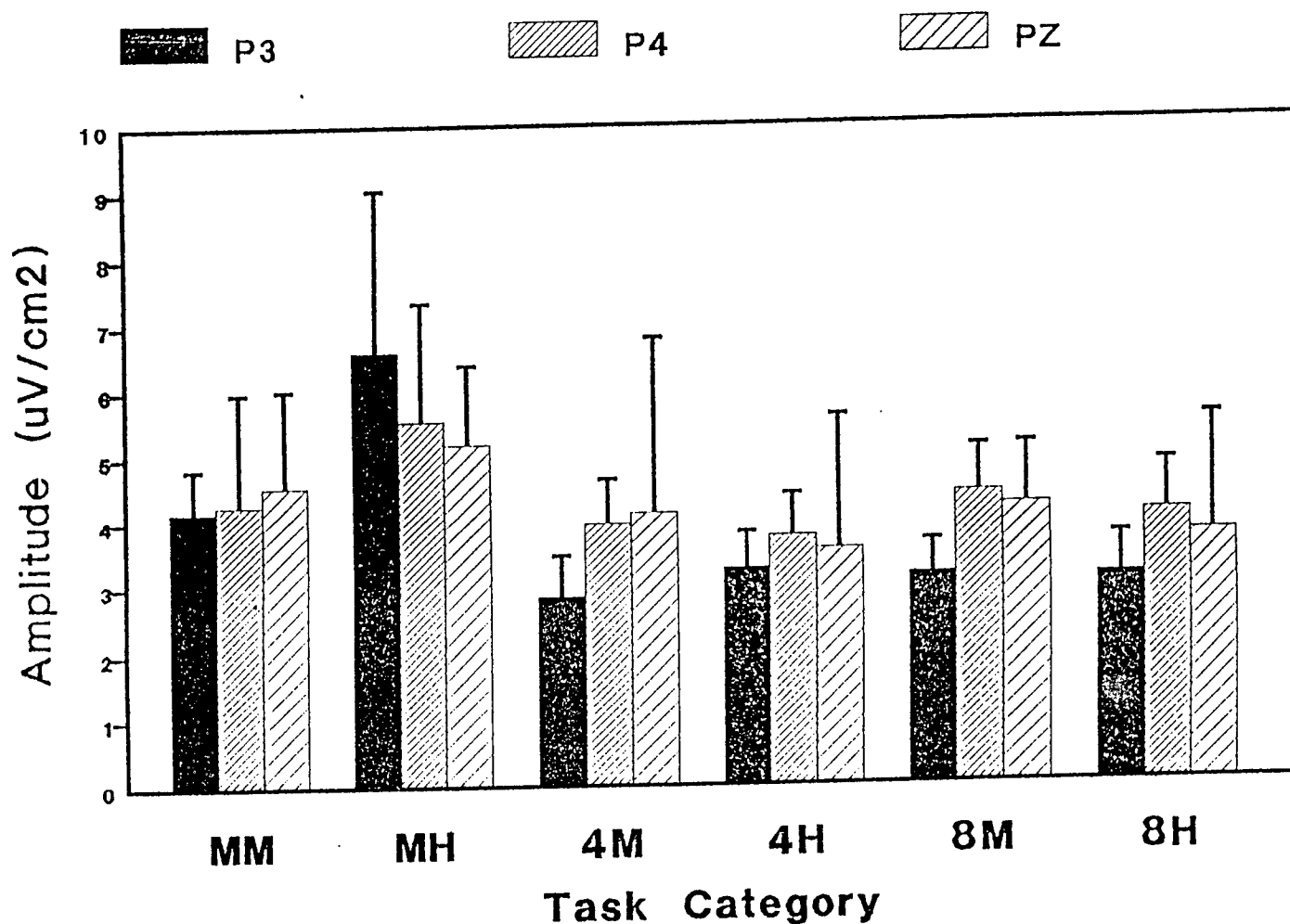


Figure 3. P300 amplitudes across the parietal electrode sites for the six experimental conditions: MM = medium difficulty cognitive arithmetic single task; MH = high difficulty cognitive arithmetic single task; 4M = medium difficulty cognitive arithmetic with 4 lbs. force tracking dual task; 4H = high difficulty cognitive arithmetic with 4 lbs. force tracking dual task; 8M = medium difficulty cognitive arithmetic with 8 lbs. force tracking dual task; and 8H = high difficulty cognitive arithmetic with 8 lbs. force tracking dual task.

ECG

Heart period from the six experiment conditions in which P300s were collected was available for five subjects. As can be seen in Figure 4, group mean heart period is shorter (i.e., faster heart rate) in the high (MH) than in the medium (MM) difficulty cognitive arithmetic single tasks consistent with the P300 results. Also like the P300, heart period was faster for the dual tasks than for the single tasks. The results for heart period across the dual tasks were different from the P300 results, however. While heart period was faster for the high than the medium difficulty cognitive arithmetic task, it was also faster for 8 lbs. force (high physical demand) than for the 4 lbs. force (low physical demand) condition. Further, the difference between high and medium difficulty cognitive arithmetic tasks was considerably reduced in the 8 lbs. force than in the 4 lbs. force conditions. This interaction illustrates the difficulty of using heart period to isolate MWL effects from background physiological activity driven by physical demand. Since heart period is sensitive to both mental and physical demand, the size of the MWL effect is reduced when physical demand is high.

Conclusions

Even with this small sample, it can be concluded that the experimental manipulations effectively established conditions in which our questions regarding central/autonomic nervous system interactions can be answered. Future analyses of the ZCG, heart rate variability, and other measures will provide a greater understanding of when and why psychophysiological MWL measures dissociate. This study will also help to clarify theoretical issues with regard to the effects of environmental variables such as physical workload upon cognitive processes. These effects are especially critical in operational settings where the environment is constantly changing. To be effective in the field psychophysiological measures of MWL must be robust across dynamic environmental conditions. This study will provide important information about how to increase the sensitivity and diagnosticity of psychophysiological MWL measures.

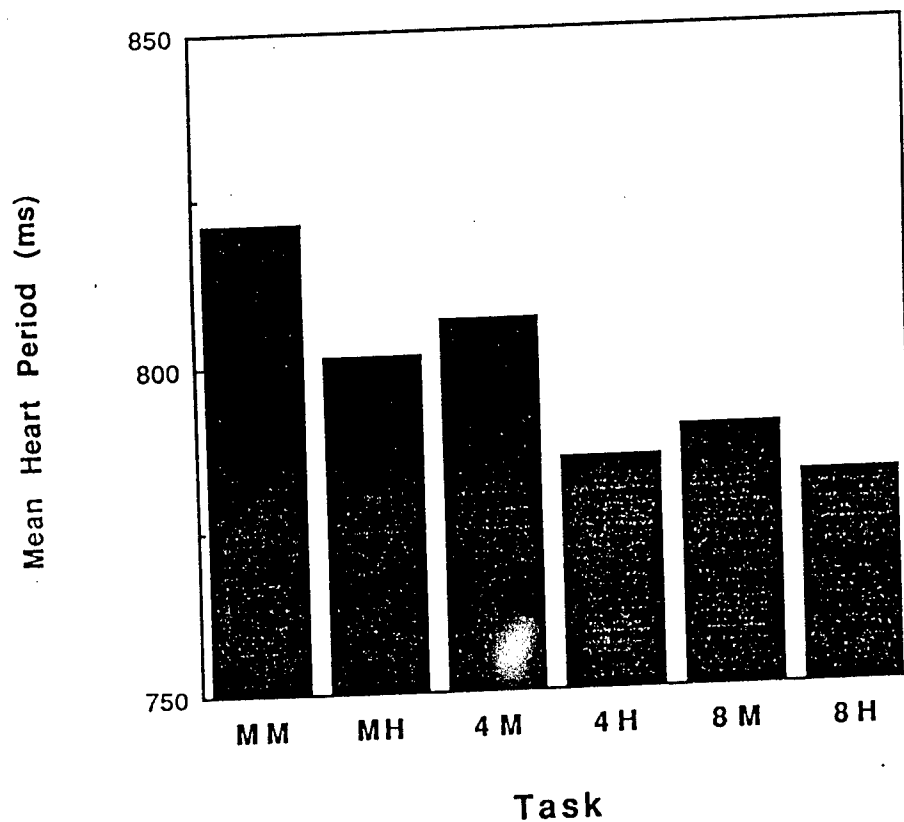


Figure 4. Mean heart period for the six experimental conditions in which P300 data were collected: MM = medium difficulty cognitive arithmetic single task; MH = high difficulty cognitive arithmetic single task; 4M = medium difficulty cognitive arithmetic with 4 lbs. force tracking dual task; 4H = high difficulty cognitive arithmetic with 4 lbs. force tracking dual task; 8M = medium difficulty cognitive arithmetic with 8 lbs. force tracking dual task; and 8H = high difficulty cognitive arithmetic with 8 lbs. force tracking dual task.

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EFFECT OF TYROSINE SUPPLEMENTATION ON COGNITIVE PERFORMANCE
AND FATIGUE IN SLEEP-DEPRIVED HUMANS

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Abstract

This study was designed to examine the effect of tyrosine (TYR) supplementation on fatigue induced decrements in cognitive performance during sleep deprivation. Nine healthy, male subjects participated in two sleep deprivation sessions separated by two weeks. Each subject ingested 75 mg/kg body weight of either TYR or placebo during the TYR session (T) and placebo session (P), respectively. In each session, subjects reported to the laboratory following a full day of work and were tested for 16 hours throughout the night beginning at 1800 h. Each subject completed a cognitive performance battery, subjective fatigue rating and recorded oral temperature hourly. Every other hour a POMS Mood Scale was completed. Intake of energy nutrients was similar for two days prior to the sleep deprivation trials, however mean TYR intake was greater ($p < 0.05$) for the two days prior to P (2988.0 ± 942.13 mg) than for the two days prior to T (2085.5 ± 678.09 mg). All fatigue scores and temperature were lowest between 0300 and 0600 h. Cognitive performance was also lowest at this time. Oral temperature was significantly higher ($p = 0.02$) for T (36.93 ± 0.330 °C) than for P (36.23 ± 0.414 °C). Subjective fatigue ($p < 0.11$) and POMS fatigue ($p < 0.06$) each showed a tendency to be less for T than for P. None of the variables analyzed by the cognitive performance battery were different between T and P. While the TYR dosage failed to affect the decline in cognitive performance in this study, future studies utilizing more stressful conditions to increase catecholamine depletion and/or larger doses of TYR would seem to be warranted.

EFFECT OF TYROSINE SUPPLEMENTATION ON COGNITIVE PERFORMANCE AND FATIGUE IN SLEEP-DEPRIVED HUMANS

Kevin Tipton

INTRODUCTION

Sustained military operations often involve sleep deprivation which may result in high levels of fatigue and fatigue-induced stress. For example, many USAF operations depend on a limited number of highly trained and skilled pilots to fly multimillion dollar aircraft on extended missions. The Air Force Inspection and Safety Center (AFISC/SERD, Norton AFB, CA) reported that a total of 66 class A mishaps (accidents involving more than \$1,000,000 damage and/or a fatality) occurred between January 1978 and March 1989 in which sleep deprivation and stress were the suspected causes of the accidents. Seventy-eight fatalities resulted from these accidents. These peacetime losses would probably increase during actual combat situations. Ground personnel may also be exposed to sleep deprivation and its associated problems during sustained operations.

Accidents which result during sleep deprivation may occur for many reasons. Sleep deprivation causes decrements in mood and performance in humans (Hartmann et al., 1977; Angus et al., 1985). Vigilance decrements have resulted from sleep deprivation (Holding, 1983) and the greater the sleep loss, the earlier in the task the vigilance declines (Williams et al., 1959). Fatigued subjects performing simulated flying tasks demonstrated a narrowing of attention to items of central importance and increased response variability (Bartlett, 1943). More recently, Moore-Ede (1993) reported a three-fold increase in pilot error during night-time sleep deprivation. Simulated landing performance declined following 20 hours of simulated flights by airlift crews (Hartman, 1965). Reaction time is also increased by periods of sleep loss (Williams et al., 1959). Subjective fatigue (SF), which may account for a person's acceptance of greater risks for a savings in effort (Holding, 1983), increases with sleep deprivation and has been frequently associated with performance decrements (French et al., 1990, 1993; Neville et al., 1993). Declines in any of these conditions may result in mishaps during military operations, possibly resulting in damages and fatalities.

Countermeasures which would decrease performance decrements due to fatigue and

associated stress resulting from sleep deprivation would therefore be extremely valuable for preventing accidents in military operations. Performance decrements due to fatigue may result from one, or a combination of, several biological mechanisms. Depletion of catecholamine neurotransmitters, dopamine (DA) and norepinephrine (NE), in the brain may contribute to fatigue related deficits in performance. Many animal studies have demonstrated that various types of stress, including sleep deprivation (Tsuchiya et al., 1969), cold-swim stress (Roth et al., 1982), and exercise (Stone, 1971), deplete catecholamines in the brain. Additionally, stress-induced catecholamine depletion in the rat brain has been closely related to reductions in performance in avoidance/escape tasks (Anisman et al., 1980) locomotor activity and swimming (Weiss et al., 1981; Lehnert et al., 1984). These results from animal studies support the idea that catecholamine depletion may be related to performance decrements in stressful situations.

Fatigue has also been associated with the serotonergic systems in the brain. Evidence for the influence of serotonin (5-HT) on sleep and the state of tiredness is presented by Young (1986). Montgomery et al. (1991) demonstrated that motivation in humans is reduced by increased brain 5-HT. Additionally, fatigue during exercise in both rats (Bailey et al., 1993a) and humans (Davis et al. 1993) and decreased mental performance in humans (Blomstrand et al., 1991) is associated with increased 5-HT in the brain. It is possible that the increases in 5-HT and associated increases in fatigue may be related to the catecholaminergic system. Bailey et al. (1993b) demonstrated that rats given a 5-HT agonist had decreased levels of DA which were associated with fatigue during exercise. Additionally, increased levels of melatonin (MEL), a 5-HT derivative and pineal hormone, have been related to fatigue and decreased cognitive performance (Lieberman et al., 1985a).

DA, NE and 5-HT, along with acetylcholine, are neurotransmitters which can be influenced by the dietary intake of their precursors. The catecholamines, DA and NE, are derived from the large neutral amino acid (LNAA) tyrosine (TYR). Similarly, tryptophan, another LNAA, is the precursor for 5-HT and ultimately, MEL. Administration of TYR has been shown to increase production of brain catecholamines (Wurtman et al., 1974). The level of brain 5-HT is dependent on the level of free TRP (Fernstrom and Wurtman, 1971) and the levels of other LNAA in the blood. All LNAA are transported into the brain by a

common carrier (Pardridge and Oldendorf, 1975). Therefore, brain TRP levels change when either blood TRP levels change or when levels of one or more of the other LNAAs change (Fernstrom and Wurtman, 1972).

It is possible that the competitive interactions of these LNAA may make feasible the use of nutritional countermeasures to fatigue-induced performance decrements. Supplementation of TYR may influence fatigue induced performance decrements by providing the precursor for depleted catecholamines. Animal studies have demonstrated that TYR ingestion may have a positive effect on performance associated with stress (Brady et al., 1980; Lehnert et al., 1984). This positive effect seems to depend on the level of depletion of the catecholaminergic neurons. Bandaret and Lieberman (1989) reported that soldiers exposed to a combination of altitude and cold which stressed their catecholaminergic system responded positively to a dose of TYR. Alternatively, TYR supplementation may influence performance by altering the production of 5-HT, and thus MEL, in the brain. MEL follows a well-defined circadian pattern (Lynch et al., 1975) which is related to fatigue Lieberman et al., 1985a). It is possible that this pattern may be altered by ingestion of TYR which will compete with TRP for entry into the brain. The purpose of this study was to examine the effects of TYR supplementation on the circadian pattern of MEL and on cognitive performance measures during fatigue induced by sleep deprivation.

METHODS

Nine healthy males volunteered to participate in the study after being fully informed of the risks and signing informed consent. Each subject participated in two sleep deprivation trials separated by a two week washout period. Treatments were randomly administered in a double-blind, cross-over design. In the experimental trial (T), subjects ingested 75 mg/kg body weight TYR. In the placebo trial (P), subjects ingested 75 mg/kg body weight flour. Either the T or P dose was mixed in 450 ml of unsweetened applesauce and ingested at 1950 h on the evening of the sleep deprivation trials.

For each sleep deprivation trial, subjects reported for testing at 1730 h following a regular work day and remained in the testing facility without sleeping until 0930 h the next morning. Oral temperature was taken with a digital thermometer (Becton Dickinson Co.,

Rutherford, N.J.). The School of Aerospace Medicine (SAMS) fatigue scale which ranges from 1 to 7 (Table 1) was used to evaluate SF in the subjects. This fatigue scale was derived from the SAM 10-point fatigue checklist (Pearson and Byars, 1956). Temperature and SF were obtained hourly beginning at 1800 h. In addition, Profile of Mood States (POMS) questionnaires were taken every other hour starting at 1800 h.

A battery of five cognitive performance tests was performed every hour beginning at 1800 h. The five tests were generated on color monitors by IBM compatible personal computers. The tests were presented in a battery that took approximately 13 minutes to complete. The tests were selected from a standard Tri-Service/NATO performance test battery. These tests included the Matrix, Continuous Recognition (CR), Switching (SW), Grammatical Reasoning (GR) and Unstable Tracking (UT) tests. Each test took two minutes to complete, with the exception of the switching test, which took four minutes. The Matrix test evaluated short term memory for spatial patterns. The complexity of the analysis of the Matrix test prevented the inclusion of these data here. The CR test was designed to measure short term memory for numbers. Dual processing ability was measured by the SW test. During SW, attention was divided randomly, back and forth, between the Manikin test of spatial relationship and a mathematical reasoning test, depending on an indicator at the bottom of the screen. The GR test assessed the ability to evaluate logical relationships. Hand-eye coordination was tested by the UT test using a mouse attached to the personal computer to keep the cursor in the center of the screen. Subjects practiced each test at least seven times during the four days prior to their initial sleep deprivation trial.

Subjects recorded their dietary intake for two days prior to the sleep deprivation trials. In an attempt to regulate dietary intake between the two trials, each subject was given a copy of the diet record from the first trial prior to their second sleep deprivation trial, and asked to duplicate the dietary consumption from the first trial. Dietary intake was analyzed for each subject utilizing the Nutritionist IV nutrient analysis program (N² Computing, Salem, Oregon) and Bowe's and Church's Food Values of Portions Commonly Used (Pennington, 1993).

All data are presented as means $\pm$ se except where indicated. Data were analyzed by repeated measures ANOVA with zero between and two within levels (treatment, time).

Significance was set at the $p < 0.05$ level. Differences between means were identified using Duncan post-hoc test. All statistical analyses were performed using the SAS data analysis package.

RESULTS

Dietary intake for five of the eight subjects for two days prior to each sleep deprivation session is summarized in Table 2. Percentage of kilocalories consumed as fat was greater ($p < 0.05$) prior to P than T. There was no difference in the dietary intake two days prior to testing between dose sessions for total calories, carbohydrate, protein or alcohol consumption. However, TYR consumption was greater for the two days prior to P than for T ($p < 0.001$).

Temperature response to sleep deprivation in the two trials is presented in Figure 1. Temperature decreased during both treatments with a nadir at about 0400 h, then rising until completion of data collection. Temperature was higher for T ($36.93 \pm 0.330^{\circ}\text{C}$) than for P ($36.23 \pm 0.414^{\circ}\text{C}$) over all times combined ($p = 0.02$). Mean SF scores from the SAMS Fatigue Scale increased over time from 1800 h to around 0500 to 0600 h (Figure 2). Difference between the means of the SF scores for T vs. P were not significantly different ($p < 0.11$).

POMS Fatigue scores increased with time, peaking at 0600 h (Figure 3). There was a trend ($p = 0.06$) for the mean POMS Fatigue scores for T to be less than for P (42.95 ± 8.748 vs. 46.88 ± 10.536). Mean scores for POMS depression, confusion, tension and vigor all changed over time, however there was no effect of treatment on any of these variables. Depression, confusion and tension all peaked and vigor was lowest at 0600 h. There was no change in POMS anger scores over time or between doses.

There were no significant effects of TYR supplementation on any parameter measured in any of the cognitive performance tests. Performance on several of the tests did decrease with time. Performance on the UT decreased, as evidenced by increasing control losses and average distance from center. These values peaked between 0400 and 0600 h. The performance of the subjects was also at its worst between 0400 and 0600 h for percent correct responses on GR. Switching performance also followed a similar pattern as

demonstrated by reaction time and percent correct on the manikin and reaction time for transition to the manikin. Percent correct responses was lowest and reaction time during CR was greatest between 0400 and 0600 h. Figure 4 illustrates the pattern of change over time for the cognitive performance tests utilizing throughput, the speed-accuracy product, during CR as an example. Performance on all the cognitive tests followed a similar pattern.

DISCUSSION

The results of this study demonstrate that a 75 mg/kg body weight dose of tyrosine does not attenuate the decline in performance associated with sleep deprivation induced fatigue. However there seems to be an effect of TYR administration on body temperature. Although not statistically significant, there was a trend toward a decrease in subjective feelings of fatigue following TYR consumption.

In the present study, performance on the cognitive tasks was lowest for most measured variables between 0400 and 0600 h. These results are in accordance with those which have been reported by others. Gillooly et al. (1990) reported performance on most variables in a battery of cognitive tasks, similar to the one used here, to be lowest between 0230 and 0630 h. Other tasks including psychomotor performance, reaction time, symbol cancellation, digit summation, performance on a flight simulator, grip strength, time estimation, tapping and personal tempo tests have all been demonstrated to have a similar pattern with performance lowest between 0300 and 0600 h (Aschoff et al., 1972; Hockey et al., 1972; Klein et al., 1968; 1970; 1972). An overall feeling of fatigue is often associated with performance decrements on cognitive tests (French et al., 1993; Neville et al., 1993). Not surprisingly, the decreased performance on the cognitive test battery was associated with increased feelings of fatigue. Body temperature rhythms were also similar to those reported previously (French et al., 1990; Klein et al., 1972).

A TYR dose of 75 mg/kg body weight increased the oral temperature readings in these subjects. TYR supplementation would cause an increase in blood TYR levels (Glaeser et al., 1975) which would alter the TRP/LNAA ratio. Since brain TRP and, ultimately, 5-HT levels are dependent on the TRP/LNAA ratio (Fernstrom and Wurtman, 1972), the TYR dosage may have affected brain TRP and 5-HT by competing with TRP for carriers across

the blood-brain barrier (Pardridge and Oldendorf, 1975). The precursor for MEL is 5-HT, so TYR administration may also have altered MEL production. Since MEL values are not reported here, it is not possible to confirm any alteration of MEL levels. However, if MEL rhythms were altered, this may account for the slight increase in temperature measured during the sleep-deprivation period when TYR was consumed.

An alteration in MEL production may also account for the possible effect of TYR supplementation on feelings of fatigue. There was a trend toward a dose effect of TYR on both subjective fatigue and POMS fatigue values. Increased MEL values have been associated with increased fatigue and decreased cognitive performance (Lieberman et al., 1985a), although no effect on performance in the cognitive test battery was observed in the present study. This trend towards an effect on fatigue makes it tempting to speculate that a larger dose of TYR may have had an impact on fatigue and perhaps even performance. Increasing the TYR dosage given to the subjects would lead to a further increase in blood TYR levels and thus alter the competitive interactions at the blood-brain barrier. Increasing TYR dose from 100 mg/kg to 150 mg/kg increased the TYR/LNAA ratio from 0.28 to 0.35 in humans (Glaeser et al., 1979). If the subjects in the present study had greater blood TYR levels which would have been associated with a greater dose, then fatigue, and possibly performance, may have been affected. Bandaret and Lieberman (1989) found that a TYR dose of 100 mg/kg increased cognitive performance and improved mood in humans exposed to cold and altitude stressors. Others have found an effect of 100 mg/kg of TYR supplementation on vigor (Wurtman, 1979) and Hamilton Depression Scale scores (Gelenberg et al., 1980).

An alternative explanation for the lack of an effect of TYR on cognitive performance may be associated with the diet of the subjects. A significantly greater amount of TYR was consumed for the two days prior to P (2988.0 ± 942.13 mg) than T (2085.5 ± 678.09 mg). While both of these values are within a normal intake range for TYR (LSRO, 1992), an increased intake of TYR should increase blood TYR levels (Fernstrom et al., 1979). If blood TYR levels were maintained at a higher level due to increased consumption prior to P, the dose of TYR (75 mg/kg) which was consumed during the T session may not have been enough to raise blood TYR to levels significantly greater than those experienced in the P

session. Therefore, any possible difference between the two sessions would not be readily apparent. Unfortunately, no blood samples were taken to determine plasma TYR.

Others have tested TYR supplementation under different conditions and found no effect of TYR supplementation on cognitive performance or mood in humans under daytime laboratory conditions. Leathwood and Pollet (1983) investigated the effect of 100 mg/kg TYR on mood in 60 volunteers. There was no difference in sedation/stimulation ratings or mood, from an 11 item questionnaire, between TYR and placebo. POMS vigor and fatigue, as well as reaction time, grooved pegboard test, Thurstone tapping test, and Visual analog mood scales (VAMS) were compared between a TYR dose of 100 mg/kg and placebo by Lieberman et al. (1985b). There were no differences between the two treatments for any of the measured variables.

The results of the present study and those reported by Leathwood and Pollet (1983) and Lieberman et al. (1985b) are in disagreement with those reported by Bandaret and Lieberman (1989). However, the level of stress to which the participants were exposed was quite different in the various studies. The subjects in the Leathwood and Pollet (1983) and Lieberman et al. (1985b) studies were tested in a quiet, laboratory setting during daylight hours, thus minimally stressing them. In the present study, increased stress was introduced by having the subjects perform the tests throughout the night without sleep. In contrast, the subjects in the Bandaret and Lieberman (1989) study were exposed to severe stress in the form of extreme cold and simulated altitude. Subjects responded to the exposure with different physiological manifestations and those with the most severe reactions responded to the TYR supplementation. This led the authors to conclude that TYR supplementation will be successful in preventing performance decrements only in subjects who are severely stressed. TYR is the precursor for the catecholamine neurotransmitters. Severe stress has been demonstrated to deplete catecholamines in rat brains (Tsuchiya et al., 1969; Stone, 1971; Roth et al., 1982) leading to decreases in performance (Anisman et al., 1980; Irwin et al., 1980; Weiss et al., 1981; Lehnert et al., 1984). Therefore the stress induced by the studies in which TYR administration did not successfully improve performance may not have been enough to deplete the catecholamine levels in the brains of the subjects and so additional TYR would not cause any improvement. Although 46 hours of sleep deprivation did cause

brain catecholamine depletion in rats (Tsuchiya et al., 1969), the 27 hours of sleep deprivation experienced by the subjects in the present study may not have depleted catecholamine levels. This may explain why no improvement in cognitive performance was seen with TYR consumption. If further stresses were introduced to humans during sleep deprivations, such as those which may be experienced in a combat, or simulated combat, situation, the stress may be enough to warrant the usage of TYR to combat performance decrements. Alternatively, TYR may be totally ineffective in preventing cognitive performance decrements during sleep deprivation.

In conclusion, there was a slight effect of 75 mg/kg TYR dosage on temperature and a possible effect on subjective fatigue, but no effect on any cognitive performance variable measured. It is possible that these subjects were not stressed enough to deplete catecholamine levels or that the TYR dose was not large enough to see differences from the placebo. Future studies involving 100-150 mg/kg doses of TYR and more strenuous testing conditions would seem to be warranted to resolve this question.

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Table 1. Subjective fatigue scale used hourly during sleep deprivation.

SUBJECTIVE FATIGUE

Write the number of the statement which describes how you feel RIGHT NOW.

- 1 = Fully Alert, Wide Awake, Very Peppy
- 2 = Very Lively, Responsive, Not at Peak
- 3 = Okay, Somewhat Fresh
- 4 = A Little Tired, Less Than Fresh
- 5 = Moderately Let Down
- 6 = Extremely Tired
- 7 = Completely Exhausted, Unable to Function

Table 2. Dietary intake of five subjects for two days prior to both tyrosine supplementation (T) and placebo (P) sleep deprivation trials (mean $\pm$ sd).

	T	P
Total calories	2593.6 $\pm$ 636.92	2590.90 $\pm$ 619.10
Carbohydrates (%)	54.2 $\pm$ 5.46	49.8 $\pm$ 7.52
Fat (%)	30.0 $\pm$ 5.18	34.6 $\pm$ 6.41
Protein (%)	13.8 $\pm$ 1.68	14.6 $\pm$ 1.36
Alcohol (%)	1.6 $\pm$ 3.20	0.6 $\pm$ 1.20
Tyrosine (mg)	2085.5 $\pm$ 678.09	2988.0 $\pm$ 942.13

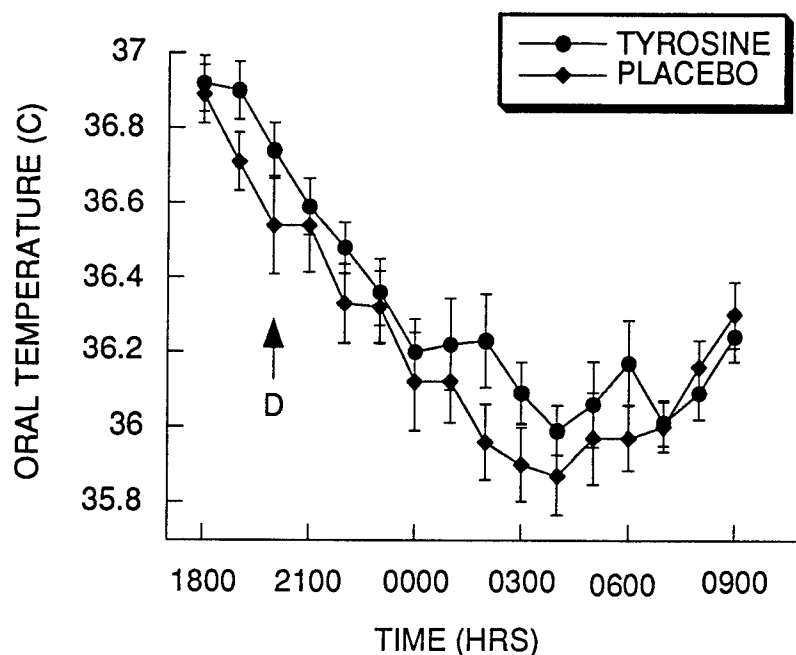


Figure 1. Oral temperature values measured in 9 subjects from both tyrosine and placebo sleep deprivation sessions beginning at 1800 hours. Either 75 mg/kg of tyrosine or placebo was given at 2000 hours (D). Temperature was significantly greater for tyrosine than placebo ($p < 0.05$) over all times combined.

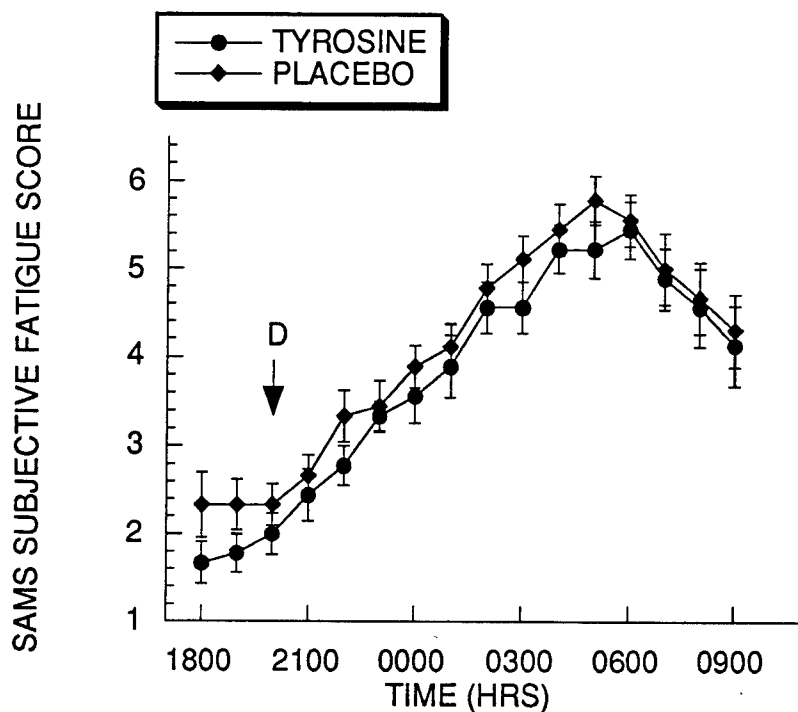


Figure 2. Average subjective fatigue reported from the SAMS scale by 9 subjects in both tyrosine and placebo sleep deprivation sessions beginning at 1800 hours. Either 75 mg/kg of tyrosine or placebo was ingested at 2000 hours.

**THE EFFECTS OF FRAMING ON HUMAN PERFORMANCE
IN A DYNAMICALLY COMPLEX WORK ENVIRONMENT**

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**Final Report for:
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Abstract

In order to better understand and integrate developments in man-machine work environments and multi-faceted information technology, the new field of cognitive engineering has emerged from research in the fields of cognitive psychology, computer sciences, and human factors. Our focus is to emphasize the contribution that one sub-area of cognitive psychology, judgment and decision making, can make to cognitive engineering (Nygren, 1993). For simple decision contexts, it has been shown that how a choice task is framed to individuals in terms of gains or losses can drastically alter their final judgements and choices (see Kahneman & Tversky, 1979). We propose that such framing effects will consequently have an observable impact on the actual performance of the task as well. To examine this hypothesis, we conducted an initial study of the effects of framing in the dynamically complex work environment of the flight operator. Our results showed that framing does produce an effect on the operator's performance; however, the actual effects and influences of framing in this dynamic decision-making environment appear to be more task specific than in a simple choice task.

**THE EFFECTS OF FRAMING ON HUMAN PERFORMANCE
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Introduction

In order to better understand and integrate developments in man-machine work environments and multi-faceted information technology, the new field of cognitive engineering has emerged from research in the fields of cognitive psychology, computer sciences, and human factors. Our focus has been to emphasize the contribution that one sub-area of cognitive psychology, judgment and decision making, can make to cognitive engineering (Nygren, 1993). It is known that cognitive components drive the way that information about relevant outcomes and events are perceived, integrated and used; however, little research has focused on the affective components that are influential. For simple situations, it has been shown that how a task is framed to individuals in terms of gains or losses can drastically alter their judgments and choices (see Kahneman & Tversky, 1979). We propose that such framing effects will consequently have an observable impact on the actual performance of the task as well. To examine this hypothesis, we conducted an initial study of the effects of framing in the dynamically complex work environment of the flight operator. This paper presents the results of that study and the implications of our findings for work environments with significant dynamic decision-making components.

The concept of "framing" has been recognized as an integral element of many decision situations because it operates upon the components of the choice alternatives. Tversky and Kahneman (1979) have shown repeatedly that between a pair of competing alternatives, individuals actually make opposite choices when the situation is merely framed differently to them. Specifically, researchers have found that when choice alternatives are framed in terms of what could be gained, people

are generally risk-averse. However, when the same situation is framed in terms of what could be lost, people are often risk-seeking. The risk-averse person will often choose a sub-optimal sure gain over a gamble in order to avoid or reduce risk, while the risk-seekers are willing to take a chance on a sub-optimal gamble in order to avoid a sure loss. Comparable results have been found for many different types of simple gambling tasks.

Our study was designed to examine whether these strong differences that have been previously found in more restrictive laboratory settings can be generalized to decision strategies made in a dynamically complex work environment with real-time constraints, particularly to the behavior of a flight operator. In this experiment, the performance measure was described to the human operators in terms of a positive or a negative frame. In the positive frame, operators were informed on how they can gain performance points in order to achieve an overall high performance score. In the negative frame, subjects were instructed on how to avoid losing performance points so that they could achieve a high performance score.

To simulate various aspects of the complex environment of the flight operator, the Multi-Attribute Task (MAT) Battery (Arnegard & Comstock, 1991; Comstock & Arnegard, 1992) was used for this study. In the battery, the human operators performed four sub-tasks simultaneously: tracking, system monitoring, auditory communications, and resource management. Each task in this dynamically changing "work environment" requires continual vigilance on the part of the operator. Hence, it is argued that the MAT task has at least some face validity with tasks performed by commercial pilots when flying their aircraft. Our objective in this study was to examine how framing affects performance on tasks like those found in the MAT battery and subsequently on operators' subjective workload ratings for the task.

In this battery, three of the tasks (tracking, monitoring, and

communications) were perceptual tasks which means that they require the human operator to notice the occurrence of a specific event and to make the appropriate response. However, the resource management task was a more cognitively challenging task which requires planning and strategic decision making to fulfill the task objective. We propose that on perceptual tasks, framing should not affect task performance; however, on cognitive tasks framing should have an influence because of the numerous alternative choices available to the operator.

An aspect in this simulated work environment that real flight operators contend with is the choice to set a task on automatic mode. Studying the use of the automatic mode is important because the role of the flight operator is changing from that of a worker to that of a supervisor, strategist, and decision maker. In our experiment, we suggested that the operator set either or both the tracking task and the resource management task to automatic mode if he feels that his overall performance is suffering or if he experiences a heavy workload. The choice to use the automatic mode, we propose, is influenced by framing. Specifically, when the task is framed in terms of losses, operators will be more risk-seeking (move above their ideal level of workload) and will put tasks in manual mode more than during their training session. When the task is framed in terms of gains, operators will be more risk-averse (move down from their ideal level) and will put tasks in automatic mode more than during their training session. Hence, the loss/negative frame will elicit more risk-seeking behavior and more use of the manual mode than the gains/positive frame.

Method

Subjects

Eighty-two undergraduate students from the Ohio State University volunteered to participate in this study as a partial fulfillment of the requirements for their introductory psychology course. The subjects were not given any monetary incentive for their performance, but they were

told that they could possibly win prizes (cassette tapes) based on their performance.

The subjects were randomly and approximately equally distributed to one of the two framing conditions. They were run in groups of three to four, but they were individually seated in front of PC work station. All stimulus materials and responses were presented and recorded on these computers.

Apparatus

Task Battery

The Multi-Attribute Task (MAT) Battery simulated several aspects of the relatively complex work environment of the flight operator. This PC-based program required that the operator perform four tasks simultaneously. Each task had its own separate window on the computer

Insert Figure 1 about here

screen, its own set of command keys on the keyboard, and its own output file that recorded the operator's actions. In the tracking task, the objective was to maintain the circular target in the center of the tracking window by movements with a mouse. Every 15 seconds, the amount of deviation from the center was recorded to the output file. In the system monitoring task, four vertical gauges and two warning lights were monitored for abnormalities. These abnormalities were the absence of a green light, the presence of a red light, and significant deviations in the gauges. If the operator did not detect and respond to an abnormality, then after a preprogrammed time out period the signal or gauge would be reset to normal status. The monitoring output file recorded when an event took place (i.e., red light was on) and the response time to the abnormality. For the auditory communication task, the operators listened to a series of pre-recorded audio messages which were presented to them through headphones. Each message began with a six-digit call sign, and the operators were to only attend to those

messages bearing their assigned call sign. When a message was directed at them, they were to follow the message's instructions by moving the up or down arrow keys to the specified channel (which could be one of four channels), and then they were to change the frequency of the channel to the prescribed setting using the left or right arrow keys. The output file for this task contained information on when an event took place (i.e., operator switched on a new channel) and if the frequency of the channel was changed. The three tasks just described are relatively simple tasks that require a specific response to a certain event. The following task allows for a wider range of response patterns. In the resource management task, the objective was to maintain the levels of two primary fuel tanks (tanks A and B in Figure 1) at the set level of 2500 gallons each by turning on or off any of the eight pumps. The rate of fuel loss for the primary tanks was 800 gallons a minute. Each primary tank could be feed from two auxiliary tanks. The feeding rate of one auxiliary tank (tanks C and D respectively) was 800 gal/min and the other was 600 gal/min. The former auxiliary tank contains a 1000 gallons of fuel at the begin of the experiment, and in order to refill this tank, fuel has to be transferred from the other auxiliary tank to it at a rate of 600 gal/min. Also, the primary tanks were connected to each other such that fuel could be transferred between them at a rate of 400 gal/min. To increase workload on the operator, planned pump failures occurred which required the operators to use alternative pumps to move fuel. These failures occurred for a limited amount of time, then the pumps were reopened. The output file for this task recorded all pump activity and the level of the tanks periodically.

In addition to the four tasks, the MAT presented the subjects with a modification of the NASA Task Load Index (TLX) (Hart & Staveland, 1988) after every six minutes on the task battery. The TLX consisted of six scales: confidence, mental demand, temporal demand, stress demand, riskiness, and performance. The rating scales for the first five

measures went from low (0) to high (100), while the performance scale was from good (0) to poor (100). The operator's responses on the TLX were recorded in its own output file.

Matana

Matana, MAT Analysis computer program was written by Carina Tornow and Thomas Nygren.¹ This program summarizes the MAT output files for each subject. For each task, it calculates overall means for certain variables and counts the total number of responses for specific variables. Also for each six-minute intervals, data was summarized for that specific time period. The summarized information for all the subjects can then be analyzed by numerous statistical packages.

Procedure

Each subject came in for two 30-minute sessions on MAT. After every six-minutes on the battery, the program paused, and the computer monitor displayed the modified NASA Task Load Index (TLX) to be completed by the subject. Each session was therefore comprised of five six-minute intervals.

The subjects were distributed into one of two groups whose instructions differed in the framing of the descriptions of the performance measure. The positive framing group was instructed on how to gain points by making fewer errors, keeping tracking close, and using better resource management. The negative framing group was instructed to avoid losing points which would occur if they had many errors, poor tracking, and poor resource management.

The instructions were similar for each day, and the MAT program script remained the same for both days. The purpose of the first session was to train and familiarize the subjects with MAT and with the keyboard controls for each task, and also to allow them time to try different strategies. They were informed that their performance on each of the four tasks would be recorded by the computer; however, their practice performance would not affect their test performance scores, but they were

instructed to do their best in order to maximize their chances on the test session. The instructions informed the subjects about the option to set the tracking task and/or resource management task on automatic mode if they experienced a heavy workload and/or if their performance was suffering.

The second session was the test session which was administered on a different day. The subjects were given similar instructions as in their practice session. They were warned that the use of the automatic mode would affect the rate at which they gained or lost performance points. Their objective was to perform their best in order to achieve a high performance score.

Results

Data was collected and stored by the MAT program for each of the four tasks of monitoring, tracking, communications, and resource management. Because of technical limitations in the presentation of the auditory information to subjects, it was not possible to present the communications signals at exactly the same time during the MAT script for each subject. Thus, the communications task was used primarily for the purpose of including an auditory task that would add significantly to the operators' workload. Therefore, communications data was not analyzed in this experiment.

Of the remaining three tasks performed, framing had a most noticeable effect on performance of the resource management task. A multivariate analysis of variance revealed that the positive framing group deviated significantly less in maintaining each of the primary tanks at the set level of 2500 gallons, Tank A: $F(1,72)=5.18$, $p=.026$, Tank B: $F(1,72)=10.14$, $p=.021$. The MANOVA analysis also showed that the mean-deviation scores were significantly different across the time intervals for Tank A: $F(4,288)=19.965$, $p<.001$, and for Tank B:

Insert Table 1 & 2 about here

$F(4,288)=10.14$, $p<.001$. However, the final levels of the primary tanks at the completion of the 30-minute experiment were not significantly

Insert Table 3 & 4 about here

different between the two framing groups. Another significant groups effect was found in the number of actions performed (i.e., turning on a pump), $F(1,78)=6.94$, $p=.01$. Overall, the negative framing group took fewer actions ($\bar{M}=60.30$) than the positive framing group ($\bar{M}=85.37$).

Framing had an effect on only one variable in the monitoring task. The reaction time means to the presence of a red signal varied significantly between the groups, $F(1,80)=4.25$, $p=.043$. The reaction times of the positive framing group ($\bar{M}=1.79$ sec) were longer than those in the negative framing group ($\bar{M}=1.58$ sec). However, each group responded to most of the occurrences of the red signal, so the number of responses to the presence of a red signal did not vary between the groups.

On the tracking task, framing had no effect on performance. Tracking performance was only influenced by time spent on the task. The target deviation scores decreased significantly over the time intervals, $F(4,316)=7.60$, $p<.001$; while use of the automatic mode increased across the time intervals, $F(4,316)=4.47$, $p=.002$.

On the TLX rating scales only two measures were affected by framing. The temporal demand ratings from the positive framing group ($\bar{M}=63.79$) were statistically greater than the ratings from the negative framing group ($\bar{M}=55.02$), $F(1,80)=5.1$, $p=.027$. The other main effect was in the confidence ratings, $F(1,80)=5.54$, $p=.021$. The confidence ratings from the negative framing group ($\bar{M}=96.4$) tended to be higher than the ratings from the positive framing group ($\bar{M}=51.03$). Confidence ratings differed significantly across the time intervals in a linear increasing trend, $F(4,320)=9.11$, $p<.001$. Performance ratings varied only across the time intervals in a linear decreasing fashion which means that the

subjects thought that they were doing better, $F(4,320) = 3.23$, $p = .013$. For stress demand ratings, an interaction between the groups and the time intervals was close to significant, $F(4,320) = 2.35$, $p = .054$, such that the subjects in the positive frame were possibly experiencing more stress at a faster rate than those in the negative frame.

Conclusion

From the analyses, it appears that framing does have a significant influence on performance of some tasks like those found in the MAT battery. On the cognitive task of resource management, framing appears to influence performance such that individuals in the losses/negative frame took fewer actions and tended to deviate more from the task objective than the operators in the gains/positive frame. However, on the perceptual tasks the operators' performance was not affected by framing. This evidence supports our hypothesis that the effects of framing are task specific in the complex work environment.

Through further analysis, we would like to investigate whether framing influences the amount of attention partitioned to a certain sub-task. Operators in one condition may possibly segregate between sub-tasks in order to maximize performance on specific tasks, while in the other condition operators are equally devoted to performing well on all the tasks. In this study, operators in the losses/negative frame did not perform as well as those in the gains/positive frame on the resource management task. However in the monitoring task, these operators in the losses/negative frame did react faster to the presence of a red signal. Implications as to which group segregates is not obvious. However in a study by Ritov, Baron, and Hershey (1993), when subjects are given the choice between options that achieved the same overall reduction, subjects in the losses frame selected to reduce the two risks equally while the subjects in the gains frame preferred to largely reduce only one risk. Therefore in this study, it is possible that the operators in the gains frame placed more weight on performing well on the resource management

task than the others.

Our other hypotheses which conjectured that framing would influence the use of the automatic mode on a task were not borne out in this experiment. In fact, operators did not vary in their use of the automatic mode. This may have resulted because the task was not excessively demanding, and the program script did not change between the two days; therefore, the operators did not feel the need to use the automatic mode. Further studies should be conducted to observe if framing affects the use of automation under differing workload levels.

The type of choice behavior present in simple situations, that is people in the gains frame tend to be risk-averse while people in the losses frame tend to be risk-seeking (Tversky & Kahneman, 1979), was not noticed in this complex environment. A reason for this may be derived from the subjective ratings. The operators in the positive frame felt more of a time constraint on their performance, so in order to accumulate the highest number of performance points within the time period, they may have felt the need to take greater chances and become risk-seeking. Time constraints were never a factor in the simple situations, but it appears to hold great significance in the complex work environment. This time constraint factor may also explain why the operators in the gains frame were not as confident in their performance as the subjects in the negative frame.

In conclusion, findings from simple laboratory studies on framing may not be generalizable when a time-constraint is placed on a dynamically changing task. Our study presents interesting results and suggests the need for further investigations of the effects of positive and negative framing, stress, and time constraints on performance in high workload environments.

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Footnote

The program, MATANA, that is used to summarize subject data files has been written in Fortran, and it can be obtained by writing to:

Thomas Nygren
The Ohio State University
Department of Psychology
142 Townshend Hall
1885 Neil Avenue Mall
Columbus, OH 43210-1222

Table 1**Mean Deviation From the Set Level of 2500 Gallons for Tank A**

Frame	Time Interval				
	Int 1	Int 2	Int 3	Int 4	Int 5
Positive (N=38)					
Mean	-318.18	19.14	1.40	-98.39	-15.96
Median	-276.70	19.25	5.45	1.00	6.80
Std Dev	333.45	400.06	417.56	479.92	342.10
Negative (N=36)					
Mean	-602.60	-293.09	-253.57	-326.43	-171.04
Median	-488.25	-100.95	- 72.75	-109.75	- 19.80
Std Dev	505.78	580.16	680.60	726.46	702.30

Table 2**Mean Deviation From the Set Level of 2500 Gallons for Tank B**

Frame	Time Interval				
	Int 1	Int 2	Int 3	Int 4	Int 5
Positive (N=38)					
Mean	-236.49	3.18	- 80.17	- 70.19	- 13.62
Median	-186.85	1.05	- 0.20	12.45	9.20
Std Dev	355.11	401.06	496.52	442.05	389.54
Negative (N=36)					
Mean	-575.78	-434.88	-284.97	-227.78	-236.48
Median	-416.15	-203.85	- 94.50	- 70.20	- 71.90
Std Dev	523.43	659.81	755.25	729.79	729.86

Table 3**Final Level of Tank A**

	Frame	
	Positive	Negative
N	41	39
Mean	2523.63	2366.33
Median	2516.00	2517.00
Std Dev	397.15	752.82

Table 4**Final Level of Tank B**

	Frame	
	Positive	Negative
N	41	39
Mean	2559.37	2361.00
Median	2507.00	2468.00
Std Dev	421.73	635.73

Multi-Attribute Task Battery

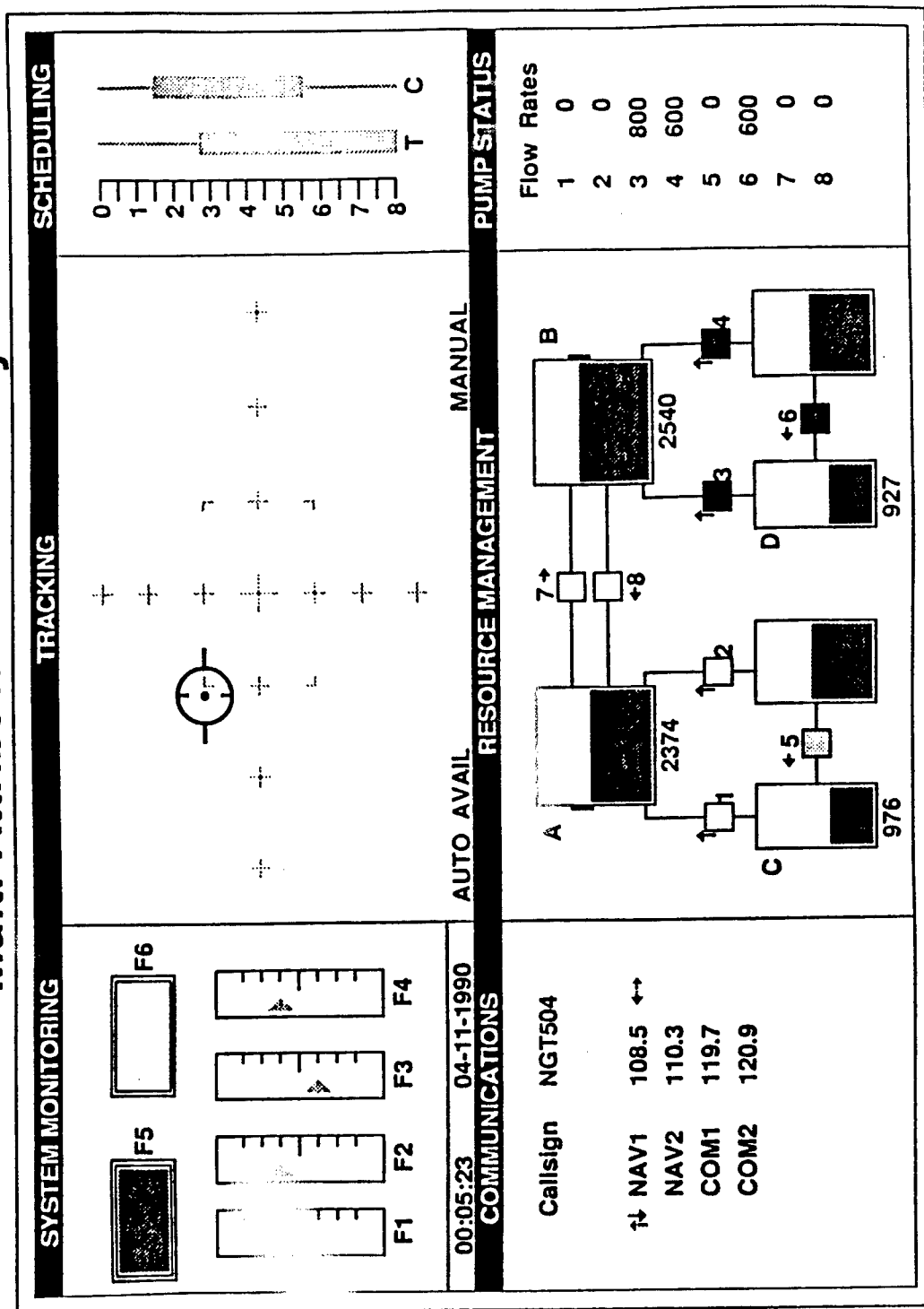


Figure 1

A MODEL OF THE SHOULDER, ELBOW, HIP, KNEE AND ANKLE JOINTS FOR THE
ARTICULATED TOTAL BODY MODEL PROGRAM

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Abstract

The Articulated Total Body Model program (ATB) is a computer program which has the ability to describe the three dimensional response of a body in different types of dynamic environments. The model contains a series of coefficients and equations, which work together to describe the response of the different segments and joints of a body, when specific loads are applied. In many cases a coefficient is able to describe certain response characteristics quite accurately, however, there are cases when an equation would be much more accurate. As the experimental data becomes available, equations may be derived to be used in place of coefficients. This report explains how experimental data containing the resistant force and resistant torque characteristics of the shoulder, elbow, hip, knee, and ankle was manipulated. The data was manipulated to form characteristic equations that would be suitable to replace the corresponding coefficients in the ATB program.

A MODEL OF THE SHOULDER, ELBOW, HIP, KNEE AND ANKLE JOINTS FOR THE ARTICULATED TOTAL BODY MODEL PROGRAM

Christine D. Whitmore

INTRODUCTION

The Articulated Total Body model (ATB) is a computer program which has the ability to describe the three dimensional response of a body in different types of dynamic environments. The ATB model is used mainly to evaluate the dynamic response of the human body while it is in some type of moving vehicle. The model contains a series of coefficients and equations, which together, help describe the response of the different segments and joints of the body, when specific loads are applied. Both the coefficient and the equations describing the response characteristics, are derived from experimental data obtained for certain characteristics, for a specific segment or joint of the body. In many cases a coefficient is able to describe a certain characteristic quite accurately. Presently, there are cases where a coefficient is being used to describe a characteristic, but the equation which describes the characteristic would be much more accurate.

When experimental data is obtained which may be used to derive characteristic equations for certain segments and joints of the body, the manner in which the data was taken must be considered very carefully. A full understanding of how the data was obtained and what is being used as a reference point when obtaining the data, is very important. Once this information is understood, the data may be put into the correct format and then be placed into the ATB model. Information must be placed into the ATB model describing the reference systems, if they are different from the axis system used in the ATB model to describe the location of the joint.

The response characteristics of the joints are presently being expressed in the ATB program in the form of coefficients. Using the Human Articulation and Motion - Resistive Properties report, written by Ali E. Engin and Huenn-Muh Chen and the Measurement of Resistive Torques in Major Human Joints report, written by Ali E. Engin, resistant force data was obtained for the shoulder, hip, elbow, knee, and ankle joints and resistant torque data was obtained for the shoulder and the hip. This data was then used to derive equations which

describe the resistant force or resistant torque characteristics of the specific joint.

The data found in the two reports were in very different forms. In the Human Articulation and Motion - Resistive Properties report, data was found for the shoulder and the hip. The results contained in this report did not consist of actual experimental data. The results were in the form of expansion coefficients that fit into a general equation. There were different general equations and coefficients for different characteristics of the joint. The two characteristics given in this report that were used for the ATB model, were the voluntary complex sinus characteristic and the resistant force characteristic. The general equation could not be directly placed into the computer model mainly because they were not given in the correct form and they did not have the correct orientation. The Measurement of Resistive Torques in Major Human Joints report gave resistant force characteristic data concerning the ankle, knee, and elbow and the resistant torque data concerning the shoulder and hip. The results of this report were given in the form of actual experimental data, which was collected and then plotted for each of the subjects involved in the experiment.

METHODOLOGY OF THE DATA ANALYSIS

In the experiment corresponding to the Measurement of Resistive Torques in Major Human Joints, the subject was placed in a rotating restraint system resembling the basic shape of a chair. This system has yaw, pitch, and roll capabilities so that the subject may be placed in any desired position. This allows the body segment under investigation to stay at a constant elevation while the at the same time being placed in different positions relative to other segments of the body. While the muscles are totally relaxed, so there will be no active muscle motion, a horizontal force is applied to the body segment. This type of force application eliminates the gravitational component of the joint moment. Forces were applied to the segment using a global force applicator containing high precision potentiometers. The GFA provided information on both the position of the segment and the force and torque resistance. Plots of the experimental data were given for the three subjects used in this experiment. The angle of the joint (degrees) was used to describe the position of the joint and the passive resistant moment (N-m) was used to describe the resistant force. The resistant torque characteristic motion was

described using joint angles (degrees) and the passive torque (N-m).

Numerical data was taken from the given data points on the resistant force plots. These sets of data points represented the experimental data obtained from each subject. The data points for each subject were plotted on their own plot and curve fitted so that the best curve fit was obtained. This resulted in an equation describing the characteristic behavior of the specific joint for each subject. These resulting equations were used to produce their characteristic curves for each joint. Data was found using, the equations from the curve fit, at every ten degrees. The data at every ten degree increment was averaged so that an average characteristic curve for each of the joints was obtained. The average characteristic curve was then oriented so that the start of the curved area of the characteristic plot was located at 0 degrees and 0 in-lb. This average, shifted characteristic curve was then curve fitted to the third order. The ATB program requires that the equations describing the characteristic curve only contain a cubic, quadratic, and linear coefficient. The resulting third order equation was then used in the ATB program using only the cubic, quadratic, and linear coefficients.

Only two equations were needed to describe the characteristic behavior of the elbow, ankle, and knee joints. All three joints are designated as pin joints. A pin joint is defined as a joint with only one independent coordinate. The angle formed between the two segments attached by the joint is the only angle needed to describe the position of the joint. The joint however, usually has a characteristic curve which is curved at one end, flat in the middle, and curved at the other end. These two curves are usually quite different and need to be defined using different equations. The flat area in the middle is considered the 'deadband' zone. In the deadband zone is the section of the curve in which there is no passive resistant force over a specific range of angles. Instead of trying to define this complicated curve using one equation, the ATB model program lets the user describe the curve using two equations. The center of the deadband zone is designated as the 'center' of the curve. The values of the center angle relative to the joint axis system, is specified in the program. The length of the deadband zone, in radians, from the center to one end of the zone is referred to as the stop angle. The stop angle is specified in the program relative to the center of the zone, not relative to the joint axis system. The two curves at the end of the characteristic curve are then defined as individual curves starting at the position 0,0. The curve that describes the characteristic resistant force when the joint is flexed, when the outer segment

is moved toward the inner segment attached to the joint, is designated with a phi angle of 0 degrees. The curve that describes the characteristic resistant force when the joint is extended, when the outer segment attached to the joint is moved away from the inner segment, is designated with a phi angle of +180. Whether the motion is flexion or extension is relative to the center angle of the deadband zone. The phi angle describes the motion that occurs around the z axis. Since the joints were described as pin joints, and therefore have no motion around the z axis, the designation of 0 or 180 phi properly describes which side of the deadband zone the characteristic equation belongs with. The curves are both curve fitted and the cubic, quadratic, and linear coefficients are used in the ATB program.

The passive torque characteristics for the shoulder and hip joints were also found using the Measurement of Resistive Torques in Major Human Joints. For the torque characteristic, only one equation could be used having a cubic, quadratic, and linear coefficient. The method used for finding the average characteristic curve for the passive resistant torque was the same as the method used to find the passive force resistance characteristics. The average curve was then split into half at the center of the curve where the torque value was zero. The curves were then shifted into the first quadrant and averaged. The average curve obtained was then curve fitted. The resulting equation was used in the ATB program using the specified coefficients.

The experiment used to find the data corresponding to the Human Joint Articulation and Motion - Resistive Properties report was very similar to the experiment described for the previous report. The subject was placed the same type of rotating restraint system with a horizontal force being applied to the segment of the body. In this experiment however a sonic digitizing process was used to measure the position of the joint. The sonic digitizer has the ability to convert position data via sound waves to digital values that may be easily used. The axis system used on the moving body was found on a record by record basis. This 'most accurate' axis system was found using sonic digitizer readings. Two types of information were obtained from this report on both the shoulder and the hip joints. The first was the voluntary complex sinus of the joint. Since both joints are defined as being global joints, having two independent coordinates, phi and theta. Axial rotation is not considered in this experiment. The voluntary joint sinus was found for each subject, averaged and then curve fitted. The equation for the voluntary complex sinus is in terms of the angle theta dependent on phi. So the

equation, when a certain phi is put into it, will give the average maximum theta angle that the shoulder or hip will move voluntarily. The general form of this equation is the following:

$$\Theta(\phi) = \Sigma \cos^{n-1} \phi (C_{2n-1} + C_{2n} \sin \phi)$$

The expansion coefficients were given for this equation for both the shoulder and the hip. This equation gives the stop angles needed for each degree of phi for which a characteristic equation will be put into the ATB program. The second type of information contained data on the passive resistant moment characteristics. The moment data was collected for each of the ten subjects, at specific positions, plotted, and then curve fitted. The general equation describing the resistant moment of both the shoulder and the hip is a function of both theta and phi. The general form of this equation is the following:

$$f(\phi, \Theta) = (C_1 + C_2 \cos \phi + C_3 \sin \phi) \Theta + (C_4 \cos^2 \phi + C_5 \cos \phi \sin \phi + C_6 \sin^2 \phi) \Theta^2 + (C_7 \cos^3 \phi + C_8 \cos^2 \phi \sin \phi + C_9 \cos \phi \sin^2 \phi + C_{10} \sin^3 \phi) \Theta^3$$

The expansion coefficients were also given for this equation. It should be noted that two types of coefficients were given, space based and subject based. The space based coefficients were derived using data that was described with respect to the joint axis system of each subject. The space based coefficients were derived using data that was described with respect to a common mean joint axis system for all subjects. From this equation, a series of equations were needed describing the passive resistant moment characteristic depending on the angle theta for certain angles of phi. It was determined using the stop angle plots that defining phi in increments of 30 degrees would give a very good representation of the characteristic relationship between phi and theta. So for increments of 30, the values of phi were placed into the general equation giving a series of 12 equations

dependent on theta. These equations could not be put into the ATB program. The program needs equations describing the moment characteristic starting from the edge of the deadband zone, defined by the stop angle. Each equation was plotted in increments of 10 degrees theta starting with the stop angle. This curve was then shifted both horizontally and vertically so that the first point on the curve was 0,0 , or 0 N-m at 0 degrees theta. The curve then has an offset of the stop angle and the force that the equation defined there. The ATB program defines the deadband zone to be an area where the moment force is zero. The angle of the joint placed into the system will have the stop angle subtracted from it before any calculations are made. This value of theta will then be placed into the characteristic equation for the specified phi. The modified curve was then curve fitted to the third order. The quadratic, cubic and linear coefficients of this equation were used in the program.

The equations put into the ATB program were described with respect to the mean joint axis systems. The ATB program needs to know the location of this axis with respect to a fixed body axis. The theta and phi angles that locate the joint axis with respect to the designated fixed body axis are given for both joints. The location of the reference axis system are not needed for the elbow, knee, or ankle. This is because at the location defined as zero degrees for all three joints in the equations, is the location where the two segment coordinate systems and the joint coordinate system are aligned along all three axis with each other. Due to the relationship of the two segments joined together by the pin joint, the z axis are both along the long bone axis. The x and y axis do not need to be defined since the torsional motion of the arm does not affect this study.

ANALYSIS OF THE SHOULDER JOINT

The passive resistant moment characteristic of the shoulder was found and modified using the Human Joint Articulation and Motion-Resistive Properties report. The general equation and expansion coefficients were given for the relationship between the angle phi and the average maximum value of the angle theta, or the stop angle. The general equation and the expansion coefficients were used to plot the characteristic relationship between the two angles. This equation was the following:

$$\Theta(\phi) = (1.49896 - 0.09266 \sin \phi) + \cos \phi (-0.24693 - 0.29213 \sin \phi) + \cos^2 \phi (0.18903 + 0.58438 \sin \phi) + \\ \cos^3 \phi (0.4545 + 0.00604 \sin \phi) + \cos^4 \phi (-0.43012 - 0.4672 \sin \phi)$$

Values of phi, in increments of 30 degrees were input into this equation so that the value of the stop angle of theta could be determined. The angles obtained from the equation defined the characteristics of the right shoulder. The relationship between the angles is slightly different in the left shoulder due to the difference in the joint coordinate systems. The z axis in both the right and left shoulder coordinate systems are aligned with the long bone axis of the upper arm segment. This causes the y axis in the left shoulder's joint axis system to be directed in the opposite direction of the y axis in the right shoulder's joint axis system. The x axis is oriented in the same direction in both joint axis systems. The result is that when the position of the joints looks the same, the values of phi for the two systems will not be equivalent. The values of phi will actually be the same in magnitude but the direction of the angles are the opposite of each other. Using the symmetry of a circle and the above relationship, the values of the stop angles for the different increments of phi were easily found using the stop angles calculated using the equation. This data may be found in Figure 3.

The relationship between the resistant force and the angles phi and theta was also expressed using a general equation and expansion coefficients. This relationship for the shoulder is the following:

$$f(\phi, \Theta) = (-21.498 - 2.089 \cos \phi + 2.139 \sin \phi) \Theta + (19.70 \cos^2 \phi + 5.07 \cos \phi \sin \phi + 15.699 \sin^2 \phi) \Theta^2 \\ + (-1.3626 \cos^3 \phi - 3.98 \cos^2 \phi \sin \phi + 2.233 \cos \phi \sin^2 \phi - 0.577 \sin^3 \phi) \Theta^3$$

Phi angles in increments of 30 degrees were input into the equation above. Using increments of 10 degrees for the angle theta, the characteristic curve for each value of phi was plotted. The values of theta used for this curve started with the value of the stop angle for that value of phi. The curve was first shifted horizontally so that the

RIGHT SHOULDER**STOP ANGLE (RAD./DEG.)**

<u>PHI = 0</u>	-12.06008x ³ +121.37742x ² +224.67518x	1.4654/ 83.96
<u>PHI = 30</u>	-21.23372x ³ +95.97214x ² +195.69639x	1.397/ 80.04
<u>PHI = 60</u>	-11.68411x ³ +119.98884x ² +189.09152x	1.3478/ 77.22
<u>PHI = 90</u>	-5.12107x ³ +117.34594x ² +189.09152x	1.4063/ 80.58
<u>PHI = 120</u>	-10..19562x ³ +75.20777x ² +188.21787x	1.7328/ 99.28
<u>PHI = 150</u>	-6.46771x ³ +117.34608x ² +225.9482x	1.4834/ 84.99
<u>PHI = 180</u>	12.06009x ³ +212.39799x ² +234.46380x	1.0503/ 60.18
<u>PHI = 210</u>	21.30807x ³ +258.15159x ² +326.86077x	1.1515/ 65.98
<u>PHI = 240</u>	11.68770x ³ +217.72164x ² +356.61525x	1.4392/ 82.46
<u>PHI = 270</u>	5.11969x ³ +163.41026x ² +272.00868x	1.5916/ 91.19
<u>PHI = 300</u>	10.18323x ³ +176.16988x ² +258.27399x	1.5575/ 89.24
<u>PHI = 330</u>	6.47994x ³ +176.3638x ² +288.37937x	1.5633/ 89.57

LEFT SHOULDER**STOP ANGLE (RAD./DEG.)**

<u>PHI = 0</u>	-12.06008x ³ +121.37742x ² +224.67518x	1.4654/ 83.96
<u>PHI = 30</u>	6.47994x ³ +176.3638x ² +288.37937x	1.5633/ 89.57
<u>PHI = 60</u>	10.18323x ³ +176.16988x ² +258.27399x	1.5575/ 89.24
<u>PHI = 90</u>	5.11969x ³ +163.41026x ² +272.00868x	1.5916/ 91.19
<u>PHI = 120</u>	11.68770x ³ +217.72164x ² +356.61525x	1.4392/ 82.46
<u>PHI = 150</u>	21.30807x ³ +258.15159x ² +326.86077x	1.1515/ 65.98
<u>PHI = 180</u>	12.06009x ³ +212.39799x ² +234.46380x	1.0503/ 60.18
<u>PHI = 210</u>	-6.46771x ³ +117.34608x ² +225.9482x	1.4834/ 84.99
<u>PHI = 240</u>	-10..19562x ³ +75.20777x ² +188.21787x	1.7328/ 99.28
<u>PHI = 270</u>	-5.12107x ³ +117.34594x ² +189.09152x	1.4063/ 80.58
<u>PHI = 300</u>	-11.68411x ³ +119.98884x ² +189.09152x	1.3478/ 77.22
<u>PHI = 330</u>	-21.23372x ³ +95.97214x ² +195.69639x	1.397/ 80.04

Figure 3

curve was offset by the value of the stop angle. The curve was then shifted vertically so that the force value at the stop angle θ would be equal to zero. This shift was quite small for all of the angles of ϕ . The shifted curve was then curve fit to the third order. The ATB program needs only the quadratic, cubic and linear coefficients of these equations. These results may be seen in Figure 3. The same procedure was used for the left shoulder. It may be observed that the equations for the relationship between the resistant force and θ are the same in the left shoulder for ϕ angles that are the opposite of each other.

The value of the coefficients for each of the equations were input into the ATB program with the corresponding ϕ angle. The information was input into program using the E.7 cards. The data for the remaining joint was input into the program using a program manager called Dynaman. It should be noted that the coefficients input into the E.7 cards are in units of in-lb per radian. The stop angles specifying the limits of the deadband zone are in units of degrees. After the data was input, changes were made to the D.1 cards so that the program would use the new data.

The ATB program uses the upper torso axis system for the fixed body axis system for the shoulders. The location of the joint axis system in both shoulders in the fixed body axis system was needed in the ATB program. The values were given in the report to be $\phi = 59.29$ degrees and $\theta = 79.08$ degrees for the right shoulder. The corresponding angles for the left shoulder are then $\phi = -59.29$ degrees and $\theta = 79.08$ degrees.

After the program was programmed for proper use of the data, trial runs were run using the Dynaman program to check the output. Certain angles of ϕ and θ relative to the fixed body axis system in the upper torso were input into the input preprocessor. A simulation was run for each of the 4 positions. The output consists of the values of the total resulting force in the joint, the position of the joint relative to the joint axis system, and the components of the resulting force relative to the upper torso axis system and the upper arm axis system.

The resistive torque characteristics of the shoulder joint were found using the Measurement of Resistive Torques in Major Human Joints report by Ali E. Engin. The data in this report was in the form of three plots each containing the characteristic curve for the resistive torque for individual subjects. No actual experimental

data was contained in this report. Data was pulled from each plot and plotted again on separate plots by subject. The data in each plot was curve fitted to the best fit. Angular values in increments of 10 degrees were input into each equation to obtain a series of data points. The data points corresponding to a specific angular value were averaged and the series of average values were then plotted to obtain a characteristic curve. Since the motion being defined is about a single axis, there needs to be only one equation to describe the motion. There was also no deadband zone.

Near the middle of the average curve describing the characteristic of the resistant torque there is an angular value which has a resistant torque value of approximately zero. The curve is shifted horizontally so that the point of zero resistant torque is positioned at a value of zero degree. At this zero resistant torque point, the curve is split into two curves. The first section of the curve is rotated 180 degrees so that it is positioned in the first quadrant. Each of the two curves, both in the first quadrant, are then curve fitted to the best fit and data points are taken from each equation in increments of 10 degrees. These data points are averaged for every increment of 10 degrees and the average curve was plotted. This average curve was then curve fitted to the third order and the quadratic, cubic, and linear coefficients were used in the ATB program. If the information had been input directly into the ATB program the coefficients would have been placed in the B.3 cards. The Dynamman program manager was used instead. It should be noted that the coefficients used for the resistant torque had the units in-lb/ degree. The equations may be found in Figure 5.

ANALYSIS OF THE HIP JOINT

The analysis of the hip joint was performed in exactly the same way as the shoulder joint. The values of the stop angles for the right hip were found by plugging in the expansion coefficients into the general equation. This equation was the following:

$$\Theta(\phi) = (0.523 + 0.002\sin\phi) + \cos\Theta(0.0026 - 0.138\sin\phi) + \cos^2\phi(0.354 - 0.044\sin\phi) + \cos^3\phi(-0.0052 - 0.257\sin\phi) + \cos^4\phi(0.0113 + 0.183\sin\phi)$$

Values of phi in increments of 30 degrees were plugged into the above equation and the corresponding values of theta were found for the right hip. The relationship between the joint axis coordinate systems for the right and left hip is the same as the relationship between the right and left shoulder joint axis coordinate systems. The z axis for both the right and left hip joint axis follows along the long bone axis of the corresponding upper leg. Since the x axis in both systems is directed in the same direction, the y axis of the two systems point in opposite directions. This results in the phi angle of the left hip being equal in magnitude but opposite in direction to that of the right hip when the two joints are in what appears to be the same position. This relationship along with circular symmetry allowed the stop angles and their appropriate phi angles be found for the left hip using the data for the right hip. This data may be seen in Figure 6.

The relationship between the resistant force and the angles phi and theta was found using the general equation and the expansion coefficients given exclusively for the hip joint. This equation is the following:

$$F(\phi, \Theta) = (-8.41 - 1.66\cos\phi + 22.32\sin\phi) + (59.44\cos^2\phi + 57.39\cos\phi\sin\phi + 95.11\sin^2\phi)\Theta^2 + (0.76\cos^3\phi - 1.11\cos^2\phi\sin\phi + 2.57\cos\phi\sin^2\phi - 10.63\sin^3\phi)\Theta^3$$

Using increments of 30 degrees for the angle phi, a series of equations were found using the above relationship. Values of theta were then input into each of the equations in increments of 10 degrees and the characteristic curves were plotted. The curves were shifted in the same manner as the curves found in the analysis of the shoulder. The shifted curves were curve fitted to an order of 3. The quadratic, cubic, and linear coefficients were taken from these equations and input into the ATB program using the Dynamman program manager. This data may be also found in Figure 6. The same procedure was used for the left shoulder.

The ATB uses the lower torso segment coordinate axis system as the fixed body axis system for the hip.

The location of the joint axis system in the lower torso coordinate system was needed. This location was given in the report for the right hip joint axis system to be $\phi = 43.19$ and $\theta = 56.85$. Using the relationship between the left and right hip coordinate systems, circular symmetry, and anatomic symmetry, the location of the left hip joint axis system to be $\phi = -43.19$ and $\theta = 56.85$.

Once all of the data concerning the hip was input into the ATB program, four trial runs were simulated for both the right and the left hip. Certain positions relative to the lower torso axis system were input using the Dynaman program manager and a simulation was run. The output, again, consisted of the values for the total resulting force in the joint, the position of the joint relative to the joint axis system, and the components of the resulting force relative to the upper torso axis system and the upper arm axis system.

The resistant torque characteristics of the right and left hip joints were also found using the Measurement of Resistive Torques in Major Human Joints report. The data was again contained in plots of the resistant torque characteristics for each subject tested. The analysis used to find the characteristic curve of the resistant torques characteristics of the left and right hip was exactly the same as that for the left and right shoulder. The data was taken off the plots, curve fitted, and an average curve representing all three subjects was found. This average curve was shifted, split, and the first section of the curve was rotated 180 degrees so that it was placed in the first quadrant. These two curves were curve fitted, averaged, and a final average characteristic curve was found. This curve was curve fitted to the third order and the quadratic, cubic, and linear coefficients and used in the ATB program. The equations may be found in Figure 5.

ANALYSIS OF THE KNEE JOINT

The Measurement of Resistive Torques in Major Human Joints report was used as the source of experimental data for the resistant force characteristics of the knee joint. The experimental data on the resistant force characteristics was presented in the same way as the experimental data of the resistant torqued was presented. A plot described the characteristic curve of the resistant force characteristic for each of the three subjects. After much consideration the knee data of the first subject was disregarded during the analysis. The

characteristic curve of the first subject was very different than the other two subjects. This discrepancy was thought to be due to experimental error, not variation in the characteristic behavior of the knee joint.

Data was taken from the two remaining curves and plotted on separated plots and curve fitted. Angular values in increments of 10 degrees were input into the equations so that a series of data points was obtained. The corresponding data points were averaged so that an average characteristic curve was found. This average curve contained a deadband zone between the angles of 35 degrees and 95 degrees when the curve was positioned so that the beginning of the curve started at 0 degrees. The center of the deadband zone was 65 degrees so this became the new origin. When describing the position of the aligned positioning of the joint axis system with the fixed body axis system, the upper arm, relative to the fixed body axis, the position will be 65 degrees about the y axis.

The curve was split into three sections, the curve prior to the deadband zone (defining extension of the joint), the deadband zone, and the curve after the deadband zone (defining flexion of the joint). The curve segment prior to the deadband zone was shifted horizontally so that the point that it has in common with the deadband zone is located at 0 degrees and 0 in-lb. This is done because the ATB program will subtract the corresponding stop angle from all input angles. The length of the deadband zone, where the resistant force is zero, has a length of 30 degrees on each side of the origin. Therefore, the stop angle for both extension and flexion will be 30 degrees.

The equations must define the motion as though the angle and the resistant force are zero at the beginning of the defined motion, at the end of the deadband zone. The curve was then rotated 180 degrees so that it would be located in the first quadrant. This was done because the ATB program also converts all the theta angles to positive values. Whether the theta angle is defining flexion or extension, the theta angle will be defined in the program as being positive. The program will note the difference by using the phi angle. If the theta angle is describing a flexion of the joint, the phi angle is equal to 0 degrees. If the theta angle is describing an extension position of the joint, the phi angle is equal to 180 degrees.

The rotated curve was then curve fit to the third order and the quadratic, cubic and linear coefficients were used in the ATB program. This data may be seen in Figure 8. The analysis of the second curve, the

flexion curve, was performed in the same way except a rotation of the curve was not necessary. The curve was already located in the first quadrant. Please note that the flexion curve was shifted so that the first point of the curve was located at 0 degrees. Four simulations were run using both sets of coefficients.

ANALYSIS OF THE ELBOW

The analysis of the elbow was done using exactly the same procedure that was used in the analysis of the knee. The characteristic curve of the third subject was disregarded during the analysis. The characteristic curve was very different than the curve of the other two subjects. This difference was accounted for through experimental error, not variation in the characteristic of the joint. Data was taken from the plots of the first two subjects and curve fitted. The curve fit equations were used to find an average characteristic curve. The deadband zone was located from 30 degrees to 90 degrees on the curve when the beginning of the curve was located at 0 degree. The new origin was then 60 degrees since this was the center of the deadband zone. This gave stop angles for both flexion and extension of 30 degrees. The flexion and extension curves were separated from the deadband zone. The extension curve was rotated 180 degrees so it would be located in the first quadrant. The curves were shifted so they began at 0 degrees and curve fitting was done separately on each curve to the third order. This data may be seen in Figure 8. The quadratic, cubic, and linear coefficients were put into the ATB program and four simulations were run.

ANALYSIS OF THE ANKLE JOINT

The analysis of the ankle joint was performed in the same manner as both the elbow and the knee joints. The data for all three subjects were used in this analysis. Data was taken from the subject plots and replotted. A curve fit was taken for each curve and the average curve was then found using the three equations. The analysis of the ankle joint was slightly different than the others in that the orientation of the ankle's joint axis system was positioned differently in the experiment than in the ATB program. The orientation of the ankle's joint axis system in the experiment was rotated 90 degrees, along the y axis, differently than the ankle's

joint axis system defined in the ATB program. A point in the deadband zone was shifted horizontally to 90 degrees. The stop angle for the extension position became 5 degrees and the stop angle for the flexion position became 15 degrees. The curves were separated from the deadband zone and the flexion curve was rotated 180 degrees. The two curves were then separately curve fit and the quadratic, cubic, and linear coefficients were used in the ATB program. This data may be seen in Figure 8. Four simulations were run to verify the data.

SUMMARY

The experimental data describing the characteristics of the resistant force and torsion of the shoulder, elbow, hip, knee, and ankle was presented in two different ways. Two different methods were used to obtain the experimental data in the form that was needed. Once values that were very close to the experimental data were obtained, the data was manipulated into an equation that could both describe the motion of the joint accurately and be used in the ATB program. The biggest difficulty in doing this was making sure the location of the reference axis systems used to obtain the data and their relationships to the axis systems used in the ATB program. Once these relationships were found, the data was put into the form of a third order equation, with the correct offsets. The equations were then ready to be used in the ATB model program.

HIP

SUBJECT 1

$$0.0016x^3 + 0.0092x^2 + 3.382x + 20.5$$

SUBJECT 2

$$0.00179x^3 + 0.042x^2 - 1.168x - 58.289$$

SUBJECT 3

$$0.001x^3 - 0.016x^2 + 2.24x - 31.99$$

CHARACTERISTIC EQUATION

$$0.00148x^3 + 0.00422x^2 + 1.448x$$

SHOULDER

SUBJECT 1

$$-0.0004x^3 - 0.0512x^2 - 1.34x + 17.51$$

SUBJECT 2

$$-0.00044x^3 - 0.044x^2 - 0.87x + 25.082$$

SUBJECT 3

$$-0.00279x^3 - 0.014x^2 - 0.3088x + 14.87$$

CHARACTERISTIC EQUATION

$$0.000392x^3 - 0.0005x^2 - 0.3456x$$

Figure 5

RIGHT HIP**STOP ANGLE (RAD./DEG.)**

<u>PHI = 0</u>	6.7266x ³ +544.0146x ² +861.34x	0.8882/ 50.89
<u>PHI = 30</u>	-6.1508x ³ +812.2637x ² +1138.5858x	0.6883/ 39.438
<u>PHI = 60</u>	-53.8655x ³ +897.4798x ² +1082.2875x	0.5281/ 30.258
<u>PHI = 90</u>	-94.0839x ³ +693.475x ² +929.901x	0.5255/ 30.1089
<u>PHI = 120</u>	-72.6071x ³ +390.166x ² +758.37x	0.70129/ 40.181
<u>PHI = 150</u>	-24.738x ³ +312.82x ² +716.474x	0.9735/ 55.778
<u>PHI = 180</u>	-6.7266x ³ +508.172x ² +858.641x	0.8879/ 50.876
<u>PHI = 210</u>	6.1508x ³ +836.306x ² +860.531x	0.6146/ 35.214
<u>PHI = 240</u>	53.866x ³ +1067.024x ² +829.960x	0.521/ 29.8557
<u>PHI = 270</u>	94.0839x ³ +988.7387x ² +680.9929x	0.5206/ 29.828
<u>PHI = 300</u>	72.6071x ³ +695.0266x ² +611.584x	0.6983/ 40.01
<u>PHI = 330</u>	24.738x ³ +452.0407x ² +569.458x	0.902/ 51.702

LEFT HIP**STOP ANGLE (RAD./DEG.)**

<u>PHI = 0</u>	6.7266x ³ +544.0146x ² +861.34x	0.8882/ 50.89
<u>PHI = 30</u>	24.738x ³ +452.0407x ² +569.458x	0.902/ 51.702
<u>PHI = 60</u>	72.6071x ³ +695.0266x ² +611.584x	0.6983/ 40.01
<u>PHI = 90</u>	94.0839x ³ +988.7387x ² +680.9929x	0.5206/ 29.828
<u>PHI = 120</u>	53.866x ³ +1067.024x ² +829.960x	0.521/ 29.8557
<u>PHI = 150</u>	6.1508x ³ +836.306x ² +860.531x	0.6146/ 35.214
<u>PHI = 180</u>	-6.7266x ³ +508.172x ² +858.641x	0.8879/ 50.876
<u>PHI = 210</u>	-24.738x ³ +312.82x ² +716.474x	0.9735/ 55.778
<u>PHI = 240</u>	-72.6071x ³ +390.166x ² +758.37x	0.70129/ 40.181
<u>PHI = 270</u>	-94.0839x ³ +693.475x ² +929.901x	0.5255/ 30.1089
<u>PHI = 300</u>	-53.8655x ³ +897.4798x ² +1082.2875x	0.5281/ 30.258
<u>PHI = 330</u>	-6.1508x ³ +812.2637x ² +1138.5858x	0.6883/ 39.438

Figure 6

KNEE

SUBJECT 1

Data was disregarded

SUBJECT 2

$$21.509x^4 + 27.743x^3 - 242x^2 + 330x - 184$$

SUBJECT 3

$$441x^5 - 2.54x^4 + 5790x^3 - 5770x^2 + 2350x - 354$$

EXTENSION

$$32.634x^3 + 329.82x^2 + 138.66x$$

FLEXION

$$462x^3 + 291x^2 + 107x$$

ELBOW

SUBJECT 1

$$4.51x^4 - 76.64x^3 + 199x^2 - 167x + 84.89$$

SUBJECT 2

$$-15.28x^6 + 46.09x^5 + 157x^4 - 839x^3 + 1220x^2 - 669x + 129$$

SUBJECT 3

Data was disregarded

EXTENSION

$$359x^3 + 112x^2 + 1.67x$$

FLEXION

$$366x^3 - 382x^2 + 197x$$

ANKLE

SUBJECT 1

$$-882x^4 - 286x^3 + 448x^2 - 128x - 11.137$$

SUBJECT 2

$$-2720x^5 + 68.01x^4 + 1150x^3 - 135x^2 - 252x + 9.73$$

SUBJECT 3

$$38.68x^4 - 153x^3 + 81.14x^2 - 143x + 1.186$$

EXTENSION

$$1532.03x^3 - 1069.86x^2 + 387.76x$$

FLEXION

$$5928.52x^3 - 3505.45x^2 + 740.07x$$

Figure 8

DEVELOPMENT OF NON-INVASIVE PROTOCOLS FOR INVESTIGATING
TROPHODYNAMICS OF DASYATID STINGRAYS IN ST. ANDREW SOUND

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Abstract

Dasyatid stingrays in St. Andrew Sound were studied over a 10 week period from 21 June - 28 August 93. Non-invasive methods were utilized in the removal of stomach contents from collected specimens. Rays were captured by one of three methods; otter trawl, casting net, and dip net. Capture by otter trawl was the most abusive collection method utilized. The casting net proved to be the least injurious method of collection. Four species of rays were collected: the Atlantic stingray, Dasyatis sabina, the southern stingray, Dasyatis americana, the roughtail stingray, Dasyatis centroura, and the smooth butterfly ray, Gymnura micrura. The spotted eagle ray, Aeteobatus narinari and the cownose ray, Rhinoptera bonasus were observed in the sound. D. sabina was the most common ray found, and was most often seen on sand flats either searching for food or lying on the bottom covered with sand. A method of gastric lavage was utilized in the removal of stomach contents from live rays after rays were anesthetized with MS-222. A 60 cc syringe was connected to a salem sump tube (stomach tube), filled with sea water, lubricated, and inserted through the mouth into the stomach. Water was rapidly pumped in and out of the stomach by pushing in and pulling out on the plunger of the syringe. A slurry of food and water was created in the stomach allowing food items to be effectively removed. Rays were revived and released uninjured. Several rays held in holding tanks for long term evaluation of this procedure were seen feeding on shrimp within one hour of the procedure being performed. On preliminary evaluation stomach samples were found to contain polychaetes, mysids, grass shrimp, and larval fish. These findings suggest that dasyatid stingrays are opportunistic feeders. Stomachs were removed from 13 D. sabinas for evaluation of this method. Forty-four percent of these stomachs were empty. Fifty-five percent of the stomachs contained some food items, and with one exception, no new food types were found.

DEVELOPMENT OF NON-INVASIVE PROTOCOLS FOR INVESTIGATING
TROPHODYNAMICS OF DASYATID STINGRAYS IN ST. ANDREW SOUND

Joyce Riesinger

INTRODUCTION

St. Andrew Sound is a shallow salt water lagoon located in the Florida Panhandle, just east of Panama City (fig. 1). The sound is characterized by a large seagrass meadow beginning at its mouth extending eastward. Seagrasses in the western end of the sound were lacking with sediments consisting mainly of mud and sand. The principal study site was located in the eastern end of the sound, in close proximity to the 9700 area of Tyndall Air Force Base (TAFB) (fig. 2). The waters of St. Andrew Sound are bordered by TAFB property and remain relatively undeveloped. Water depths varied from 0 m on shore to 5 meters in the channel at high tide. There was a narrow range of salinities from 32 to 36 ppt, and water temperatures ranged from 30 to 42 C. There is very little flushing in this system and fresh water influence is negligible, consisting of runoff from several small creeks.

Several species of rays were captured in St. Andrew Sound including the Atlantic stingray, Dasyatis sabina, the southern stingray, D. americana, the roughtail stingray, D. centroura, and the smooth butterfly ray, Gymnura micrura. Two other species of rays, the spotted eagle ray, Aetobatus narinari and the cownose ray, Rhinoptera bonasus were observed in the field. St. Andrew Sound is an important habitat for these rays in several ways. First it supplies the rays with several abundant food types such as shrimp, mysids, fish, and polychaetes. Second, this area serves as important pupping grounds for at least D. sabina. Two pregnant female sabinas captured with a casting net gave birth to two pups each approximately 20 minutes after being captured. Adults and young of the year were also observed in the field.

Dasyatid rays feed on polychaetes and other burrowing organisms such as shrimp, by digging in the mud, sand, or seagrasses. Water is pulled in through the spiracles and jetted out through the mouth (Gregory, 1979). This has the effect of a dredge and allows for effective capture of prey items. As water is jetted out of the mouth the pectoral fins begin to undulate so as to move dredge spoils away from the mouth of the ray. This type of activity leaves large depressions in the substrate, which are quite obvious at low tide. In a 14.6 meter square plot 50 feeding depressions were counted.

Limited feeding studies have been carried out with dasyatid rays in the past. Gilliam (1993) compared time of day and tidal phase in relation to food types and quantity. Southern stingrays were found feeding on lancelets in Tampa Bay (Stokes 1992). Turner (1982) examined the occurrence of ophiuroid discs in the stomachs of stingrays in the Indian River Lagoon system.

Previous to this investigation the accepted method of studying food types of stingrays was by killing the ray, dissecting the stomach and removing its contents. In such studies it is not uncommon to kill 100 or more rays, removing them from the population. The main objective of this study was to determine food types of stingrays using non-invasive techniques to obtain stomach contents from live rays so that they could be released uninjured. The Atlantic stingray, *D. sabina*, was the most abundant stingray captured in the sound, therefore this species was the one primarily studied.

MATERIALS AND METHODS

A total of 25 sting rays were captured alive during the ten week study period. Three methods of collection were used: dip nets, a 4 foot casting net, and a 10 foot otter trawl. Captured rays were placed in a large plastic transport box, measuring 61 x 41 x 30 cm for transport to the boat house where they were placed in holding tanks. Holding tanks consisted of a child's swimming pool measuring 6 feet by 15 inches deep. The pool was filled with sea water to a depth of 10 inches, holding approximately 160 gallons of water. Water was collected by buckets at the boat house and emptied into the holding tanks. An airstone powered by an aquarium pump was used to aerate the water. Approximately one half of the water (80 gallons) was changed daily. The maximum number of rays held in these tanks at one time was 14, but these were only held for several hours. For long-term holding, a maximum of 5 rays were held at one time whose disc widths varied between 18.9 and 25.7 cm.

Rays were anesthetized with tricaine methanesulfonate (MS-222), a common fish anesthetic that acts on skeletal muscle (Gilbert 1957). Solutions of MS-222 were mixed prior to capturing rays and allowed to aerate for 1 to 2 hours before use. Five gallons of MS-222 was mixed at a concentration of 1.25 gm of MS-222/5 gallons of sea water (0.07 gm MS-222/Liter of sea water). This was used as a bath in which rays were emersed. At this concentration anesthetization occurred within 1.5 to 2.5 minutes. Time required until optimum anesthetization was dependent on ray size. After a ray was anesthetized and removed from the bath of MS-222, it was placed on its dorsal surface and laid on a wet towel. A second wet towel was laid over the ray's ventral surface to prevent it from drying out. A 500 ml squeeze bottle containing a 1 gm/l solution of MS-222 was also prepared and aerated prior to use. If the effects of the MS-222 began to wear off, this stronger solution was sprayed over the gills to further anesthetize the ray.

The stomach pumping device consisted of a 60 cc syringe connected to a salem sump tube (stomach tubing). Two sizes of tubing were used, 14 and 18 French (FR). Tube size used was determined by the size of the ray's mouth and esophagus, and was selected by trial and error. The stomach tube was attached to the syringe, filled with sea water and lubricated with KY Jelly. The tube

was then inserted into the stomach. Prior to insertion, the distance from the ray's mouth to stomach was measured by placing the tube on the ventral surface at the mouth and extending the tip of the tubing to the point just above the cloaca (fig. 3). This point coincides with the distance from the mouth to the stomach. The tube was then marked at this length and used as a reference point during tube insertion. The tube could also be felt entering the stomach by pressing down gently on the ray's abdomen with the hand. Once the stomach tube was in place, water was quickly pumped in and out of the stomach by using the plunger of the syringe. This created a slurry of water and food in the stomach allowing food items to be pulled out of the stomach into the syringe. The syringe was disconnected from the tubing and its contents emptied into a collection bottle. Stomach contents were preserved in a 10% formalin solution and later transferred to 95% EtOH. This procedure of pumping water into and out of the stomach was repeated 7 to 10 times or until stomach contents were no longer obtained. Stomach pumping took approximately 5 to 7 minutes to perform. The longest a ray was anesthetized was 10 minutes. After completion of the procedure the ray was placed in a holding tank of fresh sea water to be revived. The ray was "swum" by holding on to its pectoral fins and moving it through the water slowly, so as to facilitate water movement over the gills. Within 5 minutes rays revived and were able to swim. Within 1 hour after the procedure rays were seen feeding on shrimp in the holding tanks. Rays were held overnight and released the next day.

To evaluate the effectiveness of the method, 13 D. sabina caught by otter trawl were killed by overdosing them with MS-222 after having their stomachs pumped. An individual's stomach was then removed and placed in a small vial which was filled with 10% formalin. The vial, along with an identification tag was then placed in the collection bottle that contained the stomach contents of that individual. Stomachs and stomach contents were latter transferred to 95% EtOH.

RESULTS AND DISCUSSION

By utilizing methods that were non-injurious gut contents were obtained from stingrays to determine their forage in St. Andrew Sound. Capture methods were evaluated to determine which were least stressful to rays. Of the three methods employed (dip net, casting net and otter trawl), the otter trawl was the most stressful and resulted in the highest mortality. This was the only collection method that can be directly attributed to ray deaths. Nearly all rays caught with the otter trawl died within 8 days of capture, with most dying 2 days after capture. This was attributed to the rays being battered while in the trawl. Even in short trawls of 5 minutes rays were injured. This method of collection was also very size and species selective. Only D. sabina with disc widths less than 26 cm were caught with the otter trawl while

rays much larger than this were caught using dip net and casting nets. Only two species of rays, D. sabina and G. micrura, were collected with the otter trawl. D. sabina, D. americana and, D. centroura were all collected with casting net and dip nets. The otter trawl was useful in collecting large numbers of small rays, but since this method of collection proved to be fatal to the rays it was only utilized when rays were to be killed for evaluation of the effectiveness of the stomach pumping technique. Trawls were pulled for 5 to 15 minutes, and their contents emptied into a plastic holding tank for sorting. Stingrays were transferred into a separate holding tank for transport to the boat house. Fish species were noted and released (table 1). These were compared to collections made in St. Andrew Sound by Collard (1992) (table 2). Several species of fish were found in ghost traps and are indicated in table 1. By comparing tables 1 and 2, a variation between fish species is seen. This could be due in part to the different collection methods employed between the two years. Also, the intensity of fish collections were somewhat higher last year than this year. This year's collections were made primarily on sand and mud bottoms, as opposed to last year when collections were also made in grass flats. Bony fish were not targeted this year and were the result of by-catch from stingray trawls.

The most effective methods of capturing rays unharmed was with the use of casting and dip nets. These methods allowed three species of rays, D. sabina, D. americana, and D. centroura, to be captured uninjured. All sizes of rays were captured using these two methods. Capture by cast net only allowed the collection of one individual at a time and was very time consuming due to the time required to locate one ray. Rays were located by walking the sand flats in water no deeper than 50 cm. The small size of the net made it ineffective at catching rays in water deeper than 50 cm. Once a ray was located it was "hit" with the casting net, the lead line tightened slightly and then worked under the ray so as to purse it from the rest of the net. The cast net and ray were then dip-netted and the ray placed in a plastic holding tank for transport to the boat house.

A dip net was also used to capture 2 rays. This seemed to be the least stressful method of capture but was only effective as the primary capturing device when rays were in seagrasses. Rays on sand flats easily avoided the dip net. Of the three collection techniques utilized the casting net was the most effective method of capture for the purpose of this study.

Rays were placed in holding tanks to evaluate the long-term effects of anesthetization and stomach pumping. Seven rays lived in the holding tanks, as controls, for up to 30 days until they were killed by myxosporidians. Prior to death, all rays were feeding well and appeared healthy. Necropsy of the rays showed no internal abnormalities, but upon examination of the gills there

rays showed no internal abnormalities, but upon examination of the gills there was found to be substantial numbers of myxosporidians. These parasites had the appearance of sand grains on the gill filaments and in most cases formed a thick coating on the gills. All necropsies appeared normal except for that of one D. centroura. The larger lobe of the liver of this individual was abnormal in that it contained many hollow, cloudy, globular like, lesions. Positive identification of these lesions has not yet been determined. Within the same liver was also found a steel fishing hook. The hook didn't appear to be bothersome to the ray. The two findings are thought to be unrelated nor the cause of death. It appears that this ray also died from myxosporidian parasites.

Two other species of rays observed in the field, but not captured, were the spotted eagle ray, Aetobatus narinari, and the cownose ray, Rhinoptera bonasus. Large numbers of R. bonasus have been reported to destroy extensive sea grass meadows in the Chesapeake Bay by uprooting seagrasses as a result of digging activities while in search of food items such as bivalves (Orth, 1975). This was not observed in St. Andrew Sound. Only solitary R. bonasus were observed.

The method of gastric lavage used to extract stomach contents from live rays was beneficial in that it allowed stomach contents to be removed and analyzed in a way that was non-injurious to the rays. This allowed for their release without injury, so as not to remove them permanently from the existing population. The insertion of tubing down the esophagus and into the stomach was easily facilitated without any resistance from the ray. No force was needed for tube insertion. It was quite obvious when too large a tube was inserted into the mouth because it wouldn't pass down the esophagus. No ray encountered was too small for stomach tube insertion. In most cases food items were obtained with the first or second stomach flushing (pushing water into and pulling water out of the stomach). The maximum number of stomach flushings performed on any individual ray was 12.

The MS-222 used to anesthetize the rays was very efficient and easy to work with. Due to the short amount of time required for anesthetization and revival, less stress was placed on the rays, which enhanced their chance of survival. It also allowed more examinations of rays per day.

Examination of collected food items revealed that rays had been feeding on polychaetes, mysids, grass shrimp, and in one case larval fish. Of 18 stomachs pumped, food was obtained from 78% (table 3). Thirty nine percent of the stomachs contained polychaetes which were the food item most often seen. Five percent of the stomachs contained mixtures of mysids and larval fish or polychaetes, mysids and grass shrimp. These two groups represented the smallest percentage of food items seen. No correlation was observed between

disc width, mouth size, and food types but this was most likely due to the small sample size and to the fact that most rays were of similar size. But there should be a relationship between these 3 parameters; The smaller the ray, the smaller the mouth size, the smaller the prey item, and vice versa. No measurements were made on mouth sizes, so no definite connection can be made between the three factors. Relationships between mouth size and food types have been examined between the lesser electric ray, Narcine brasiliensis and D. sabina (Funicelli 1975).

It became obvious, even with such a small sample size, that dasyatid stingrays are opportunistic feeders on a variety of food types (Funicelli, 1975; Gilliam, 1993; Hess, 1961; and Stokes, 1992). More-in depth studies of feeding behaviors in relation to mouth size and prey selection, seasonal variations in prey types, and selection of prey between different species of rays will need to be examined in the future.

Nine of the 11 D. sabina stomachs collected were dissected to determine the efficiency of the gastric lavage performed. Two of the 11 stomachs didn't preserve properly and were discarded. Forty-four percent of the stomachs examined were completely empty. Fifty-five percent of the stomachs contained small amounts of food. Of the stomachs which still contained food items no new food types were removed with the exception of one stomach which contained a large shrimp that had been torn into three relatively equal pieces. This shrimp was too large to pass through the stomach tube. No nicks, tears, or other injuries were seen in the stomachs that could have been attributed to this procedure.

During future studies larger diameter stomach tubes will have to be used for the removal of larger food items. After further refinement, this non-invasive method should prove to be a beneficial method for the removal of stomach contents from live dasyatid stingrays.

Figure 1. Map of St. Andrew Sound.

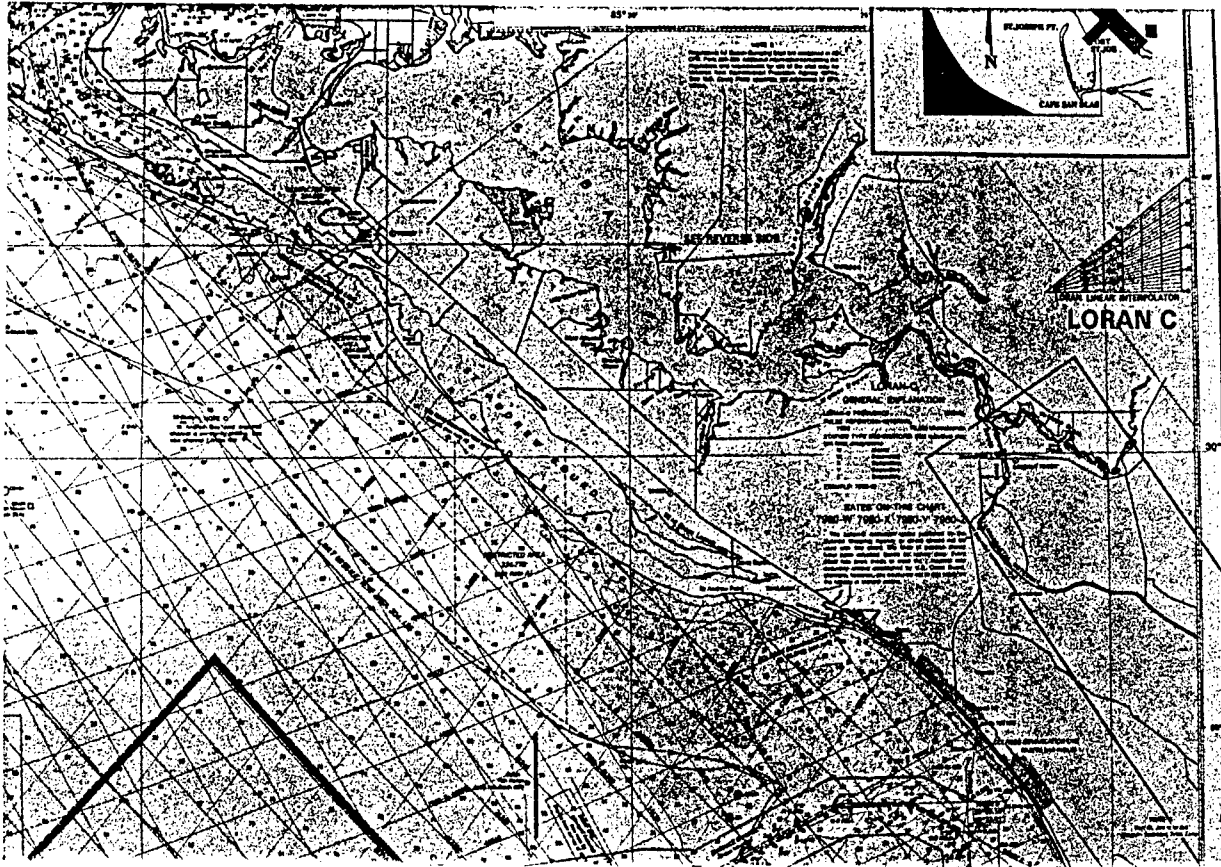


Figure 2. Aerial photo of St. Andrew Sound and Raffield Peninsula.

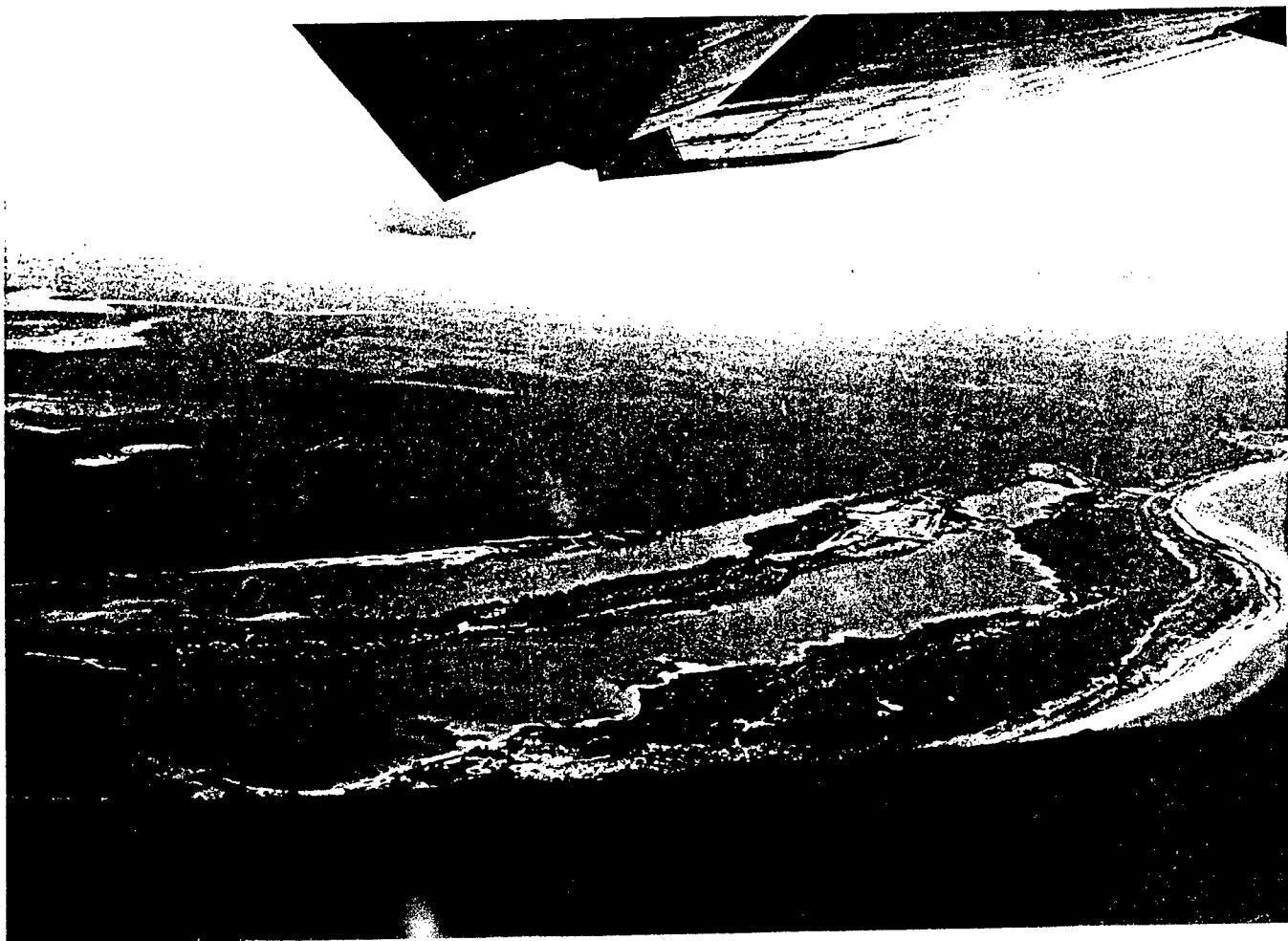


Figure 3. Distance from mouth to stomach.

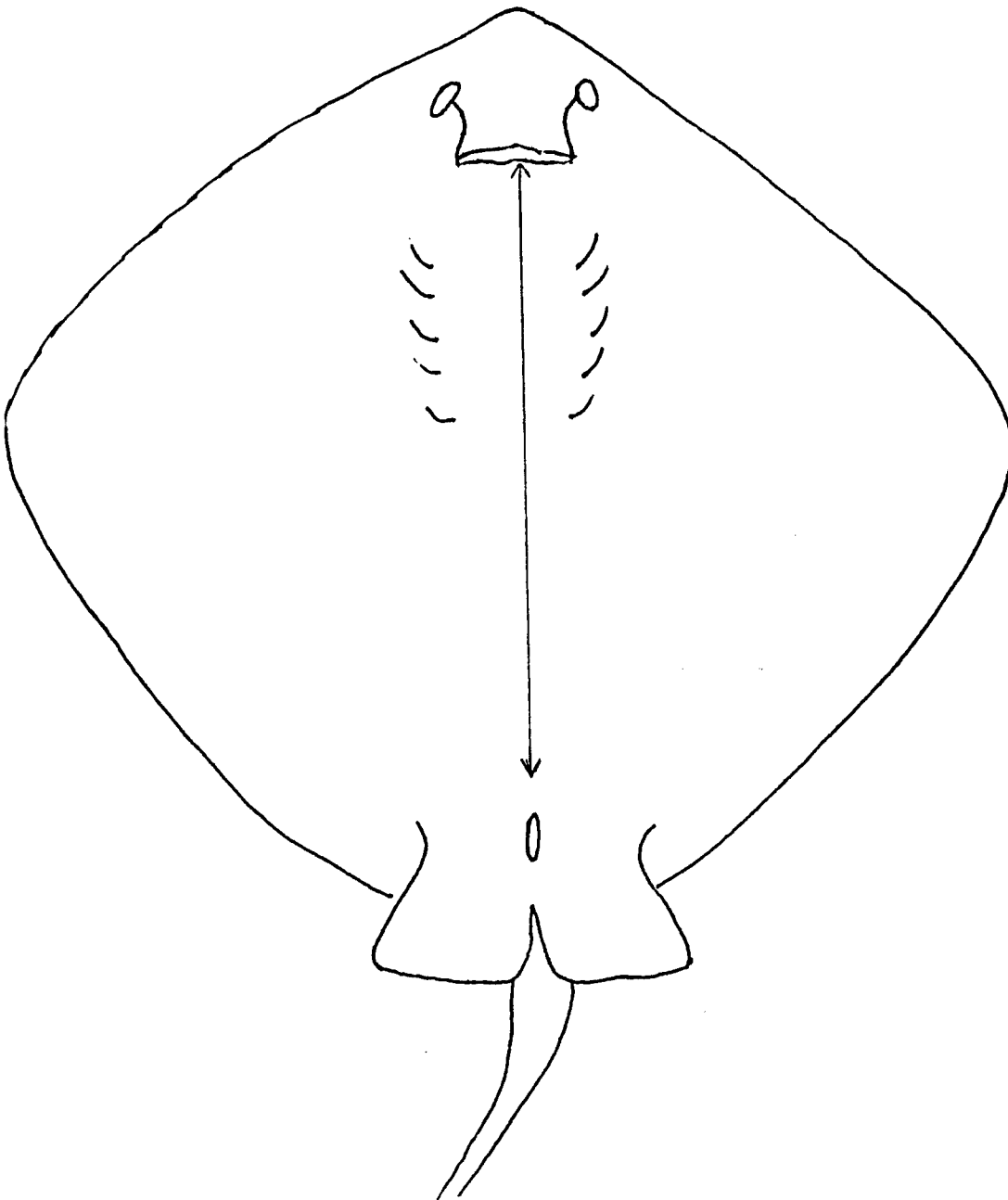


TABLE 1. FISHES COLLECTED IN CROOKED ISLAND SOUND WITH OTTER TRAWL

<u>SPECIES</u>	<u>COMMON NAME</u>
*1. <u>Lutjanus griseus</u>	grey snapper
*2. <u>Mycteroperca microlepis</u>	gag (OT; J)
3. <u>Chaetodipterus faber</u>	Atlantic spadefish (DN, OT; J, SA)
*4. <u>Paralichthys albigutta</u>	Gulf flounder (OT)
*5. <u>Lucania parva</u>	rainwater killifish (DN)
*6. <u>Sphoeroides nephelus</u>	southern puffer (OT)
*7. <u>Opsanus beta</u>	Gulf toadfish (OT, DN)
*8. <u>Diplectrum formosum</u>	sand perch (OT)
*9. <u>Cynoscion nebulosus</u>	spotted seatrout (DN; J)
10. <u>Oligoplites saurus</u>	leatherjacket (DN; J)
11. <u>Rhinoptera bonasus</u>	cownose ray (Ob)
12. <u>Cyprinodon variegatus</u>	sheepshead minnow (DN; A, J)
13. <u>Menidia beryllina</u>	tidewater silverside (BS; A, J)
14. <u>Trachinotus carolinus</u>	Florida pompano (BS; J)
*15. <u>Bairdiella chrysoura</u>	silver perch (OT, CN)
16. <u>Leiostomus xanthurus</u>	spot (OT; A)
*17. <u>Hippocampus zosterae</u>	dwarf seahorse (DN; A)
18. <u>Arius felis</u>	sea catfish (OT)
19. <u>Sparisoma radiones</u>	bucktooth parrotfish (OT; A)
20. <u>Adinia xenica</u>	diamond killifish (CN, DN; A)
21. <u>Poecilia latipinna</u>	sailfin molly (DN)
22. <u>Fundulus similis</u>	longnose killifish (CN, DN)
23. <u>Fundulus grandis</u>	Gulf killifish (DN)
24. <u>Diplodus holbrooki</u>	spottail pinfish (Ob)
25. <u>Selene vomer</u>	lookdown (DN)
*26. <u>Aluterus scriptus</u>	scrawled filefish (DN)
27. <u>Lactophrys quadricornis</u>	scrawled cowfish (DN)
28. <u>Centropristis striata</u>	black sea bass (TP)
29. <u>Dasyatis centroura</u>	rougtail stingray (CN, DN)
30. <u>Aeteobatus narinari</u>	spotted eagle ray (Ob)
31. <u>Strongylura marina</u>	Atlantic needle fish (Ob)
*32. <u>Acanthostracion quadricornis</u>	scrawled cowfish (Ob)
*33. <u>Chilomycterus schoepfi</u>	striped burrfish (OT; A)
*34. <u>Mugil cephalus</u>	striped mullet (CN; A, J)
35. <u>Orthopristis chrysoptera</u>	pigfish (OT; A, J)
*36. <u>Synodus foetens</u>	inshore lizardfish (OT)
*37. <u>Harengula jaguana</u>	scaled sardine (CN; A)
*38. <u>Lutjanus synagris</u>	Lane snapper (Ob; J)
*39. <u>Dasyatis americana</u>	southern stingray (CN, DN)
*40. <u>Gymnura micrura</u>	smooth butterfly ray (OT)
*41. <u>Lagodon rhomboides</u>	pinfish (OT; A, J)
*42. <u>Sciaenops ocellata</u>	red drum (OT; J)
*43. <u>Dasyatis sabina</u>	Atlantic stingray (CN, DN, OT; A, J)
*44. <u>Rachycentron canadum</u>	cobia (OT; J)
*45. <u>Micropogon undulatus</u>	Atlantic croaker (OT; A)

*= species seen both years. BS= beach seine; CN= casting net; DN= dip net; OT= otter trawl; TR= trap; Ob= observed. J= juvenile; A= adult; SA= sub-adult.

TABLE 2
FISHES COLLECTED IN CROOKED ISLAND SOUND WITH TRY NET AND CRAB SCRAPE

<u>SPECIES</u>	<u>COMMON NAME</u>
1.* <u>Lutjanus griseus</u>	grey snapper (early juveniles) (c) C/F
2.* <u>Mycteroperca microlepis</u>	gag (1)
3. <u>Archosargus probatocephalus</u>	sheephead (2 adults)
4. <u>Paralichthys albigutta</u>	Gulf flounder (c)
5.* <u>Lucania parva</u>	rainwater killifish (vc) Z/E
6. <u>Sphoeroides nephelus</u>	southern puffer (f)
7.* <u>Opsanus beta</u>	Gulf toadfish (vc)
8. <u>Diplectrum formosum</u>	sand perch (f)
9.* <u>Cynoscion nebulosus</u>	spotted seatrout (fc) C/F
10. <u>Ancylopsetta quadrocellata</u>	ocellated flounder (fc)
11. <u>Achirus lineatus</u>	lined sole (1)
12. <u>Paralichthys squamilentus</u>	broad flounder (r)
13. <u>Trinectes maculatus</u>	hogchoker (f)
14. <u>Symphurus plagiosa</u>	blackcheek tonguefish (fc)
15. <u>Bairdiella chrysoura</u>	silver perch (c) C/M
16. <u>Cynoscion arenarius</u>	sand seatrout (f)
17.* <u>Hippocampus zosterae</u>	dwarf seahorse (vc)
18.* <u>Hippocampus erectus</u>	lined seahorse (1) Z/E
19.* <u>Syngnathus springeri</u>	bull pipefish (c)
20. <u>Anarchopterus criniger</u>	fringed pipefish (r)
21.* <u>Syngnathus scovelli</u>	Gulf pipefish (c) Z/E
22.* <u>Syngnathus louisianae</u>	chain pipefish (c)
23.* <u>Syngnathus floridae</u>	dusky pipefish (fc)
24. <u>Myrophis punctatus</u>	speckled worm eel (1)
25. <u>Monacanthus hispidus</u>	planehead filefish (r)
26. <u>Aluterus scriptus</u>	scrawled filefish (r)
27. <u>Gobiosoma robustum</u>	code goby (c) Z/E
28. <u>Gobionellus hastatus</u>	sharptail goby (fc)
29. <u>Chasmodes saburrae</u>	Florida blenny (fc)
30. <u>Hypsoblennius hentzi</u>	feather blenny (fc)
31. <u>Ophioblennius atlanticus</u>	redlip blenny (f)
32. <u>Acanthostracion quadricornis</u>	scrawled cowfish (f)
33. <u>Chilomycterus schoepfi</u>	striped burrfish (f)
34. <u>Pomatomus saltatrix</u>	bluefish (c) (gill net)
35. <u>Mugil cephalus</u>	striped mullet (vc) OD
36. <u>Orthopristis chrysoptera</u>	pigfish (vc)
37. <u>Synodus foetans</u>	inshore lizardfish (fc)
38. <u>Sardinella anchovia</u>	Spanish sardine (r)
39. <u>Harengula jaguana</u>	scaled sardine (c)
40. <u>Lutjanus synagris</u>	Lane snapper (f) juveniles
41.* <u>Eucinostomus argenteus</u>	spotfin mojarra (f)
42. <u>Dasyatis americana</u>	southern stingray (vc) (dip net)
43. <u>Gymnura micrura</u>	smooth butterfly ray (f) (seen)
44.* <u>Menidia peninsulae</u>	tidewater silverside (vc) (dip net)
45.* <u>Lagodon rhomboides</u>	pinfish (vc) OD
46. <u>Mugil curema</u>	white mullet (c) OD (gill net)
47. <u>Sciaenops ocellatus</u>	red drum (f) (gill net)
48. <u>Scomberomerus maculatus</u>	Spanish mackerel (f) (gill net)
49. <u>Eucinostomus gula</u>	silver jenny (c)
50. <u>Dasyatis sabina</u>	Atlantic stingray (f?)
51. <u>Rachycentron canadum</u>	cobia (ling) (1) (hook and line)
52. <u>Micropogon undulatus</u>	Atlantic croaker (c?) (hook and line)
53. <u>Carcharhinus limbatus</u>	blacktip shark (3) (hook and line)
54. <u>Sphyrna barracuda</u>	great barracuda (r?) (juvenile)
55. <u>Gobiidae</u>	3 unidentified species (c)
56. <u>Sciaenidae</u>	1 unidentified species (r)
57. <u>Gobiesox</u> sp.	damaged-in aluminum can

[vc = very common; c = common; fc = fairly common; f = few; r = rare (1-3)]
 [H = herbivore; OD = omnivore-detritivore; Z/E = zooplanktivore/epifaunal
 invertebrates; C/M = carnivore/micro-macro invertebrates; C/F = carnivore/
 fishes and macrocrustaceans (Gilmore, 1987, Table 4)] [* SAV-dependent]

Note: Ogren and Brusher (1977) identified 128 species of fishes from bi-weekly day and night trawl hauls at 12 stations in the St. Andrew Bay system during the period September 1972 through August 1973. Day-only collections at the study site in CIS were made on 30 July, 7 August, and 11 August using a 2 m try net and a 1 m crab scrape. On 11 August the trawl was towed through grass beds by hand. Gill net and hook and line records were personally observed by the author.

TABLE 3
 ANALYSIS OF STINGRAY STOMACH CONTENTS OBTAINED BY GASTRIC LAVAGE

Stomach contents	% of total	# of rays	Disc widths	avg. DW
Polychaetes	39	7	20.5-25.7	22.9
Polychaetes and Mysids	28	5	18.5-26.5	22.1
Mysids and Larval fish	5	1	18.9	18.9
Mysids, polychaetes, shrimp	5	1	---	---
Empty stomachs	22	4	---	---
Total	99	18	---	---

Note: Disc widths (DW) given in cm.

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**DETERMINATION OF THE REDOX CAPACITY OF SOIL SEDIMENT
BY SPECTROELECTROCHEMICAL COULOMETRIC TITRATION**

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Abstract

The oxidative redox capacity was determined for size-fractionated soil sediment samples by the method of spectroelectrochemical coulometric titration. This method involves the measurement of absorbance of sediment particle slurries at the wavelength absorption maxima of the optically detectable mediator-titrant (reporter) molecules resorufin and methyl viologen as a function of the charge passed in a constant-potential coulometric titration. Titrations were carried out on diluted samples of gravitationally sedimented particle fractions containing particles smaller than 2 micrometers average diameter. The fraction containing particles of size < 2 micrometers was 0.115 % by weight of the initial sample slurry, which was 4.3 % solids by weight. The total organic content of the < 2 micrometer solids was 3.5 % organic carbon by weight. Titration was carried out at a diluted sediment particle concentration of 0.0128 % by weight. Resorufin was reduced first, followed by an irreversibly reducible sediment component which was consistently observed to titrate between resorufin and methyl viologen, and finally methyl viologen. The reducible component, which was absent from titration blanks, was not reoxidized when the methyl viologen and resorufin were electrochemically reoxidized. The sediment fraction studied had an oxidative redox capacity of 15 ± 2.5 millicoulombs, corresponding to 0.65 meq/gram per gram of sediment. The heterogeneity of the original sample was evidenced by the observation that the whole sediment slurry became reducing, whereas the fractionated < 2 micrometer particle slurry remained oxidizing.

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Introduction

The redox capacity of a sediment is a measure of the number of electron equivalents which it may donate or accept to reduce or oxidize a substance. This has become a topic of interest recently because of the large number of groundwater and aquifer sites which have been contaminated by pollutants. There is a growing need to clean up and prevent further contamination in such sites. The redox capacity is considered because of the possibility of biological or chemical degradation of pollutants by the sediment(1). Barcelona and Holm claim that "oxidation-reduction processes were mediated by natural microbial populations that catalyze electron-transfer reactions"(1). While both biological and chemical processes appear to be involved in sediment redox processes, evidence suggests that many of these processes are catalytic. Relatively large concentrations of electron donors and acceptors may be present, but their reactions with the pollutant species are sluggish relative to the catalytically active species. In this manner, the catalytic species can be recycled between reduced and oxidized states many times, until the supply of electron donors or acceptors is exhausted. The extent of possible cleanup or other transformation of the pollutant is limited by the overall redox capacity, which determines how many times the catalytic species can be turned over. If the sediment is capable of naturally degrading the pollutant, the following questions then arise. 1) Is it a biological or chemical process? 2) If it is biological, what organisms are involved? 3) Is the sediment a reducing or oxidizing agent? and 4) How much can it reduce or oxidize? Of course all of these questions cannot be answered by one experiment, but by studying the redox capacity, the last two questions can be answered, and give a good indication of the course to take to answer the others. We present here the results of a study of the oxidative redox capacity of a size-fractionated sediment sample.

Methodology

The method of determining the redox capacity in this study was by spectroelectrochemical coulometric titration. This method is an attractive one because low micromolar concentrations of spectrally visible and invisible species can be studied, and reliable quantitative data can be obtained(2). In a conventional chemical titration, acids or bases are used as the titrant. In an electrochemical titration, electrons (measured by the charge passed) are used as the titrant. In this experiment, charge was added

ed increments at a specific applied potential for both reduction and oxidation steps. The reduction potential was -0.700V vs. a Ag/AgCl/1M KCl reference electrode with SnO₂ as the working electrode, and the oxidation potential was +1.25V with Pt wire as the working electrode. Charge increments ranged from 0.25 millicoulombs to 5 millicoulombs.

The progress of the titration was monitored spectrophotometrically after each addition of charge. Two mediator titrants were used to help monitor the progress of the titration. Resorufin (concentration 10 micromolar) was used primarily as a reporter molecule, and methyl viologen (concentration 0.5 millimolar) was also used as a primary titrant to drive the reaction. The solutions of resorufin and methyl viologen were prepared in Milli-Q deionized water and a pH 7 buffer solution of KH₂PO₄ and Na₂HPO₄ (ionic strength 0.1). The buffer also served as an electrolyte, and a pH of 7 was chosen to maintain a neutral solution. Both of these titrants have different colors in their oxidized and reduced states. Resorufin is pink in the oxidized state and colorless in the reduced state, while methyl viologen is colorless in the oxidized state and a deep blue in the reduced state. Because both of these mediators can be spectrally detected, the absorbances of these substances can be studied during the titration. Resorufin exhibits an absorbance peak at 572 nm, and methyl viologen exhibits two major absorbance peaks at 396 and 600 nm. The titration began with both species in the oxidized state. The negative applied potential of -0.700V was applied, and the reduction phase of the titration was begun. Once the reduction was complete (when the absorbance peak of resorufin is extinguished, and the peaks for the viologen appear), the applied potential was changed to +1.25 V and the oxidation begun. Oxidation was complete when the viologen peaks were extinguished and the resorufin peak appeared and was restored to its initial amplitude.

Examples of spectra obtained during these titrations can be seen in Figures 1 and 2. Figure 1 represents the reduction step and Fig. 2 represents the oxidation step. "Blank" titrations involving only the resorufin and viologen (no sediment) were performed in order to determine the charge taken up by the resorufin and viologen, and to compare the titrations with and without sediment. The treatment of the sediment before the titration is explained in the following paragraph.

The sediment was collected from the Beaver Dam site in Athens, GA. The samples were collected in Mason jars, wet-sieved in air with the surface pond water by a 1 mm mesh brass sieve to remove large debris particles, and stored in sealed Mason jars. The sample to be studied was placed in an anaerobic glove box under N₂, CO₂, H₂ atmosphere where it was then fractionated by gravitational sedimentation to allow for the collection of particles smaller than 2 micrometers in diameter. The fractionation method was adopted from "Methods of Soil Analysis" according to the equation $t = 18 \eta h / [g (\rho_s - \rho_f) x^2]$ where t = time, η = viscosity (poise, g cm⁻¹ s⁻¹), h = height (cm), g = 980 cm/s² (acceleration due to gravity), ρ_s = 2.6 g/cm³, ρ_f = 1.0 g/cm³ (density of sediment particles and the aqueous medium), x = effective

particle diameter of the largest particle to clear (3,4). The settling time t was the variable solved in the equation in order to determine how long the sediment should settle to retain only particles of size < 2 micrometer in the top 10 cm of the jar. This time was determined to be 8.73 hr.

Sample containers were kept closed except during transfer. After the fractionation, samples were collected from the top 10 cm of the jar by siphoning and placed into three smaller jars which were crimped with a butyl rubber stopper and an aluminum cap. Samples were stored in the glove box until ready for use. Final solution preparation was also carried out in the glovebox. For the titration, sediment samples from these smaller jars were diluted with Milli-Q water in a 1:9 sediment to water ratio. This ratio was chosen due to the extensive light scattering of the sediment particles. The diluted sediment was mixed in a 1:1 ratio with the resorufin and methyl viologen solution described earlier, transferred anaerobically to a degassing bulb and the cell on a nitrogen/vacuum train outside the glove box, degassed, and titrated.

The total organic carbon (TOC) content of < 2 micrometer sediment slurries was determined using a Shimadzu TOC-5000 Analyzer and ASI-5000 Autosampler. A TOC calibration curve was obtained with KHP standards before the sediment samples were determined. Samples included with three < 2 micrometer fractionated sediment samples were a Milli-Q water sample and a known 50 ppm total carbon sample of KHP. The water sample showed a total organic carbon value of 0 ppm, the 50 ppm KHP sample showed a total carbon value of 54.86 ppm, and the three sediment samples showed values of 44.73 ppm, 42.77 ppm, and 42.49 ppm (average value of 43.33 ± 1.2 ppm) for the sediment samples corresponding to 3.5% of organic content referred to dry sediment weight.

The weight percent of solids was found to be 4.3% for the initial sediment slurry, and 0.115% for the fraction with particle size < 2 micrometers. These values were determined by measuring the mass of a 5 mL aliquot of undiluted sediment, placed in a previously weighed container, before and after overnight drying in an oven at 100 °C.

Samples were thoroughly degassed before the titration (to eliminate O_2) and to keep the solution as anaerobic as possible. This procedure will be explained in explicit detail in the apparatus section, because the procedure involves an explanation of the apparatus first.

Apparatus

The most important piece of apparatus is the vessel in which the titration takes place: the electrochemical cell. A diagram of the cell is shown as Figure 3(2). The main body and the electrodes are composed of Pyrex glass. The Pyrex valve at the top of the cell allows for filling and for closure after the cell has been

filled with solution. The main chamber of the cell excluding the liquid in the sidearms, and at the tip of the valve holds 1.864 ± 0.0016 mL of solution. The reference electrode holds 0.2712 ± 0.0003 mL, and the auxiliary electrode holds 0.4547 ± 0.0017 mL of solution. The magnetic stirrer is a ca. 7 mm long piece of steel paper clip encased in Pyrex glass and flame-sealed under vacuum. The Pt potentiometric electrode is fused inside the cell in order to serve as a potentiometric or working electrode; this electrode was used as the working electrode for the oxidation steps of the titration. The sidearms that house the electrodes are also composed of Pyrex and incorporate porous frits that connect to the main body of the cell. The working electrode for the reduction steps is a 2.5 cm square piece of SnO_2 glass that has been epoxied to the bottom of the main chamber of the cell with Devcon 2-Ton clear epoxy. The working electrode is also used to align the cell in a positioning recess in the optical train of the spectrophotometer. The reference and auxiliary electrodes are both made of Pyrex glass and join to the sidearms of the cell by ground-glass fittings (size 10/30). The electrodes are filled with 1M KCl solution and a Ag wire anodized in 6 M HCl to form a AgCl coating is inserted through a septum cap at the top of each electrode compartment to make the Ag/AgCl electrode. Solution contact is made via porous Vycor frits epoxy-sealed into the ends of the 10/30 joints. Light was passed through the main chamber of the cell to determine the absorbance values. Because the cell is composed of Pyrex, light is detected mostly in the visible region of the spectrum.

A schematic of the overall apparatus is shown as Figure 4. The electrochemical cell previously described was placed in the spectrophotometer on top of a water driven magnetic stirrer and a plexiglass platform that was designed to clamp the working electrode of the cell into place in order for the light beam to pass through the main body of the cell. The water was circulated through a constant-temperature bath to help thermostat the cell. A momentary contact switch connected a high-impedance digital voltmeter to the cell when the potentials of the working and potentiometric electrodes were periodically measured during the course of the titration. The spectrophotometer was a Perkin-Elmer Lambda Array 3840 UV/VIS spectrophotometer and was interfaced to a Perkin-Elmer 7000 Series computer. Spectral data were converted to ASCII format and transferred to a Sun Sparcstation for data manipulation using MATLAB software. The cell was also attached to a BAS-CV1B potentiostat which was responsible for applying the potentials during the oxidation and reduction steps. In order to determine the charge applied in each step, an absolute value amplifier converted the potentiostat current output to a positive value and fed it to a voltage to frequency converter. This conversion device was constructed in the laboratory of the investigators (2). In order to count the frequency pulses, the converter was connected to an events counter which allowed the frequency to be converted to counts of charge in millicoulombs with a conversion factor of 100,000 counts per millicoulomb (Data Precision Model 5740).

The degassing procedure is described in the following paragraph. The electrochemical cell was attached by a ground glass fitting to a cell adapter which was then attached to the degassing assembly. The degassing assembly consisted of a Ridox catalyst, a nitrogen gas line, a water-filled bubbler to saturate the gas with water, and a vacuum line trapped with liquid nitrogen. The cell could be either pressurized with nitrogen or evacuated via a two-way valve on the degassing assembly. An O_2 trap was placed on the copper-tubing gas line between the nitrogen cylinder and the degassing assembly as an extra precaution against leakage. All pieces of the degassing assembly were joined by ground glass fittings greased with Apiezon N. The two-way valve was connected to a vacuum system which consisted of a liquid nitrogen trap, a vacuum flask, and a mechanical vacuum pump. These were connected by butyl rubber tubing. Liquid nitrogen was placed in the trap and the valves and pump turned on. The two-way valve was switched to evacuate the solution degassing bulb/cell adapter and the cell for at least 15 min. The cell was then purged alternately with N_2 and the vacuum again for five cycles of 1-2 min. per cycle. The cell was then left under N_2 , purged for 2 min. and the cell stopcock closed off. The degassing bulb was placed on vacuum for 2 min. and closed off as well. A previously prepared solution of 1M KCl was degassed for 15 min. by passing N_2 from the degassing assembly through a ground glass adapter made for that purpose. Both the reference and auxiliary electrodes were degassed via a needle inserted through the septum cap while attached to the cell using this same adapter. They were evacuated and filled with nitrogen alternately for 2 min. for 5 cycles. After the electrodes had been degassed, they were filled with the KCl solution by a 1 mL microliter syringe. If a bubble appeared in the electrode, the electrode was emptied by the vacuum side and taken again through the procedure just described. Once the electrodes were filled, the solution to be studied was introduced into the degassing bulb. The amount of solution placed in the degassing bulb was 5.46 mL (half being diluted sediment, the other half being the resorufin/viologen solution). Because the degassing bulb had been placed on vacuum before closing it off, it was possible to place the solution in the side arm of the degassing bulb, and open the valve in air to transfer the solution into the bulb. The valve on the degassing bulb was then closed and connected once again to the two-way valve and the cell. A magnetic stirrer placed beneath the degassing bulb activated a magnetic stirrer in the latter's solution chamber to facilitate degassing. The two-way valve of the degassing assembly was then placed on vacuum for 10 min. and the solution was degassed before putting it into the cell. To put the solution into the cell, the valve between the degassing bulb and the cell was opened and the stirrer removed from beneath the bulb. The cell and degassing bulb were tilted downward and the cell was filled by purging both units with N_2 . To minimize bubble formation, the vacuum was applied for 5 sec. and the apparatus purged with N_2 again. This procedure was continued until no bubbles were visible inside the cell.

Results/Discussion

After the titrations were performed, plots were constructed using the absorbance data for peaks at 572 nm and 396 nm versus the total charge needed for the completion of the titration. Examples of these plots are shown as Figs. 5-8. The plot of the absorbance at 572 nm versus the total charge represents the titration of the resorufin while the plot of absorbance at 396 nm represents the titration of the methyl viologen. Figure 5 represents the reduction of a "blank" (resorufin and methyl viologen). The applied potential was -0.700V and the working electrode was SnO₂. This plot shows that it took up 13.5 millicoulombs of charge before the absorbance of resorufin was extinguished. A significant fraction of this charge appears to be due to residual oxygen. This plot also shows that once the resorufin was reduced, the viologen began to reduce with no significant break in between the two. Figure 6 represents the oxidation of the same "blank" solution. Here, the Pt wire was used as the working electrode with an applied potential of +1.25V. This plot shows that it took approximately 1.75 millicoulombs to oxidize the methyl viologen, but once the viologen was oxidized, the absorbance for resorufin began increasing. Figures 7 and 8 represent the titration of the sediment and the mediators. Fig. 7 represents the reduction step and Fig. 8 represents the oxidation step. The reduction plot of the sediment looks very different from the reduction plot of the blank. This suggests that something in the sediment is being reduced. In the reduction of the sediment, approximately 15 millicoulombs is taken up in the beginning of the titration. This can be explained by residual O₂ which most likely occurred as a result of insufficient degassing. A similar quantity of charge was observed for the blank. It can also be seen, however, that the charge consumed during reduction of resorufin is increased relative to the blank, and 15 millicoulombs is taken up between the reduction of resorufin and the reduction of methyl viologen. This significant charge uptake between titration of resorufin and methyl viologen is attributable to a component of the sediment, since this region is absent from a blank titration plot. Based on the relatively sharp transitions of the plots at the end of the titration of resorufin [$E^\circ = -40\text{mV}$ vs. normal hydrogen electrode (NHE) at pH 7], and the beginning of the titration of methyl viologen ($E^\circ = -446\text{ mV}$ vs. NHE), it appears that the nominal reduction potential of the titrable sediment component lies approximately between these values. The oxidation of the sediment looks much like the oxidation of the "blank". 1.75 millicoulombs were used to oxidize the viologen, and at that point, the peak for resorufin began to appear. This implies that the component in the sediment that was reduced was irreversibly reduced. To reinforce this idea, a second reduction was performed on the sample after the oxidation. This reduction step proved to look just like the reduction step for the "blank". Therefore, whatever component was reduced in the sediment was reduced only once and did not oxidize or rereduce. Figures 9-13 are three-dimensional representations of the data of Figs. 5-8.

The three dimensional plots were constructed to see the relationship between the three variables used for the titrations (absorbance, wavelength (nm), and charge (mcoul)). In Fig. 9 (reduction of the blank), the peak at 572 nm is reduced with the addition of charge. Once this peak is completely reduced, the peaks at 396 and 600 nm begin to appear representing the viologen. In comparison, Fig. 11 (reduction of the sediment) looks different because there is a space of about 15 millicoulombs between the reduction of the resorufin and the appearance of the viologen. Figures 10, 12, and 13, corresponding to oxidation of the blank, oxidation of the sediment, and rereduction of the sediment respectively, exhibit behavior analogous to the blank of the reduction. This implies that very little or no charge was taken up by the sediment during the oxidation or rereduction process.

A preliminary examination of the heterogeneity of the sample was carried out in an anaerobic glove box by adding an aliquot of the resorufin/methyl viologen/pH7 phosphate buffer medium to an aliquot of the original sediment slurry after storage for several weeks following removal of the < 2 micrometer particle fraction. The volumetric proportions were comparable to those previously used for the < 2 micrometer fraction. The resorufin was rapidly decolorized in the whole sediment fraction, whereas the resorufin retained its pink color when added under identical conditions to the < 2 micrometer fraction. Numerous factors may contribute to this observation. First, the total content of solids of the whole sediment was much greater than the solids content of the < 2 micrometer fraction. However, it is possible that the active species in the sediment may preferentially accumulate in particles of certain size. Further studies are being initiated to assess the origin of this particle size-based differentiation.

Conclusions

In the studies of the redox capacity of the < 2 micrometer particle size fraction of Beaver Dam sediment by spectroelectrochemical titration, it was determined that the sediment had an oxidative redox capacity of 15 ± 2.5 millicoulombs for a sample that was 0.118 % solids by weight in the sample diluted to final concentration in a titration volume of 1.864 mL. It was not a reversible process because the component could not be oxidized or rereduced. Barcelona and Holm reported in their study that oxidative capacities averaged on the order of 0.3 to 0.4 mequiv/g of solids(1). The results in this report indicate an oxidative redox capacity of the < 2 micrometer particle sediment to be 0.65 mequiv/g of solids. The differences in these two numbers possibly arise from different types of sediment studied. Barcelona and Holm worked with aquifer material which was very low in organic carbon content (ca. 0.1 % by weight), while this study was done on pond material with a higher organic carbon content (ca. 3.5% by weight). This relatively high value of 0.65 mequiv/g indicates a considerable capacity of sediment material to drive

oxidative reactions. Although much work remains, the spectroelectrochemical titration method has been demonstrated to be a viable one for the determination of the redox capacity. The approach should be useful in evaluating the feasibility of possible remediation processes. The method studied in this project can give an idea of the quantity of pollutant that can potentially be transformed by redox processes in the sediment, as well as the potential range in which such processes may be expected to occur.

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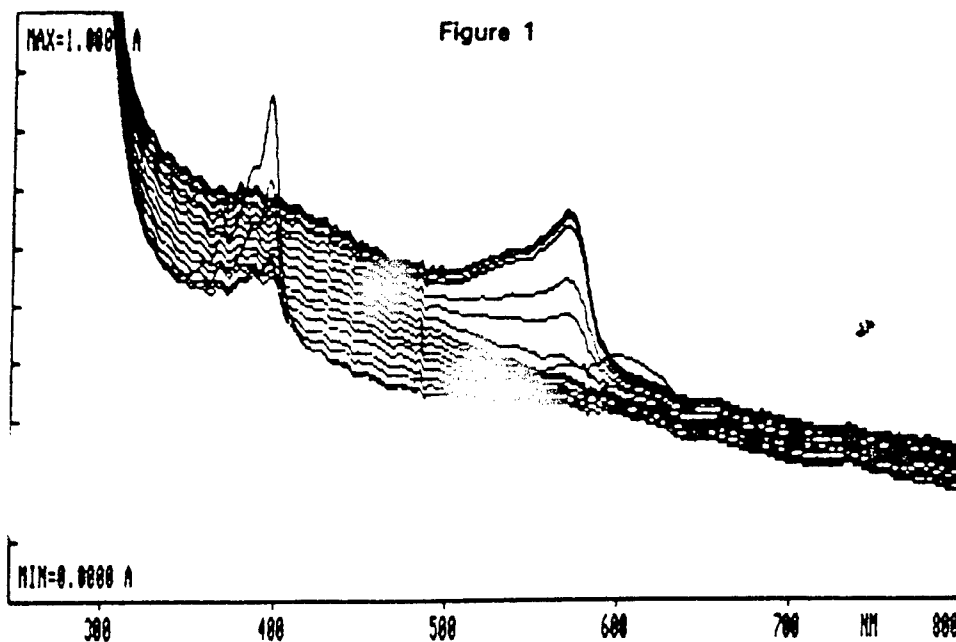
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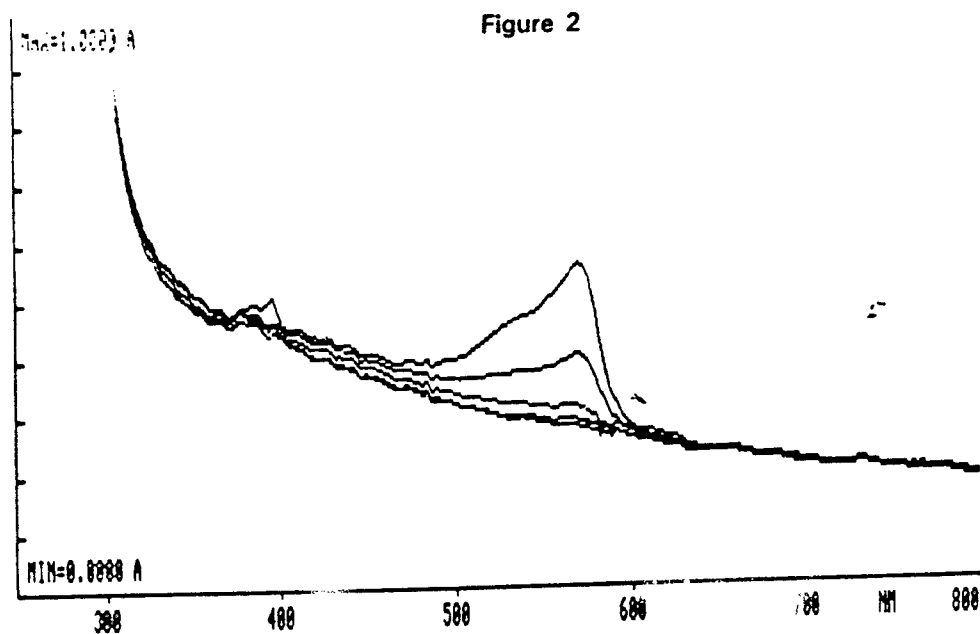
Virginia Institute of Marine Science

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REDUCTION OF BEAVER DAM SEDIMENT
8/12/93



OXIDATION OF BEAVER DAM SEDIMENT
8/12/93

Figure 3

Spectroelectrochemical Cell Used in Anaerobic Sediment Studies

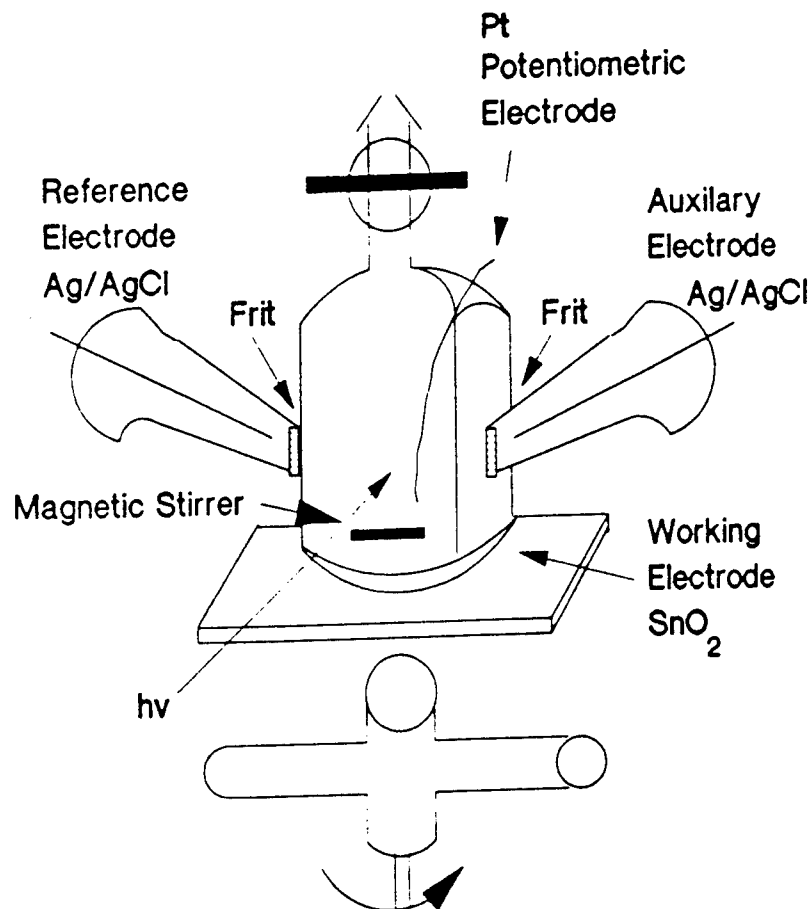


Figure 4

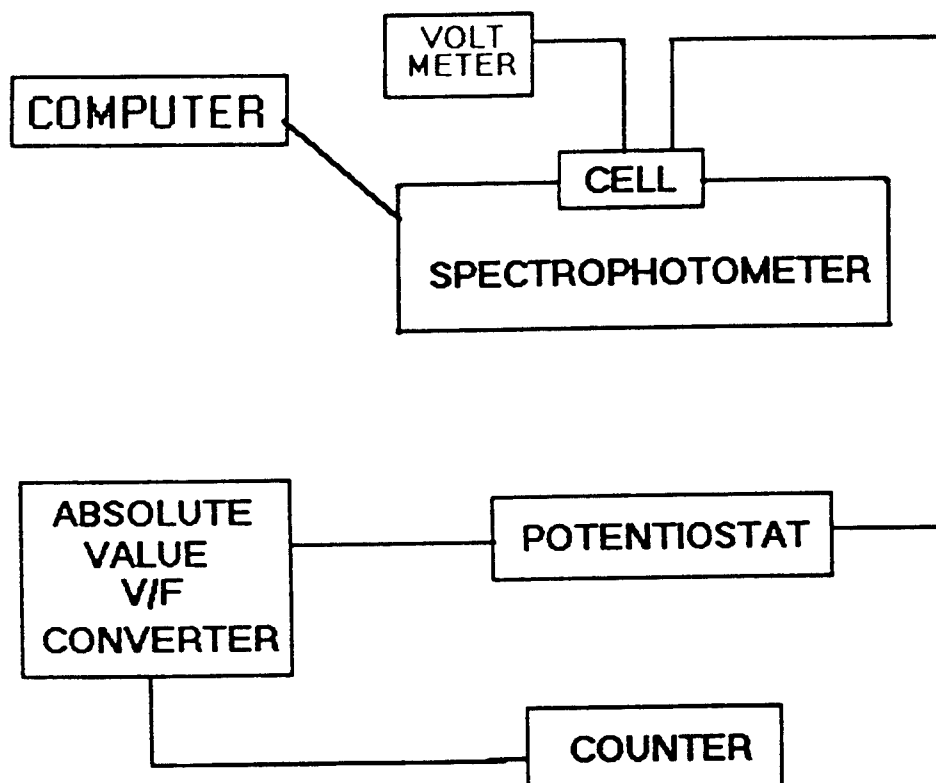


Figure 5

Reduction of "Blank" 8/12/93

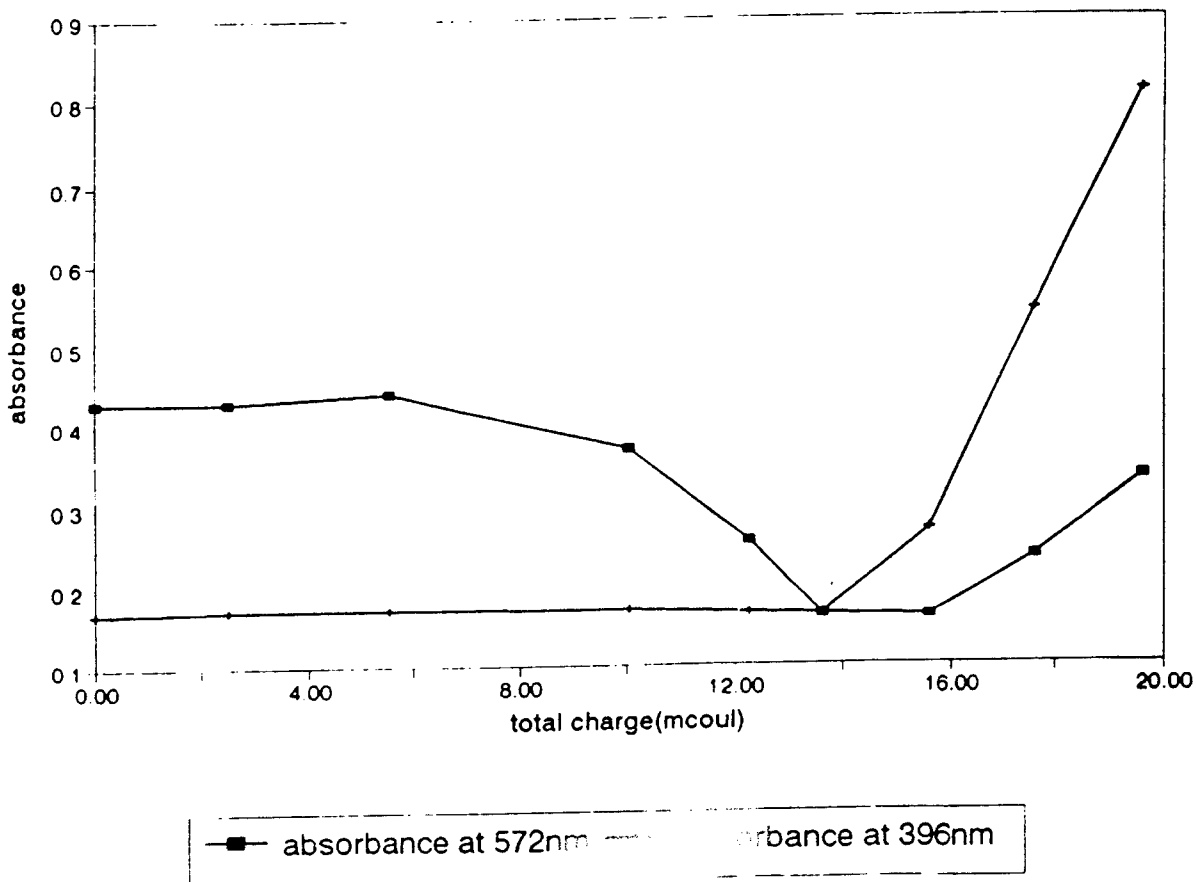


Figure 6

Oxidation of "Blank" 8/12/93

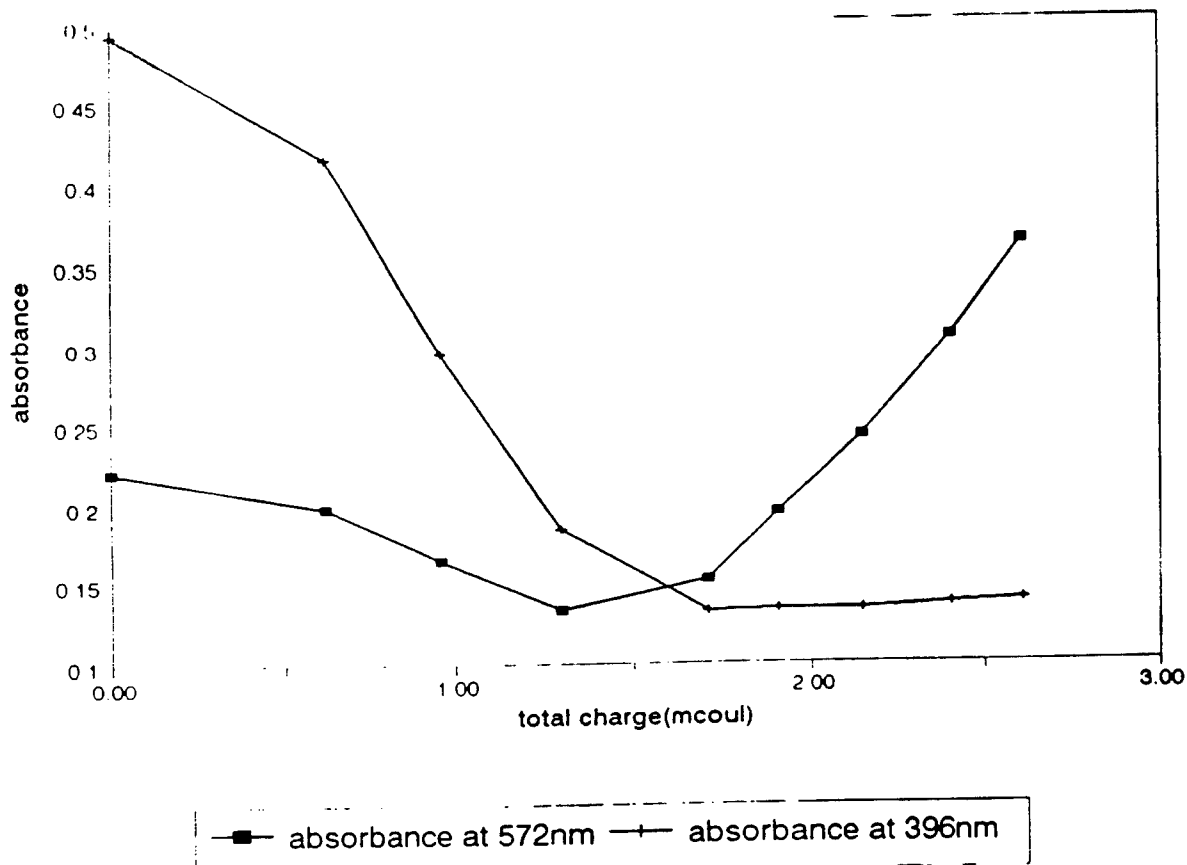
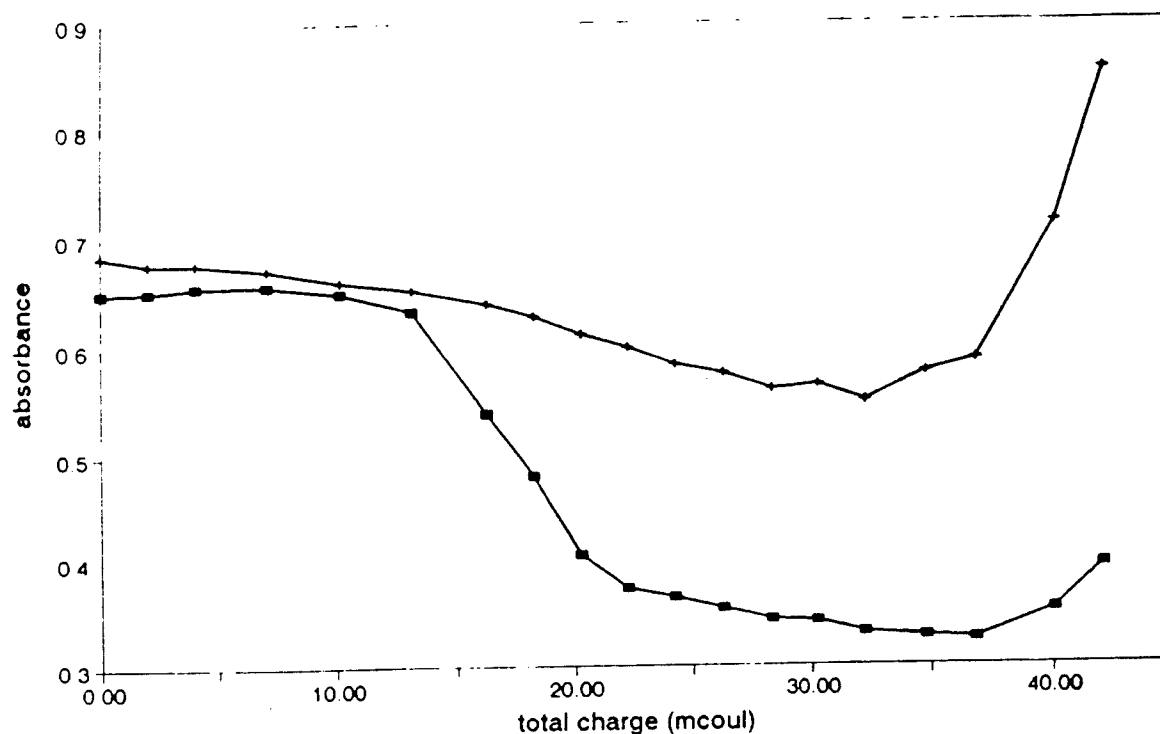


Figure 7

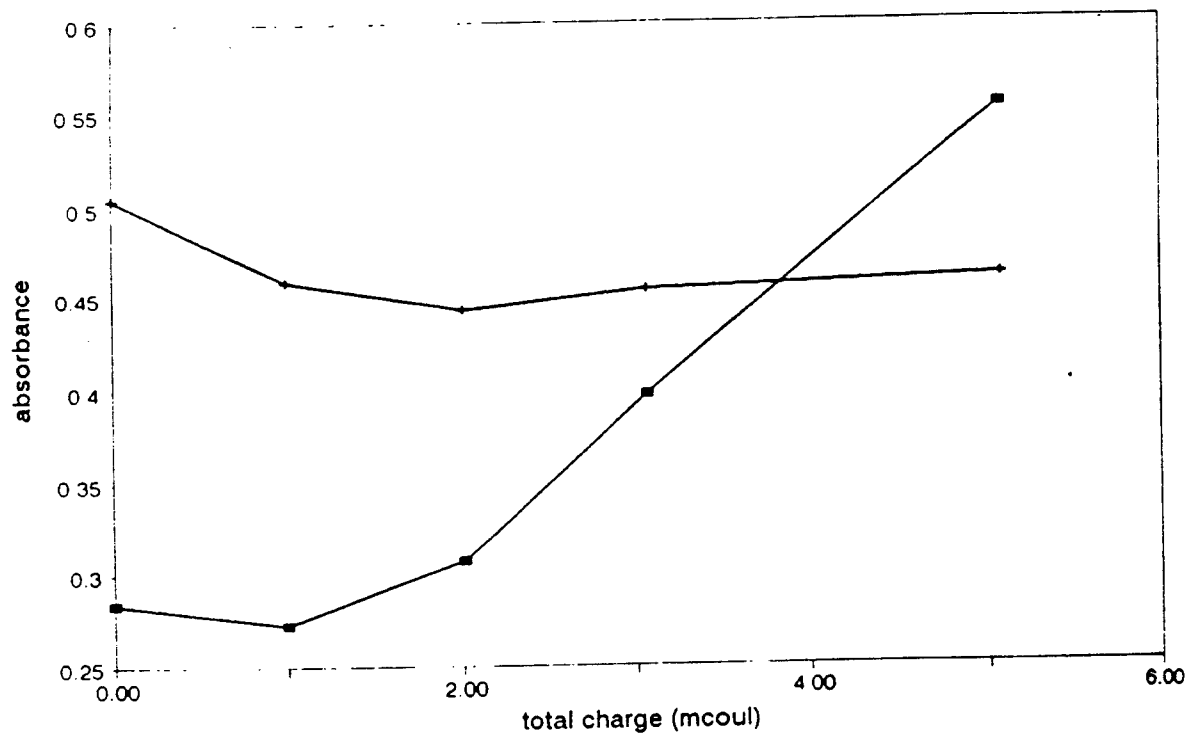
Reduction - Sediment 8/12/93



■ absorbance at 572nm + absorbance at 396nm

Figure 8

Oxidation - Sediment 8/12/93



■ absorbance at 572nm + absorbance at 396nm

Figure 9
Reduction of "Bla

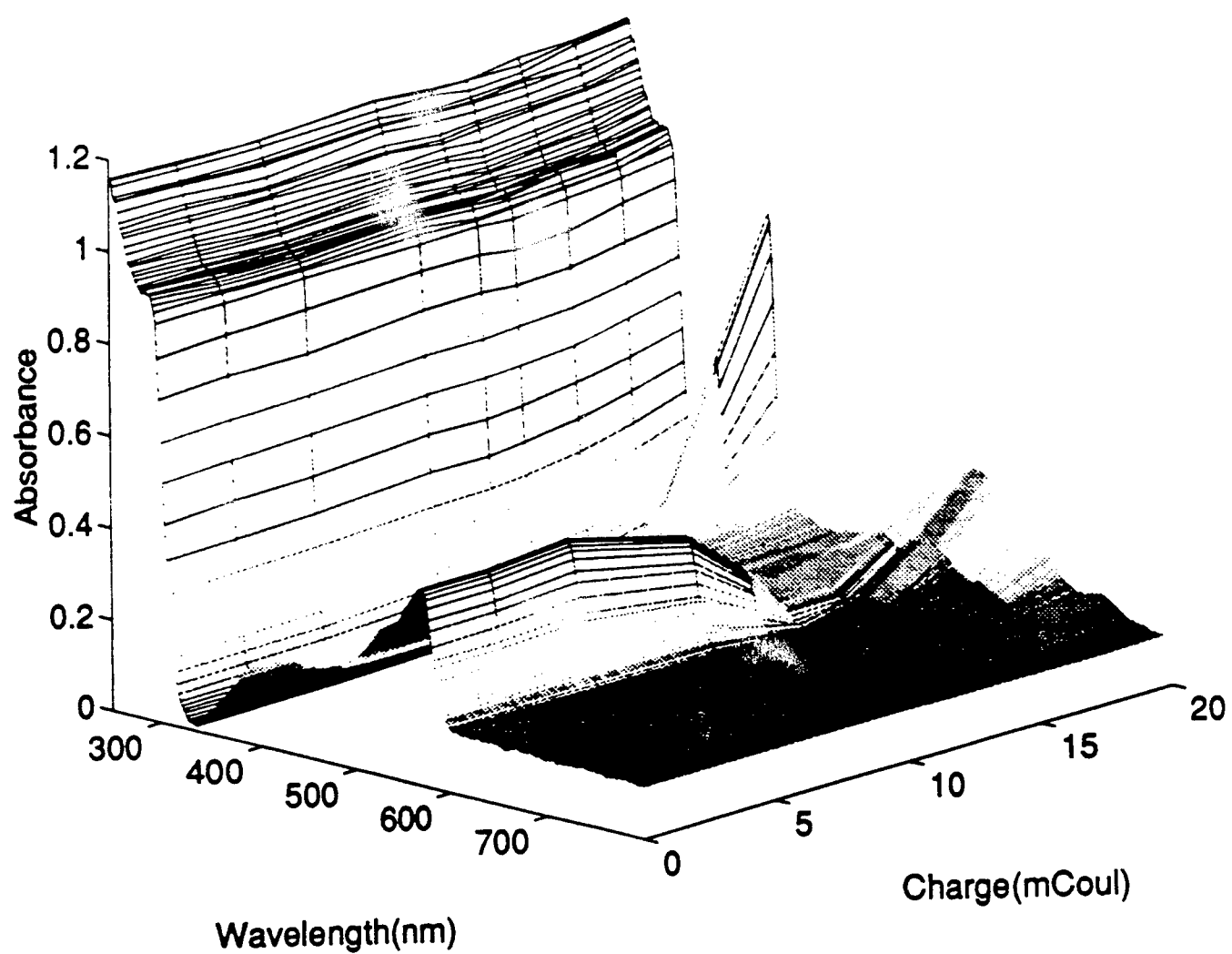


Figure 10
Oxidation of "Blank"

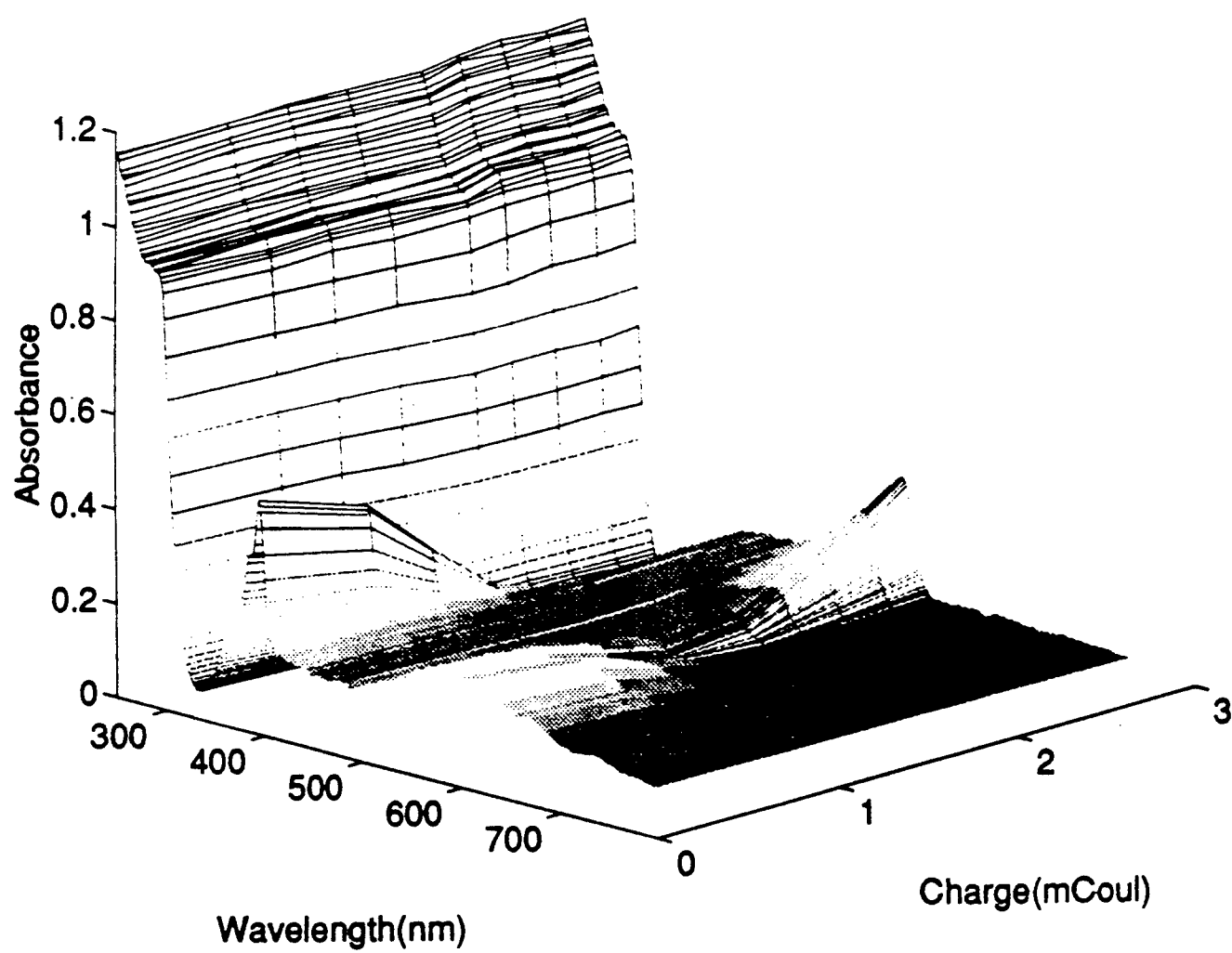


Figure 11
Reduction of Sediment

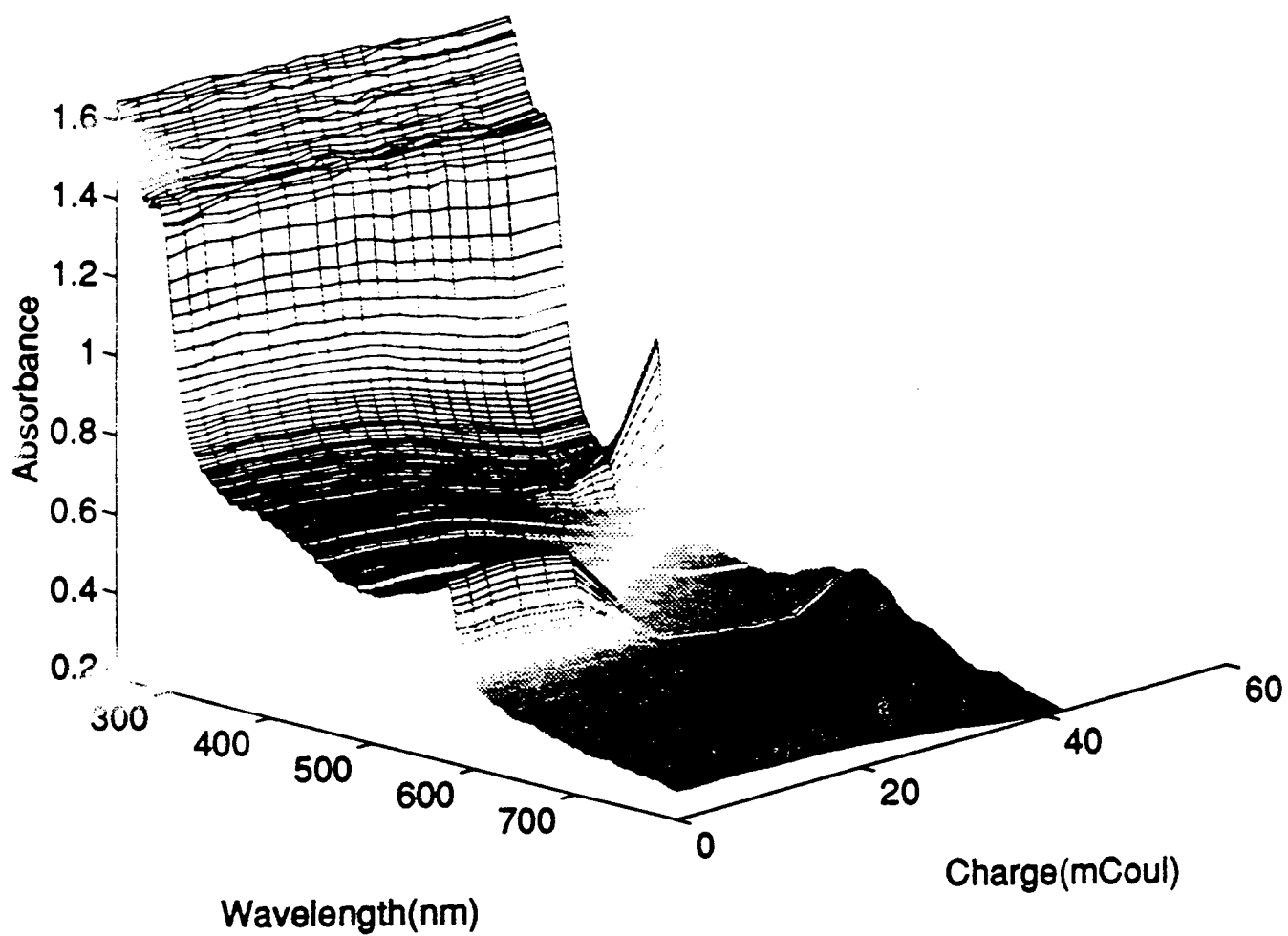


Figure 12
Oxidation of Sediment

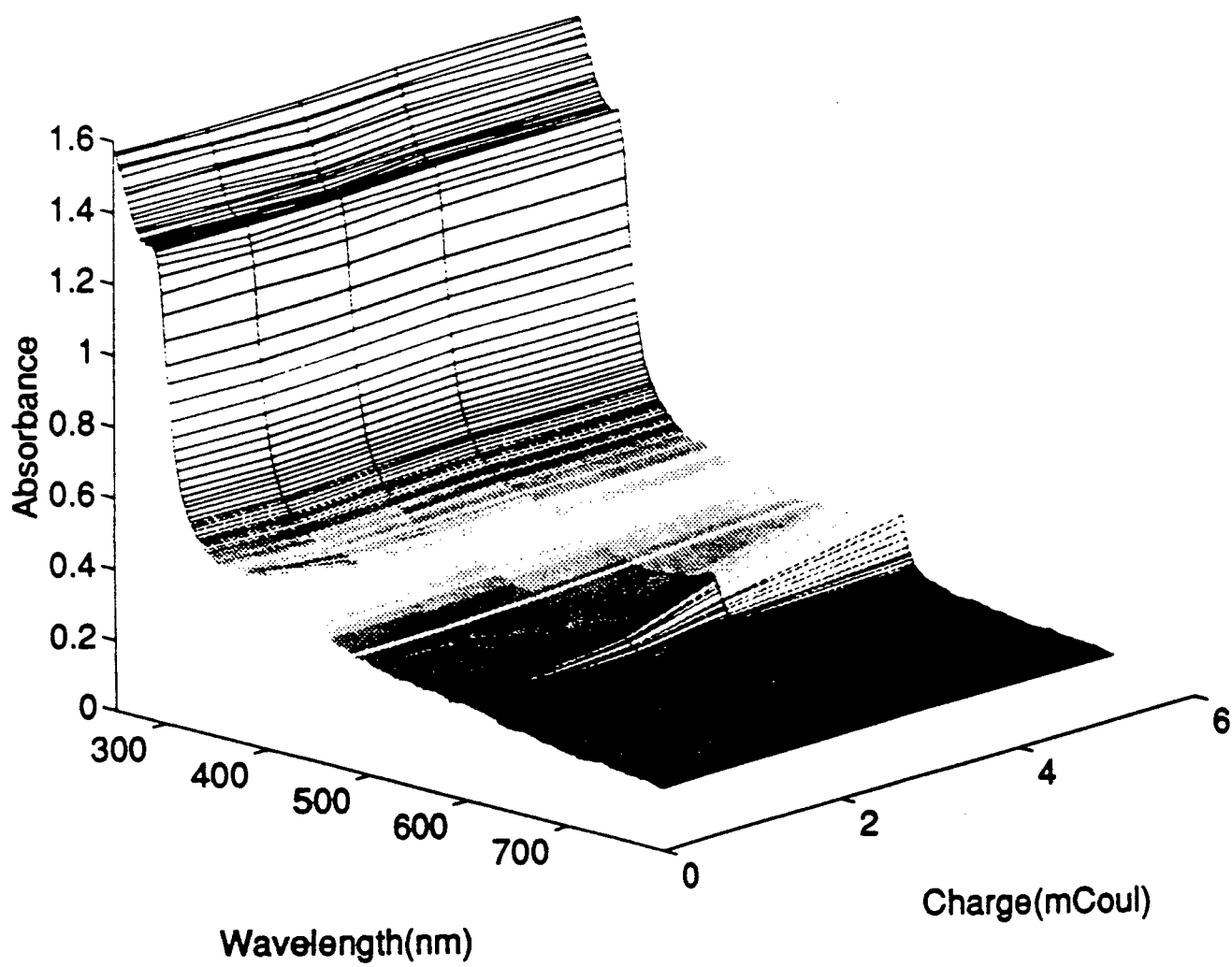
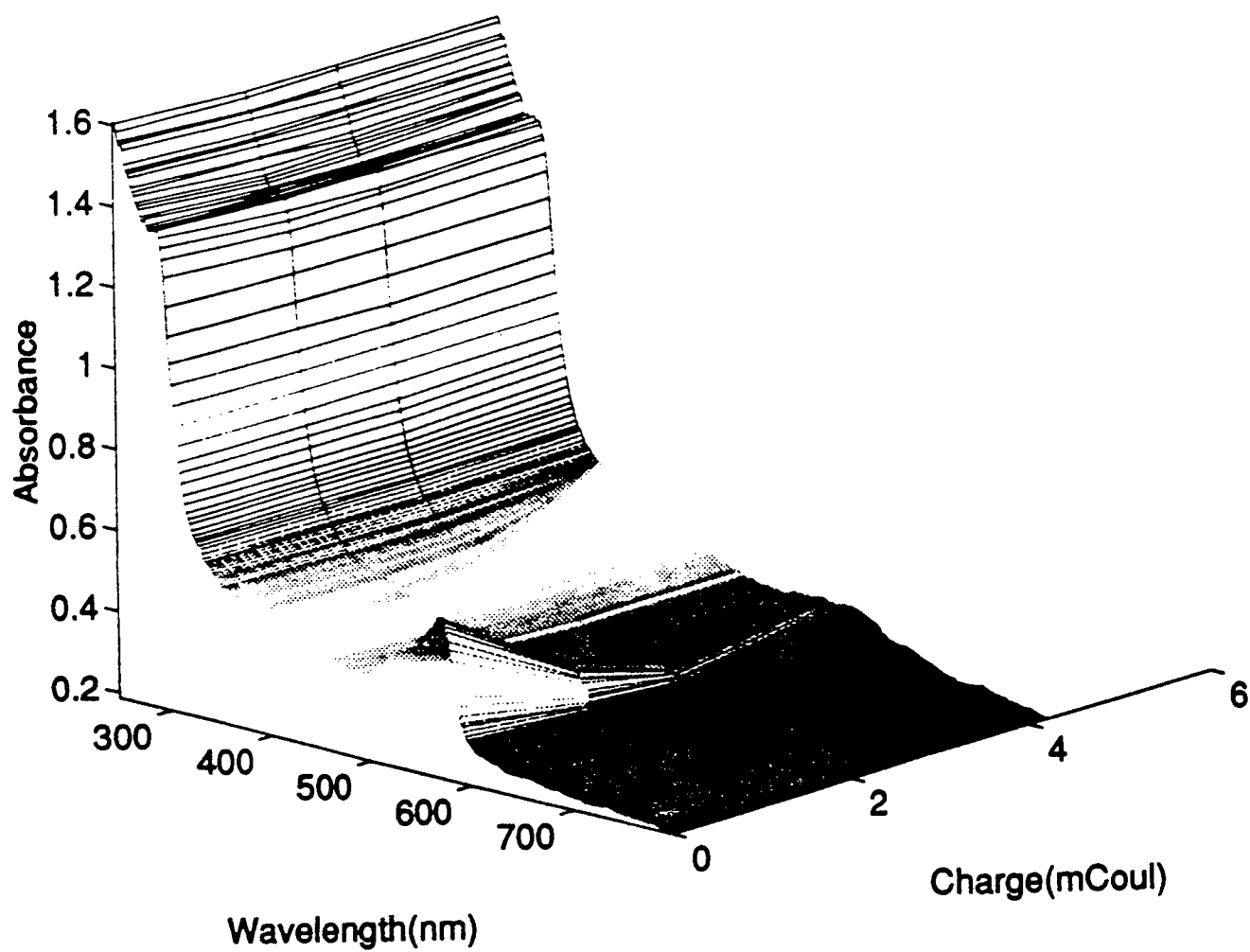


Figure 13
Second Reduction of Sediment



**EVALUATION OF AN IMMOBILIZED CELL BIOREACTOR
FOR DEGRADATION OF META- AND PARA-NITROBENZOATE**

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Abstract

Meta- and para-nitrobenzoic acid (m-NBA, p-NBA) are pollutants found in waste streams from metal-stripping processes utilizing cyanide-free solvents. The Kelly AFB Industrial Waste Treatment Plant (IWTP) is currently incapable of removing these compounds from the waste water it receives because of (1) the presence of significant quantities of ethylenediamine, a preferred substrate, and (2) an upper limit of 4.5 hours on the hydraulic residence time in the IWTP. This work describes the enrichment and preliminary characterization of a microbial consortium capable of utilizing both m-NBA and p-NBA as sole carbon sources. Experimental results indicate that m-NBA degradation involves an oxidation pathway, while p-NBA degradative proceeds through a reductive pathway. This consortium was immobilized by entrapment in alginate beads and grown in a continuous-flow airlift reactor. Single substrate and mixed substrates were fed to the reactor. Conditions were varied to simulate different waste treatment scenarios: switching from one stripping solvent batch to another, starting up of the metal stripping process, mixed solvent batches, and changing the loading rate of substrate to the bioreactor. Results indicate that the nitrobenzoate fraction of the metal stripping waste can be effectively treated in a continuous-flow, immobilized-cell bioreactor with a hydraulic residence time well below 3 hours. Furthermore, the process can be operated over long periods (>250 hours) with little diminution of performance and responds rapidly to changes in substrate.

EVALUATION OF AN IMMOBILIZED CELL BIOREACTOR FOR DEGRADATION OF META- AND PARA-NITROBENZOATE

Stuart M. Thomas and Steven W. Peretti

INTRODUCTION

Biotreatment

Proliferation of toxic organic compounds that are resistant to photochemical or biological degradation (xenobiotic) is a burgeoning threat to human health. Complications in the treatment of xenobiotics arise due to their chemical composition and the difficulty of their isolation from other compounds. Process waste streams, for example, can be isolated and their chemical composition well defined. On site treatment of these wastes is constrained by the chemical properties of the compounds present (mixed organics, extreme pH, high salinity) in the process streams. Recently, a large body of literature has accumulated regarding the use of microorganisms to degrade a wide variety of halogenated, polycyclic, and multi-substituted organic molecules heretofore thought not to be susceptible to biodegradation¹⁻⁴. While in a few cases, a single organism has been found which can mineralize a specific xenobiotic, most often a consortium of species is necessary to completely detoxify mixtures^{5,6}. Exploiting the capability of these organisms is problematic, and care must be taken to establish appropriate bioreactor design and operation. Of primary importance for these efforts will be the long-term maintenance of a population which preserves efficient biodegradative activity.

Many considerations affect the design of bioreactors for waste treatment applications. The compounds involved are often present either at low concentrations that do not support rapid growth or at high concentrations that inhibit growth. Substrate inhibition often results in growth lags, the length of which are directly proportional to the concentration of the inhibitory compound. The composition of the waste mixture may vary over time, as might the loading rates and the volumetric flow of the waste stream. Multiple organisms are usually required to effect the bioremediation of a stream containing several organic compounds.

These considerations mitigate against the use of suspended cell reactors. Cell suspensions can be grown to a limited cell density, restricting the degradative activity per unit volume. This implies that for either batch or continuous operation, the reactor must be sized such that the residence time of the fluid in the reactor is sufficient to allow degradation of the compounds. The limitation of cell growth by

high and low concentrations of the compounds will tend to cause suspended cell reactors to be rather large. Continuous flow reactors would enable a higher throughput of material than batch reactors, but the flow rates that can be accommodated are limited by the growth kinetics of the organisms. Mixed cultures can be maintained in batch reactors, but due to differences in growth rates, yields and dependence on environmental conditions, it is extremely difficult to maintain two populations in a continuous flow suspension culture, and virtually impossible to maintain three or more.

The use of immobilized cell reactors can alleviate each of the problems presented above. Immobilized cell reactors have significantly higher cell densities than suspended cell reactors, and the volumetric productivity reflects the ten- to one hundred-fold increase in cell density that can be achieved. Consequently, smaller reactors can be used. Since cell densities are higher and the cells remain in the reactor, degradation rate and cell growth rate are to a large degree uncoupled. This lessens the negative effects of the extremes of substrate concentration. Low concentrations can be fed rapidly since the cell population does not need to grow sufficiently quickly to avoid being washed out of the reactor. Substrate inhibition is also relieved. Immobilizing cells introduces mass transfer resistance due either to the immobilization matrix or the cell layers. This resistance lowers the effective substrate concentration for a fraction of the cells in the reactor, enabling them to actively degrade the compounds without a lag time.

Problem Summary

For many years the United States Armed Forces has utilized cyanide-containing stripping compounds in their metal-refinishing processes. The U.S. Air Force has undertaken a program to completely remove cyanide from these processes, and the effort has reached the demonstration stage in the plating shop of Kelly AFB. The successful demonstration of efficacy of the cyanide-free metal stripping compound CLEPO 204 led to questions surrounding treatment of the stripping wastes in the Kelly AFB Industrial Waste Treatment Plant (IWTP). The composition of spent CLEPO 204 is 33% ethylenediamine, 10% sodium nitrobenzoate and an "unidentified" red compound. Experiments were performed under the direction of the Idaho National Engineering Laboratory to determine the biodegradability of ethylenediamine(EDA) and nitrobenzoate (NBA) using sludge from the Kelly AFB IWTP.

Shake flask tests and continuous flow, bench-scale bioreactor tests were conducted using EDA or spent CLEPO 204 as the substrate. It was found that the shake flask cultures completely degraded EDA when it was the sole substrate. However, using spent CLEPO 204 as the substrate caused a reduction in EDA degradation and less than 20% degradation of NBA within 48 hours.

Continuous-flow tests with a hydraulic residence time of 5.3 hours (similar to that of the IWTP) gave only 8.8% degradation of ethylenediamine when it was the sole carbon source. Increasing the residence time to 8.3 hours led to an 88% removal of EDA but also caused ammonia levels to jump to well over 100 ppm. Spent CLEPO 204 components were not removed in the continuous-flow bioreactor with a residence time of 5.3 hours. Increasing the hydraulic residence time to 8.3 hours led to 100% removal of EDA within 30 hours and NBA within 150 hours. Concomitant with the degradation of these compounds were increases in effluent ammonia and nitrite.

The IWTP is running at or above its designed capacity with a hydraulic retention time of 4.8 hours. The residence time in the plant cannot be further increased. The preliminary conclusion drawn from these results is, therefore, that biological treatment of spent CLEPO 204 using the Kelly AFB Industrial Waste Treatment Plant is not currently feasible.

Research Objective

The objective of this work is to evaluate the feasibility of using a continuous-flow, immobilized cell reactor to remove m-nitrobenzoate and p-nitrobenzoate from aqueous streams. The effects of substrate loading rate, hydraulic residence time, substrate concentration and substrate composition on the fractional removal of nitrobenzoate have been studied. The response to starvation and rapid changes in substrate have also been investigated. These studies indicate the degree of stability of this waste treatment scheme to operational upsets and suggest other variables that might have a significant impact on the performance of this reactor system.

MATERIALS AND METHODS

Isolation of Bacteria

The organisms used in these studies were isolated from an activated sludge sample taken from the Industrial Waste Treatment Plant at Kelly Air Force Base in Austin, Texas. Inocula were diluted 1:10 v/v in Spain's minimal salts medium (SMSB)⁷ supplemented with 100 ppm m-nitrobenzoate and grown in 250 mL flasks. The

cultures were diluted 1:10 following an observable change in turbidity, which took 1-2 days following inoculation. A consortium containing at least two distinguishable species resulted from serial cultivation on m-NBA, and these species were designated Kelly 4 and Kelly 7. Kelly 4 grows rapidly on m-NBA concentrations as high as 200 ppm, following a lag period of approximately 18 hours. Nitrite is released. Kelly 4 was not observed to grow on p-NBA. Kelly 7 grows extremely slowly on p-NBA, with concomitant release of ammonia. Strains were maintained on plates containing MSB⁸, 18 g/L Bitek agar and 100 ppm nitrite.

Bacterial Immobilization

A suspended cell culture of the consortium was grown in SMSB containing 100 ppm m-NBA at 30° C. A previously autoclaved solution of 4% Na-alginate (Kelco, Manugel "GHB") dissolved in 200mM NaCl was mixed with log phase cells to give a final concentration of 3% alginate. Immobilized cell beads were formed by passing this solution through an 18-gauge needle, allowing individual droplets to fall into the airlift reactor vessel which contained 400 mL of 100mM SrCl at room temperature. Strontium was used as the cross-linking agent because it yields stronger gel beads than those formed using calcium⁹. The SrCl solution had been autoclaved and sealed so that the process was carried out under sterile conditions. The beads were allowed to cure for 4 hours with no agitation or air sparging. After curing, the reactors were flushed with SMSB to remove excess SrCl. The beads were fluidized by air sparging, and the culture allowed to grow in batch overnight in SMSB containing 100 ppm m-NBA.

Cell Culture

Suspended cell cultures, both batch and continuous, were analyzed for the more rapidly growing of the two microorganisms isolated, Kelly 4. Erlenmeyer flasks (250 mL) were used in studies to determine the maximal growth rate of Kelly 4 at 30° C in SMSB containing 100 ppm m-NBA. The concentration of m-NBA was also monitored to quantify its rate of degradation. Chemostat cultivation of Kelly 4 was also undertaken as a means to determine the dilution rate at which suspended cells would be washed out of a well-mixed reactor. SMSB amended with 100 ppm m-NBA was fed to a New Brunswick Bioflo reactor with a working volume of 500 mL. Temperature was maintained at 30° C, pH at 7.0.

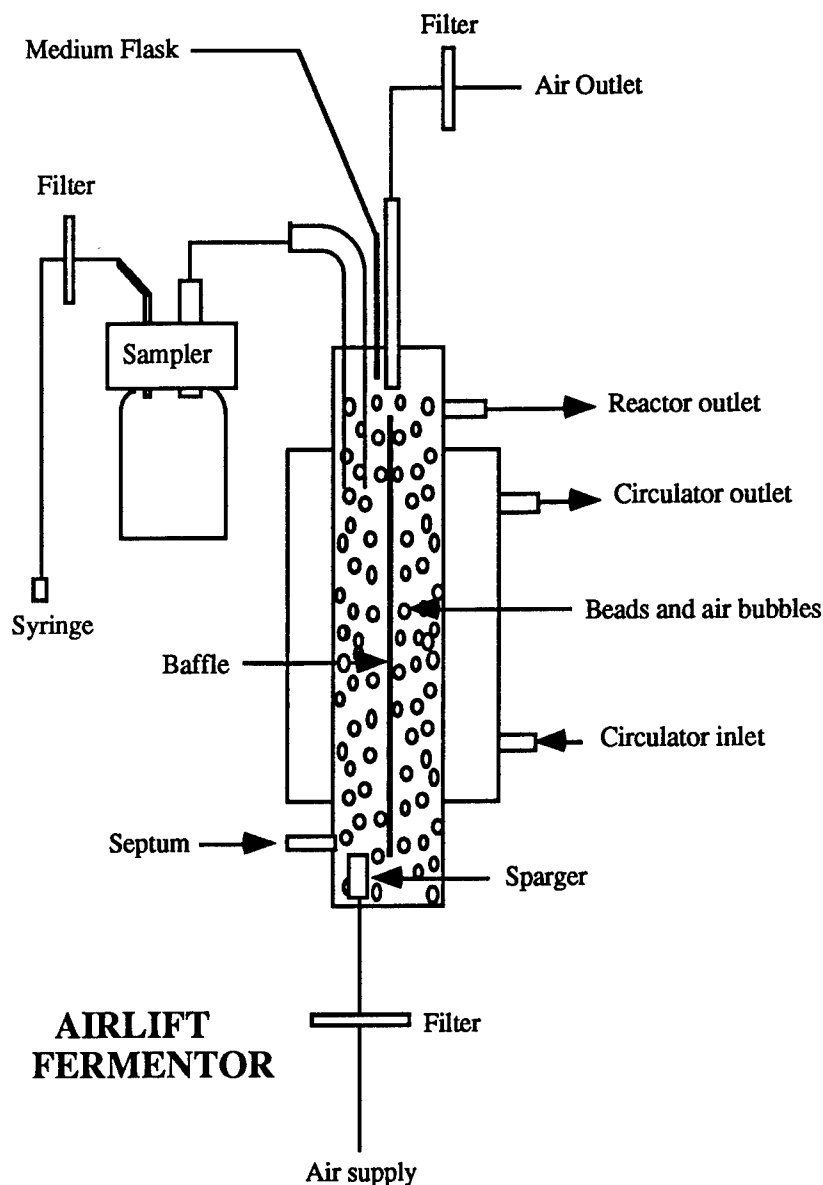


FIGURE 1

Immobilized consortium fermentations were conducted at 30°C in a pair of Kontes airlift vessels with a single central vertical baffle to promote mixing. Figure 1 is a schematic of the reactor used and its operation. Airflow was set so that the beads in the reactor (bead volume between 150 and 200 mL) were well mixed and the baffle remained free of clogging. The working volume in each reactor was maintained at 600 mL by fluid overflow. The original gel bead volume in reactor 1 was 172 mL and in reactor 2 was 185 mL. Final bead volumes were 106 mL and 115 mL, respectively. Medium and bead samples were periodically withdrawn aseptically.

The cell concentration in the medium was determined by measuring absorbance at 600 nm and using a correlation. Both reactors were in SMSB-based medium with the pH adjusted to 7.5. After batch cultivation to establish culture growth, each reactor was subjected to a different substrate and dilution rate regime. The reactor fluid volumes used to determine dilution rates are those of the total volume in the reactor at the time. All dilution rates reported have the units of reciprocal hours (hr^{-1}).

Reactor 1 was subjected to the following sequence: continuous feed of 100 ppm m-NBA at dilution rates of 0.4 and 0.6; a step change to continuous feed of 50 ppm m-NBA at a dilution rate of 1.2; batch cultivation to complete depletion of m-NBA; a step change to continuous feed of 100 ppm m-NBA at dilution rates of 0.67 and 0.33; a step change to continuous feed of 48 ppm p-NBA at dilution rates of 0.33 and 0.1; and a step change to 50 ppm m-NBA at a dilution rate of 0.31 hr^{-1} .

Reactor 2 was subjected to the following sequence: continuous feed of 100 ppm m-NBA at dilution rates of 0.7, 1.4, and 0.34; a step change to continuous feed of 50 ppm m-NBA at a dilution rate of 0.74 and 0.34; a step change to continuous feed of medium containing 40 ppm of both m-NBA and p-NBA at a dilution rate of 0.32; and a step change to continuous feed of medium containing 80 ppm of both m-NBA and p-NBA at a dilution rate of 0.31 hr^{-1} .

Analytical Methods

When a single substrate was fed to the reactor, the concentrations of m-NBA and p-NBA in the reactor samples were determined using the A266 for m-NBA and A275 for p-NBA. Calibration curves for both compounds were established in SMSB at pH 7.0. For those reactor runs containing mixed substrate feed, high-pressure liquid chromatography (HPLC) was performed using a mBondapak C8 column (3.9 mm by 30 cm; Waters Associates, Inc., Milford MA). A linear gradient was run using methanol-water (acidified with trifluoroacetic acid) as the mobile phase. The MeOH:water composition was 50:50 at the start, changing linearly to 40:60 after 3 minutes, then changing immediately to 30:70 for the next 5 minutes. This gave a peak separation time of 40 seconds and no overlap of peaks. Compounds were detected by their absorbance at 270 nm with a Hewlett Packard diode array detector. Concentrations were quantified using peak areas and calibration curves established using pure components and mixtures of m-NBA and p-NBA.

Alginate bead samples were removed from the reactor at specified times and stored at 4°C for no more than a week prior to processing. Some of the

sample was determined by decanting the residual medium, and estimating the packed bead volume using a graduated conical centrifuge tube. Approximately 1 mL of beads from each sample were dissolved with buffered saline solution⁹. The cells were precipitated by centrifugation in a Sorvall SS34 rotor at 8,000 rpm for 15 minutes. The cells were resuspended in 990 mL of 20 mM sodium phosphate pH 7.0, and lysed by adding 10 mL of 5 M NaOH and placing the samples at 100 °C for 15 minutes. A Pierce BCA total protein determination kit was used to analyze the samples. Total bead volume collected was noted throughout the course of the experiments, so that an estimate of total protein present in the reactor at any given time could be made.

RESULTS

Cell Growth

Figure 2 indicates that the maximal growth rate of Kelly 4 on 100 ppm m-nitrobenzoate in SMSB is approximately 0.36 hr^{-1} . The degradation rate of the m-NBA under these conditions is $186 \text{ mg hr}^{-1} \text{ L}^{-1} \text{ OD}_{600}^{-1}$. This implies that the washout dilution rate in a chemostat should be roughly 0.36 hr^{-1} . Figure 3 shows the results of a chemostat fermentation of Kelly 4 at 30 °C, pH 7.0. The culture is washed out at a dilution rate close to 0.2 hr^{-1} rather than the expected 0.36 hr^{-1} . The discrepancy between the performance in batch and continuous suspension culture is surprising. One possible explanation is substrate inhibition by m-NBA.

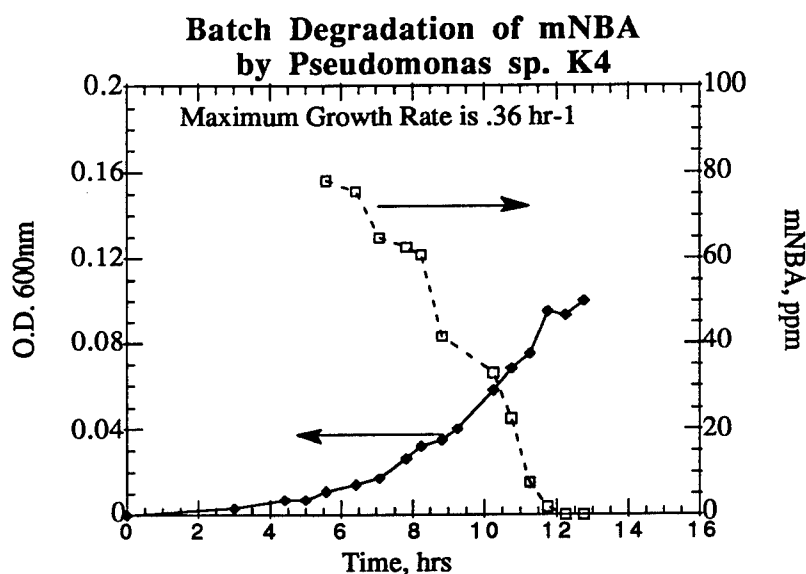


FIGURE 2

The maximal growth rate in shake culture was achieved at m-NBA concentrations in the range of 40 to 65 ppm. This hypothesis is supported by the observation that cells actively growing in shake culture exhibit some lag in growth upon sub-culturing into 100 ppm m-NBA.

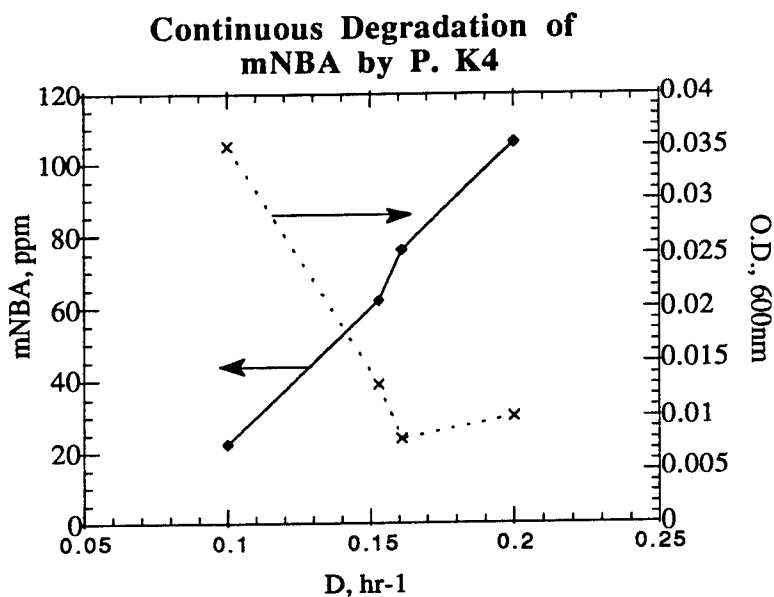


FIGURE 3

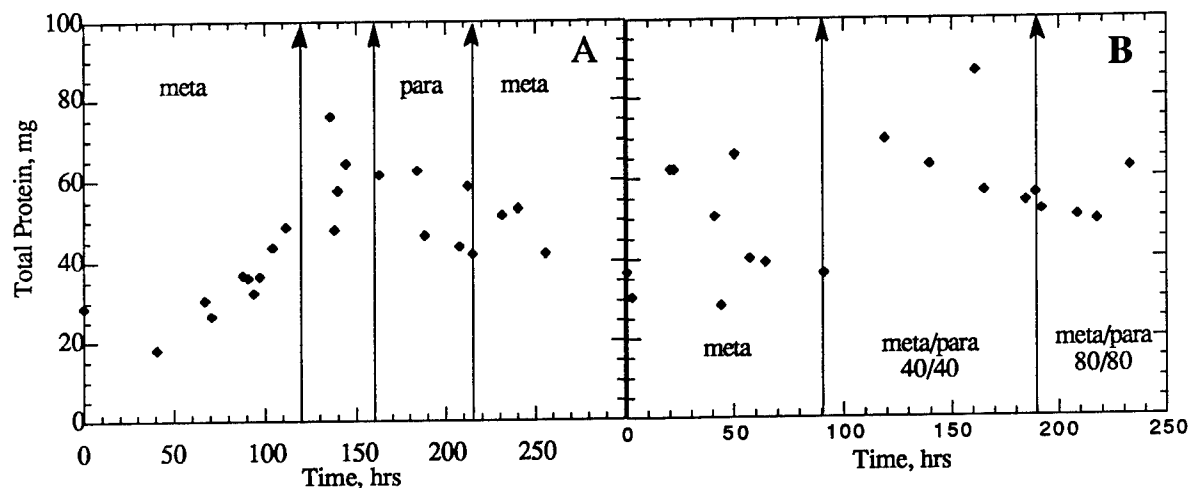


FIGURE 4

Total Protein

Figures 4A and B indicate the total protein in reactors 1 and 2, respectively, as a function of time. The results for reactor 1 indicate that it took nearly 150 hours to fully load the beads with biomass. This is supported by the observation that before

16-10

about 120 hours, the A₆₀₀ of the fermentation broth was essentially zero, indicating no cells released from the gel beads. The gradual decline in total protein in reactor 1 after 150 hours can be accounted for by the volume of beads removed during sampling. Initially, the biomass in reactor 2 grew more rapidly, probably because it was the first reactor in which beads were formed and to which growth medium was introduced. As with reactor 1, the subsequent decline in total protein in the reactor can be attributed to gel bead removal.

Reactor 1 Performance

Starvation

Figure 5 illustrates the degradative performance of the consortium in reactor 1 following a period of starvation. The solid line and diamonds represent the loading rate of m-NBA into reactor 1 as a function of time. A 15 hour period of starvation is followed by rapid pumping of m-NBA into the reactor. The outlet concentration of m-NBA increases transiently, followed by complete degradation of the compound. The transient increase in m-NBA in the reactor is due solely to mixing. There is no indication of a loss of degradative activity or of a lag phase following this period of starvation. The pH in the reactor remained roughly constant during the starvation and post-starvation periods at 6.9

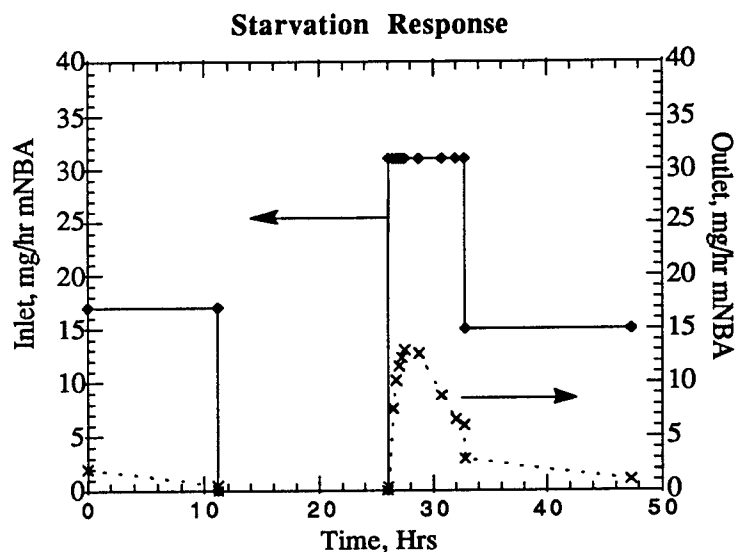


FIGURE 5

Shift from m-NBA to p-NBA

The inlet and outlet concentrations of p-NBA following a shift from m-NBA feed to p-NBA feed are shown in Figure 6. This culture was previously exposed to only m-NBA as a carbon source. The outlet concentration of p-NBA following the switch from m-NBA indicates the immediate degradation of some of the p-NBA. Subsequently, this reactor degraded about 60% of the p-NBA fed. The pH in the reactor rose to 7.5, probably due to the accumulation of ammonia released during the metabolism of p-NBA.

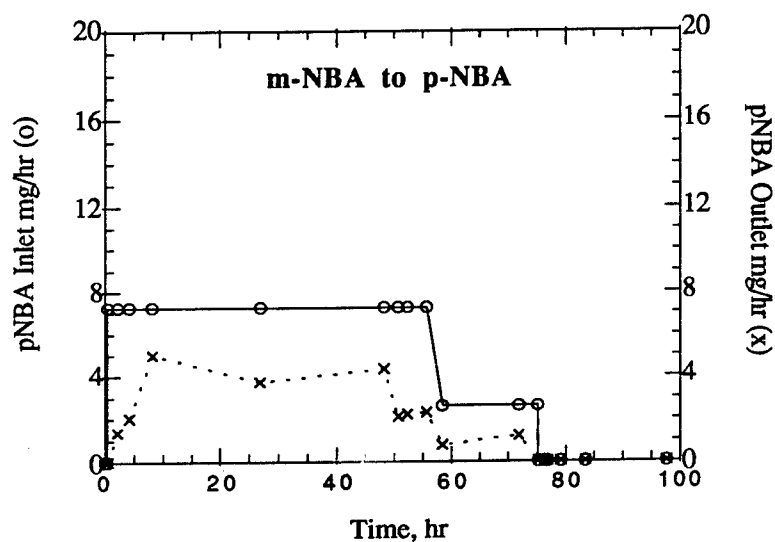


FIGURE 6

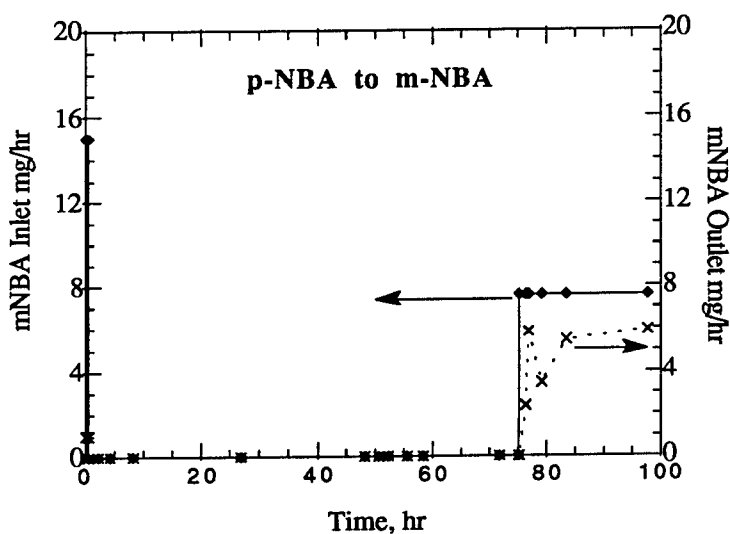


FIGURE 7

Shift from p-NBA to m-NBA

Figure 7 shows the concentration of m-NBA in the inlet and outlet streams following a switch in feed from p-NBA to m-NBA. The m-NBA feed had a pH of 7.1 instead of 7.5 due to the elevation of reactor pH caused by p-NBA degradation. Only approximately 20% of the influent m-NBA was degraded, compared to the greater than 90% degradation exhibited 50 to 100 hours earlier. The apparent inability of the reactor to exhibit the degradative performance it displayed at an earlier time may be due to the ammonia produced during the metabolism of p-NBA. The pH of the reactor was 7.5 immediately prior to the switch in substrate and took over 20 hours to drop to 6.8.

Reactor 2 Performance

Degradation of m-NBA

The carbon and energy sources provided to reactor 2 were m-NBA and mixtures of m-NBA and p-NBA. It is evident from the outlet concentrations for m-NBA displayed in Figure 8A for the straight m-NBA feed that the consortium is capable of degrading nearly 100% of the m-NBA at contact times below 3 hours. The addition of a moderate level of p-NBA (40 ppm) to the feed has essentially no effect on the degree of degradation of m-NBA. However, increasing both the m-NBA and p-NBA concentrations to 80 ppm in the feed results in a loss of the majority of the degradative activity. This is indicated by the rapid and sustained elevation in outlet m-NBA concentration following the increase in feed concentration.

Degradation of p-NBA

Figure 8B displays the inlet and outlet concentrations of p-NBA for reactor 2 during the introduction of mixtures of p-NBA and m-NBA to the vessel. Despite being fed only m-NBA for over 100 hours at the time of introduction of p-NBA to the reactor, the p-NBA was rapidly and completely degraded following a short mixing period. This performance continued unabated in the face of a concomitant doubling of m-NBA and p-NBA concentrations in the feed.

Response of Reactor 2 to Various Feed Conditions

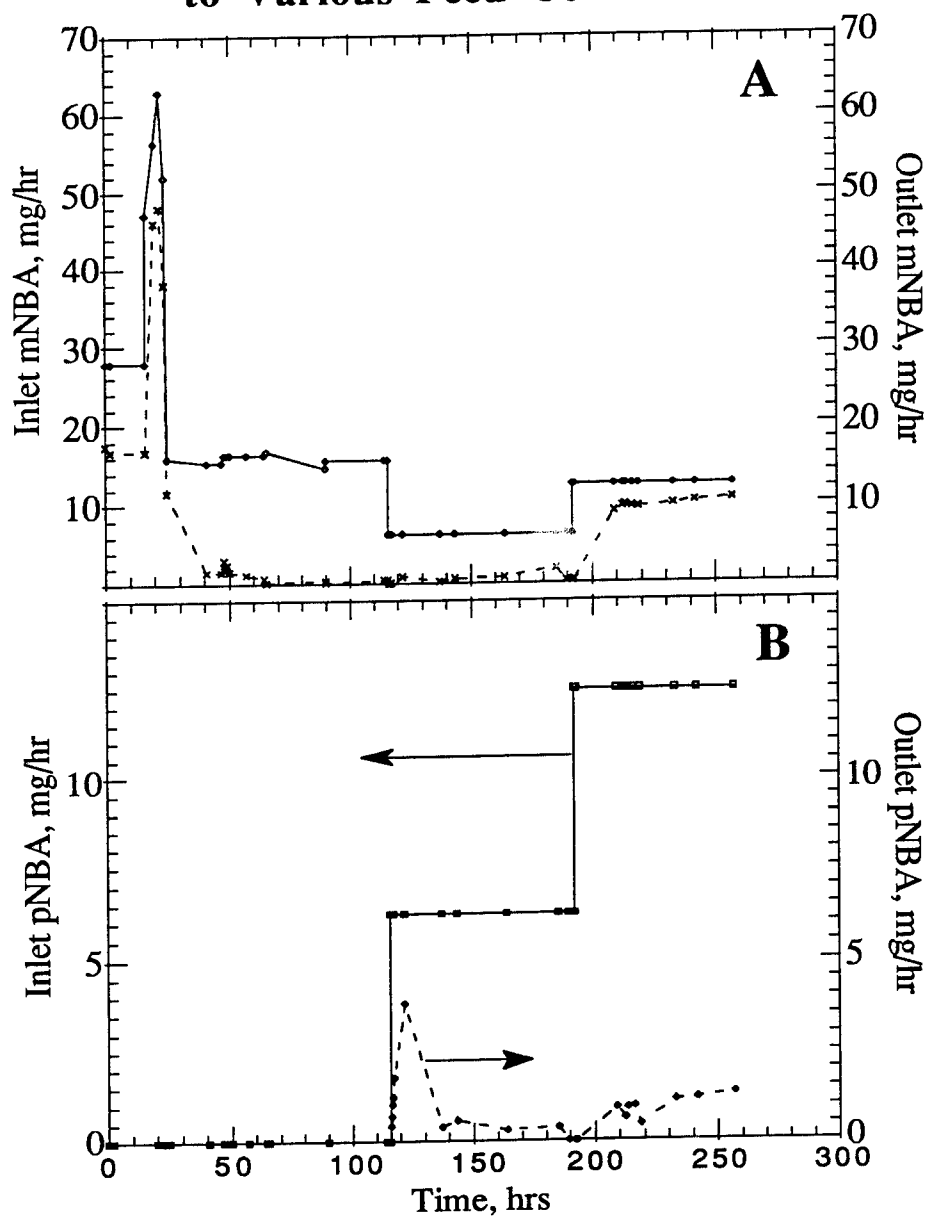


FIGURE 8

DISCUSSION

These studies indicate that the combination of organisms evaluated and the immobilized cell bioreactor represents a feasible option for the treatment of aqueous

streams containing mixtures of m- and p-nitrobenzoate. The system exhibits the capability to degrade both pure substrate and mixed substrate feeds. The system responds rapidly to shifts in substrate, exhibits stable activity over time, is insensitive to moderate starvation times (15 hours), and is effective over a wide range of loading rates.

Comparing the degree of p-NBA degradation in reactor 1 under single substrate conditions with that for reactor 2 under mixed substrate conditions suggests that p-NBA metabolism is enhanced by concurrent metabolism of m-NBA. Only about 60% of the p-NBA was degraded in reactor 1 in the absence of m-NBA, versus greater than 95% degradation in reactor 2 when both m-NBA and p-NBA were fed at a concentration of 40 ppm. Conversely, the metabolism of m-NBA appears relatively unaffected by p-NBA degradation at lower p-NBA concentrations (40 ppm) but negatively affected at higher levels (80 ppm).

It is hypothesized that each isomer is metabolized by a different organism, though Kelly 7 is expected to degrade both isomers. The interaction between Kelly 4 and 7 is probably mediated by an extra-cellular product. A plausible hypothesis for the beneficial affect of m-NBA metabolism on p-NBA metabolism involves the pH inside the gel beads. We have established that p-NBA metabolism is reductive, releasing ammonia, while m-NBA metabolism is oxidative, releasing nitrite. The medium was initially at a pH of 7.5. In the absence of nitrite generation, the release of ammonia would cause the pH within the beads to rise significantly during p-NBA metabolism, inhibiting further cell growth or p-NBA metabolism. The rate of p-NBA metabolism would then be limited by the diffusion of ammonia out of the gel beads. If nitrite were being generated as well as ammonia, the pH change would depend upon the relative rates of generation of nitrite and ammonia. Calculations based on the diffusivity of ammonia in alginate and a bead diameter of 3 mm suggests that the pH inside the bead could rise above 8.0 given the observed rates of p-NBA metabolism.

This mechanism of interaction is consistent with the observation regarding the inhibition of m-NBA metabolism at higher p-NBA levels. In this medium, ammonia is the nitrogen source for the m-NBA degrader. At low relative rates of p-NBA metabolism, the greater availability of a nitrogen source inside the bead would be advantageous to the m-NBA degraders and would alleviate the acidic inhibition of growth that is often caused by nitrite generation. As the rate of generation of ammonia increases, the pH in the beads would rise, possibly to an inhibitory level.

This would explain the observation that the rate of m-NBA degradation per unit protein in reactor 2 drops 60% when the feed concentration goes from 40:40 to 80:80.

The assertion made regarding substrate inhibition is supported by certain details of Figure 9, which shows the percentage removal of m-nitrobenzoate as a function of reactor loading rate. Squares denote a feed concentration of 50 ppm m-NBA while circles denote 100 ppm. Data are taken from both reactors during the initial periods when only m-NBA was fed. Two trends are indicated. First, percentage removal drops as loading rate increases. Second, lower feed concentration leads to higher percentage removal. Of particular interest are the two data points connected by the arrow. These points were achieved sequentially. In going from point 1 to 2, the concentration of m-NBA in the feed was changed from 100 ppm to 50 ppm and the flow rate was doubled. The jump in percent removal indicates that the degradative activity in the reactor was inhibited by the higher m-NBA concentration.

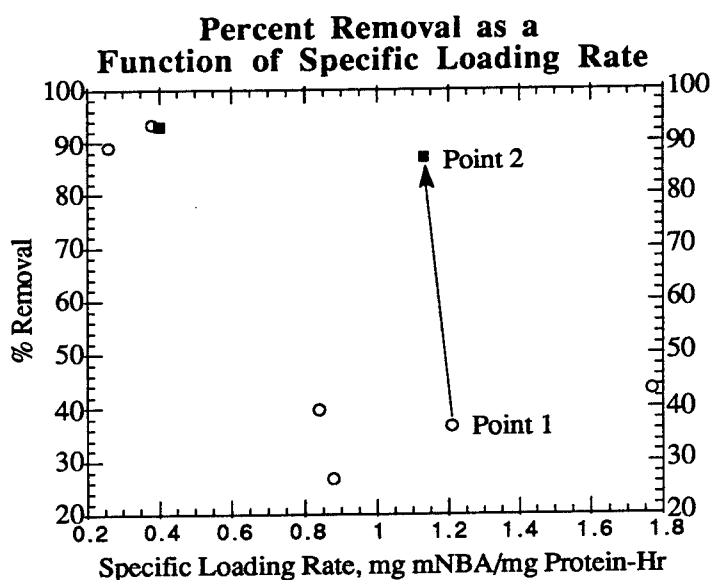


FIGURE 9

Another factor likely to contribute significantly to the behavior exhibited is the composition of the population in the reactor. As shown in Figure 6, the p-NBA concentration in reactor 1 decreases continually over time. The trend might indicate the replacement of m-NBA degraders by p-NBA degraders in the reactor. The transient is of too great a duration to be explained simply by induction of the p-NBA pathway. Population shifts could also explain the changes in degradative activity under mixed substrate feed conditions. The decline in m-NBA degradative activity

following the shift from 40:40 to 80:80 could be caused by a decline in the population most active in m-NBA metabolism.

CONCLUSION

Meta-nitrobenzoate and para-nitrobenzoate can be degraded rapidly and completely using a consortium initially isolated from the IWTP at Kelly AFB. The degradation occurs when either isomer is the sole carbon source or when both are present. Preliminary metabolic characterization of the consortium indicates that m-NBA degradation involves an oxidation pathway, while p-NBA degradative proceeds through a reductive pathway. Under certain cultivation conditions in an immobilized cell reactor, concurrent metabolism of both isomers is synergistic, leading to more rapid and complete degradation of each compound than observed in single-substrate studies. Under other conditions, the interaction appears inhibitory. Clarification of the mechanisms underlying these observations is necessary before reactor performance can be maximized. The pH sensitivity of the organisms involved is probably an important determinant and must be investigated more fully. Combined with measurements of the pH gradient inside the gel beads under different operating conditions, this information would help establish the effect of pH, nitrite and ammonia generation on degradation rates. In addition, enumeration of the population size of each species in the reactor, and elucidation of the spatial distribution of each species in the gel beads, are necessary to more clearly define the local environments these organisms experience. With such information, realistic models of reactor performance can be formulated and used to design and operate waste treatment systems effectively.

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THE DESIGN, DEVELOPMENT, AND PRELIMINARY EVALUATION OF LOADER:
A TOOL FOR INVESTIGATING THE ACQUISITION OF CONSOLE-OPERATION SKILL

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Abstract

Console-operation skill is a common requirement of many military and industrial tasks. In an effort to understand and assess the effects of instructional strategies in the acquisition of console-operation skill, the simulation task LOADER was developed. LOADER, a complex problem-solving task requiring the operation of a simulated control-panel console, is analogous to many tasks that call for knowledge of various procedures. The computer-based simulation demonstrates a number of characteristics that make it attractive for the manipulation of instructional strategies within a learning environment. This report details the features and components for use of the simulation tool and provides preliminary descriptive data from a pilot study.

THE DESIGN, DEVELOPMENT, AND PRELIMINARY EVALUATION OF LOADER: A TOOL FOR INVESTIGATING THE ACQUISITION OF CONSOLE-OPERATION SKILL

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Introduction

LOADER is a complex problem-solving task requiring the operation of a simulated control-panel console. Analogous to many military and industrial tasks that call for knowledge of various procedures, LOADER is a laboratory research tool developed to assess instructional strategies in the acquisition of console-operation skill. The computer-based simulation demonstrates a number of characteristics that make it attractive for the manipulation of instructional strategies within a learning environment. These features include an on-screen dynamic display, an expert system module, flexible feedback messages, and a student module with the ability to output extensive data for analysis. The following report will provide a complete description of the features and components for use of the simulation tool. In addition, preliminary descriptive data from a pilot study will be presented.

LOADER: A Simulation Tool

The task simulated by the LOADER environment is the operation of a remote crane control arm to load various canisters from a set of storage bins to one or more railroad cars (see figure 1 for the simulation interface). Simple tasks include the loading of a single canister whereas complex tasks require the operator to analyze the availability and capacity of railroad cars in order to appropriately position and load a number of different-sized canisters. Complexity of the task depends upon variables such as the number and size of canisters to load and the position and capacity of rail cars. Simple tasks may be performed with as few as 30 steps while complex tasks may require as many as 150 steps.

Each problem can be solved through a set of six subgoals (or procedures). These procedures are: 1) Position Rail Car to Appropriate Dock, 2) Position Crane to Appropriate Storage Bin, 3) Lift Canister from Storage Bin, 4) Position Crane to Appropriate Rail Car Receptacle, 5) Load Canister into the Rail Car, and 6) Dispatch Rail Car to Appropriate Rail Line. Each subgoal has 5 to 6 individual steps or control panel actuations. The rail yard "situation" determines the appropriate settings for various knobs and buttons as well as the correct order of sub-procedures. Complex tasks require one or more iterations through certain subgoals (see appendix 1 for an example of the steps for a simple task).

The Control-Panel Console

The control-panel console appears as set of knobs, switches, buttons, and indicators appropriately labeled and organized by function on the lower half of the LOADER interface. All interactions with the console are performed through a mouse. While most interactions occur with a simple click of the mouse, some interactions

require that the mouse button be held down for several seconds until the action is complete. The panel responds with visual feedback in the form of indicator lights, knob and switch settings with each selection. For certain actions, an audible "click" indicates the completion of the action.

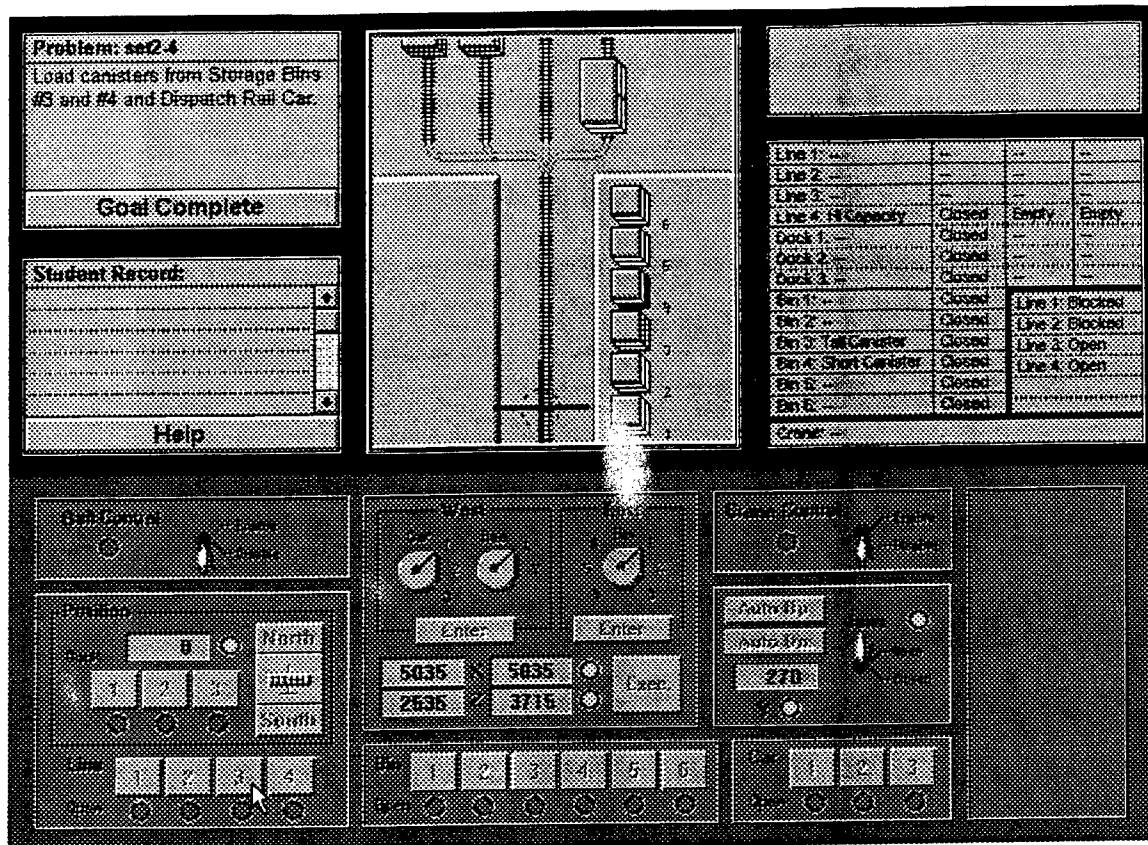


Figure 1: *The LOADER Interface.*

The console is organized into eight panels associating their related functions. The rail control, line and dock panels allow the operator to position rail cars to appropriate loading docks and dispatch cars to available rail lines. Operations requiring positioning the crane, use the centrally located panels including the crane control, crane coordinate system, and the jaws panels. Along the bottom of the screen are panels that allow storage bin doors and rail car doors to be opened. And, finally, a "breaker panel" covers a set of breakers used during "system failures."

The Dynamic Model

Located on the upper half of the LOADER interface is the dynamic graphical model. This model provides a representation of the loading task from a birds-eye-view perspective. Positions of the rail cars, storage bins, canisters, doors, the overhead crane, and the line and dock selections are indicated with a number of graphical elements. Colors aid the identification of canister sizes and rail car capacities.

The dynamic model responds to control panel interactions with animated sequences representing their outcomes. For example, engaging a selected rail car south with the button labeled "South," results in the animated motion of the rail car moving down toward the rail docks. In addition to the motion of rail cars, car doors and bin doors open and close, the crane moves above the rail cars and storage bins, and the crane lowers, grasps and lifts canisters. All of the information displayed by the dynamic graphical model (minus the motion and graphical relationship qualities) is also represented on the upper half of the screen in table form.

The Expert Help Feature

Embedded within the interactions of the problem-solving task is an expert help system. The expert help provides intelligent advice to assist in the solving of the loading task. This intelligence feature can be turned on and off by the researcher who wishes to investigate effects of types and levels of expert advice on the acquisition and performance of the task. Under the *dumb* intelligence mode, expert advice consists solely of error notification while giving the correct answer. The *smart* intelligence mode provides levels of advice often beginning with a reason why the attempted action was incorrect (see table 1 for an example of expert advice). Secondly, this mode will give a description of the subgoal that the user should be attempting. By requesting additional help, the expert advice will provide a list of the steps required to perform the subgoal with an indication of the steps already accomplished. Finally, if the user requests still more help, the system will give the correct answer. The levels of expert help (with the exception of describing the reason for an incorrect answer) are also available to the user by selecting on-screen "Help".

Error Notification:	Critical Error -- Crane cannot be lowered through the door of a closed storage bin.
Level 1 Help:	You should be attempting to: Load Canister to Receptacle 2 of Car at Dock 3.
Level 2 Help:	To Load Canister to Receptacle 2 of Car at Dock 3: * 1. Set West Car to 3 * 2. Set West Receptacle to 2 * 3. Enter West Side Coordinates 4. Set Crane Control to Enable 5. Execute Coordinates 6. Set Crane Control to Disable * Denotes Action Complete
Level 3 Help:	To Set Crane Control to Enable: Click at the button indicated with the red arrow.

Table 1: Levels of expert help while under the smart intelligence mode.

Other Display Features

In addition to the dynamic graphical model and its accompanied table, the LOADER interface includes a number of other helpful display features. Starting in the upper-left corner of the screen, the name of the problem file is displayed. Below this, a verbal description of the goal for the selected problem appears. Within the same area, a "Goal Complete" button is available for the user to select to end each problem.

Along the left side of the interface and above the "Help" button, the Student Module or Record is displayed. This window provides a record of each action that the student has made, attempted or accessed during the selected problem. Errors, error messages, and help messages displayed to the student are recorded here as well. This record can be made available to the user for on-line analysis and, as the problem is finished, this record is output to a separate file.

Researcher Controls

Many of the display features described above can be turned on or off by the researcher to study various effects in the acquisition of console-operation skill. The controls for these features are available initially on the set-up screen (see figure 2). The two main controls available to the researcher include the Intelligence of the system and the Flexibility. The Intelligence of the system can be set to smart or dumb as described above under the Expert Help System. The Flexibility of the system controls the way that the system responds to *non-critical errors* as performed by the user. Non-critical errors are those errors that identify actions as either inefficient or inappropriate. For example, the user can attempt to close the crane jaws at any time during the problem-solving process. This action may be performed by accident or in an attempt by the user to "just see what happens." While closing the jaws is at times the correct action, it is usually an incorrect action (although non-critical). Conversely, opening the crane jaws to release a canister can often result in a *critical error*. This would occur if the canister were to be released while the crane is raised or while the capacity of the car or bin is not adequate for the canister. Therefore, the distinction between critical and non-critical errors is important.

Setting the Flexibility to "on" will give the system flexibility in selecting when to provide feedback to the user, allowing the problem-solver to deviate from the single best path of responses (as determined by the expert system). In this mode, the system will not immediately respond to actions that are determined to be non-critical. Instead, these errors are simply recorded in the student record and can be used for later display or selection of instructional materials. While in the Non-Flexible mode (i.e. Flexibility set to "off") the system will respond with immediate and interruptive feedback with every action that varies from the solution path whether those actions are critical or non-critical errors.

The result is that while under the *flexible* environment, users have greater freedom to explore options and observe the results of such actions. Whereas the *non-flexible* environment operates like a "scenario machine,"

lock-stepping users into a predetermined sequence of events. Note that the flexible environment is not completely flexible in the sense that some errors (deemed critical) are always flagged with a response.

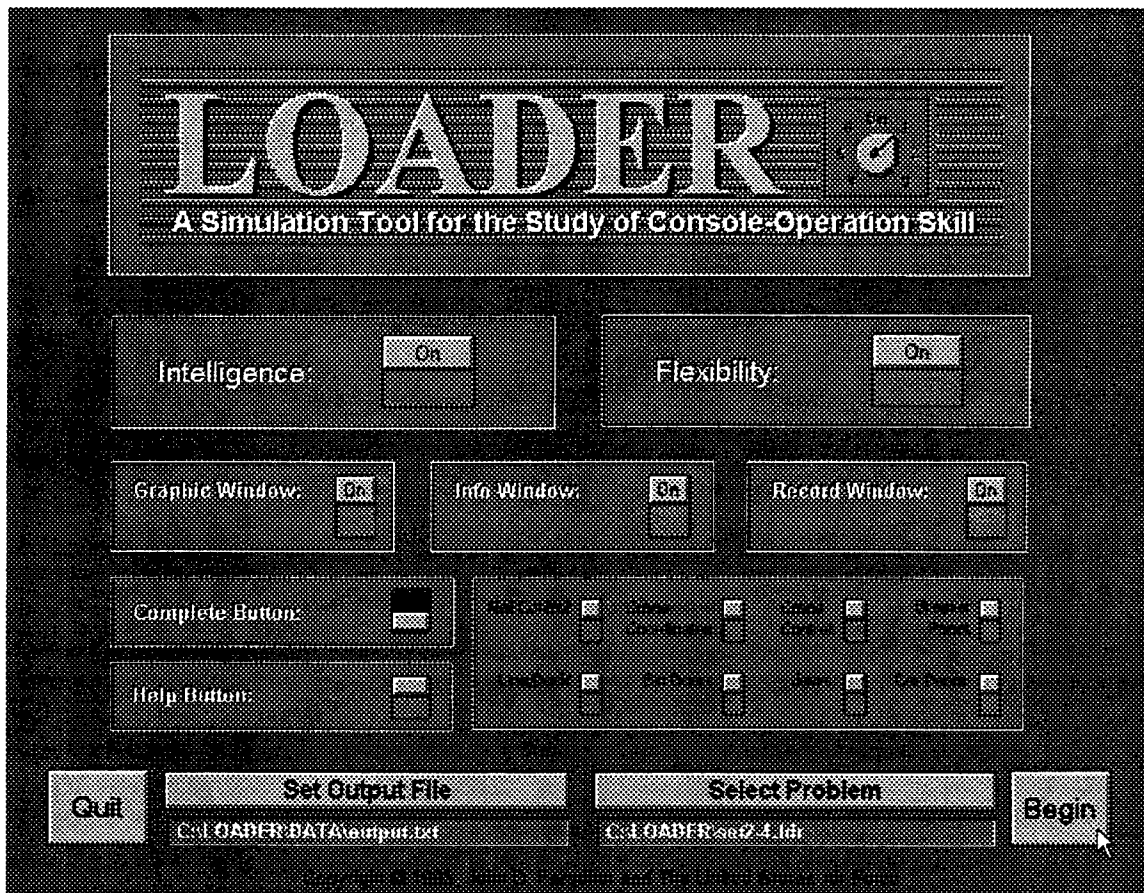


Figure 2: *LOADER Set-up screen showing researcher controls.*

Additional researcher controls include the ability to display or remove various on-screen elements. The dynamic graphical display can be turned on and off as well as the table of information and the student record window. The "Help" button can be removed from the display, and when the "Goal Complete" button option is turned off, the "Goal" button will only be displayed when the expert system determines that goal has indeed been reached. Other controls include the ability to turn on and off the display of each of the eight console panels.

Finally, on the initial LOADER screen, the researcher can set the name and location of the output file as well as selecting from a bank of LOADER problem(s). The LOADER problems are simply files in ASCII format that are executed to initialize problem elements.

Software and Hardware Specifics

LOADER is programmed entirely in OpenScript® using the ToolBook® software development environment from Asymetrix Corporation. Software requirements include MicroSoft Windows as well as ToolBook® run-time files. The program is specifically designed for '486 compatible computers with monitors of 800 X 600 screen resolution.

The Pilot Study

Design: In a 2 X 2 design, the pilot study investigated the effects of the level and timing of feedback during practice of LOADER console-operation skill. Using the LOADER software described above, the intelligence of the system was manipulated corresponding to two levels of feedback (i.e., *smart* and *dumb*). In addition, the flexibility of the system was manipulated to provide *immediate* or *delayed* feedback for non-critical errors. Where the immediate feedback condition always provided feedback within the context of the problem (interrupting and forcing correct performance), the delayed feedback condition allowed subjects to perform non-critical errors with no flagging or notification. Feedback, for the delayed condition, was provided in the form of a summary statement at the completion of each LOADER problem.

Subjects: 13 subjects supplied through a temporary employment agency were paid for their participation in the study. Selection was limited to high-school graduates between the ages of 18 and 30 years. Selection criteria maintained a subject pool representative of Air Force recruits. Since the subjects involved in this preliminary analysis were made available from other research projects, all had engaged in a LOADER-related study several days prior to this investigation.

Procedure: Subjects, occupying a laboratory of 28 computer systems, were randomly assigned to 1 of the 4 treatment conditions. After an initial briefing, subjects completed a set of increasingly complex LOADER problems at their own pace. Each problem was often preceded by a paragraph of prose and a graphic which briefly described the additional complexity presented in the problem. Subjects receiving delayed feedback also received a summary statement, detailing their performance after the completion of each problem. After completing 9 trials of LOADER procedures, subjects were given a questionnaire (see Appendix 2).

Results

This study was conducted as a pilot of the use of LOADER for the investigation of console-operation skill. During this pilot, few programming errors were noted, while subjects were engaged with the software for approximately 1.5 hours. Complete records of subject performance were successfully recorded. Because of the nature of the piloting and the small sample selected, no analyses were performed on the data. Instead, the data is presented in Appendix 3 as example of the outcomes measured.

Appendix #1

Goal: Load Canister from Bin #3, then Dispatch Rail Car to Line #2.

1) Position Rail Car on Line 3 to Dock 2.

1. Set Rail Line to 3
2. Set Rail Dock to 2
3. Set Rail Control to Enable
4. Engage Rail Car South
5. Set Rail Control to Disable

2) Position Crane to Storage Bin 3.

1. Set East Side Storage Bin to 3
2. Enter East Side Coordinates
3. Set Crane Control to Enable
4. Execute Coordinates
5. Set Crane Control to Disable

3. Lift Canister from Storage Bin 3

1. Open Storage Bin Door 3
2. Engage Crane Auto Down
3. Close Crane Jaws
4. Engage Crane Auto Up
5. Close Storage Bin Door 3

4. Position Crane to Car at Dock 2, Receptacle 2.

1. Set West Side Car Number to 2
2. Set West Side Receptacle to 2
3. Enter West Side Coordinates
4. Set Crane Control to Enable
5. Execute Coordinates
6. Set Crane Control to Disable

5. Load Canister into Rail Car.

1. Open Car Door at Dock 2
2. Engage Crane Auto Down
3. Open Crane Jaws
4. Engage Crane Auto Up
5. Close Car Door at Dock 2

6. Dispatch Car to Rail Line 2.

1. Set Dock to 2
2. Set Rail Line to 2
3. Set Rail Control to Enable
4. Engage Rail Car North
5. Set Rail Control to Disable

Table 2: *An example of a simple LOADER problem.*

Appendix #2: The Questionnaire

Directions:

Please take a few moments to respond to this questionnaire about LOADER. We will use this information to help us evaluate instructional strategies that you have encountered within the program. For each line, circle the most appropriate number on the right according to the scale provided below. Please note: negative comments are *just* as important as positive comments.

	Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree
How did you <i>use</i> LOADER?					
1. I made many mistakes.	1	2	3	4	5
2. I frequently selected "Help".	1	2	3	4	5
3. I selected "Help" whenever I was confused .	1	2	3	4	5

How do you *feel* about LOADER?

	Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree
4. I enjoyed LOADER.	1	2	3	4	5
5. I was interested in LOADER.	1	2	3	4	5
6. I was often bored with LOADER.	1	2	3	4	5
7. I was concerned about making mistakes.	1	2	3	4	5
8. I felt in control of my learning.	1	2	3	4	5
9. I found LOADER to be challenging.	1	2	3	4	5
10. I found LOADER to be frustrating.	1	2	3	4	5
11. I felt free to explore options.	1	2	3	4	5
12. I found LOADER to be confusing.	1	2	3	4	5
13. I learned how to perform the loading task.	1	2	3	4	5

About the feedback messages:

LOADER displays two different kinds of feedback messages. *Red* feedback messages indicate mistakes, whereas *blue* feedback messages provide help. *Blue* feedback messages are also available by selecting "Help." Please answer questions 14 through 20 about the *red* feedback messages, and answer questions 21 through 28 about the *blue* feedback messages.

	Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree
How did you use the <i>red</i> feedback?					
14. I always read the red feedback.	1	2	3	4	5
15. I found the red feedback to be helpful.	1	2	3	4	5
16. I felt confined by the red feedback.	1	2	3	4	5
17. I understood the red feedback.	1	2	3	4	5
18. The red feedback interrupted my train of thought.	1	2	3	4	5
19. The red feedback provided useful information.	1	2	3	4	5
20. The red feedback could be more helpful.	1	2	3	4	5

How did you use the *blue* feedback?

	Strongly Agree	Agree	No Opinion	Disagree	Strongly Disagree
21. I always read the blue feedback.	1	2	3	4	5
22. I found the blue feedback to be helpful.	1	2	3	4	5
23. I felt confined by the blue feedback.	1	2	3	4	5
24. I understood the blue feedback.	1	2	3	4	5
25. The blue feedback interrupted my train of thought.	1	2	3	4	5
26. The blue feedback provided useful information.	1	2	3	4	5
27. The blue feedback could be more helpful.	1	2	3	4	5
28. I always selected "More" from the blue feedback.	1	2	3	4	5

Time over Trials

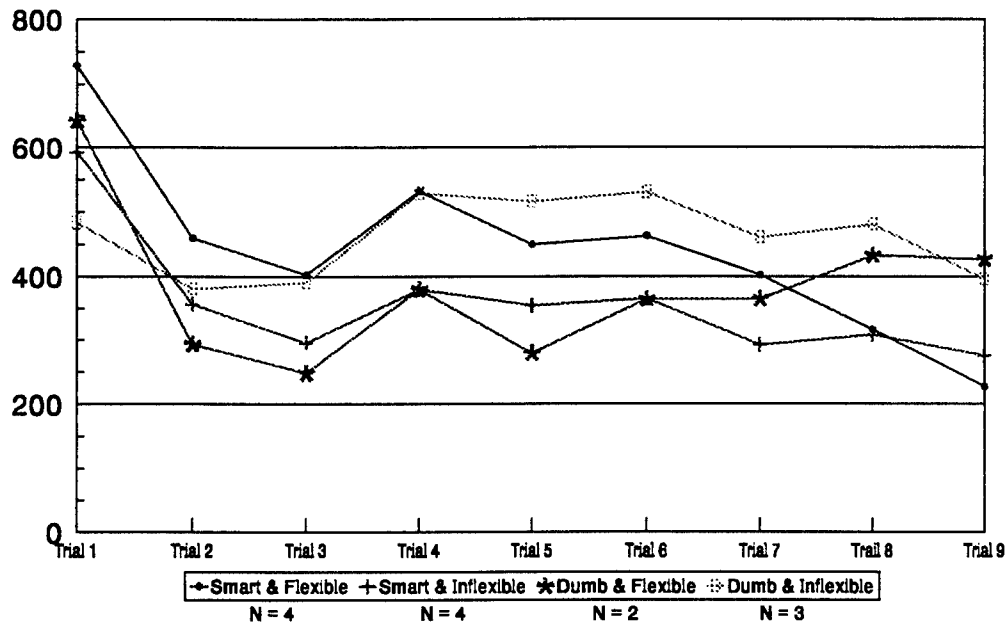


Figure 3: Time over Trials.

Steps over Trails

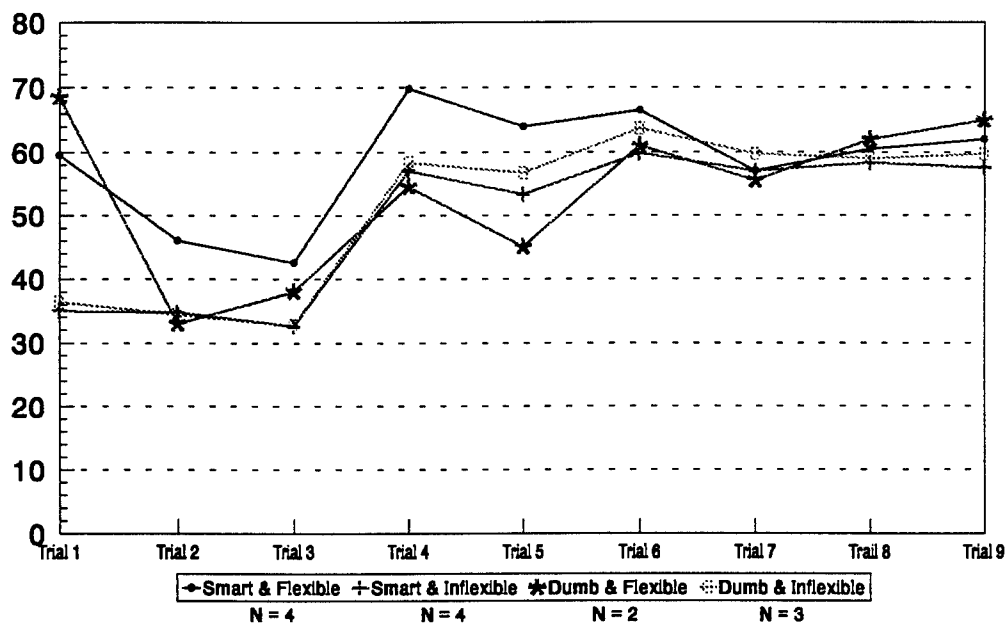


Figure 4: Steps over Trails.

Errors over Trials

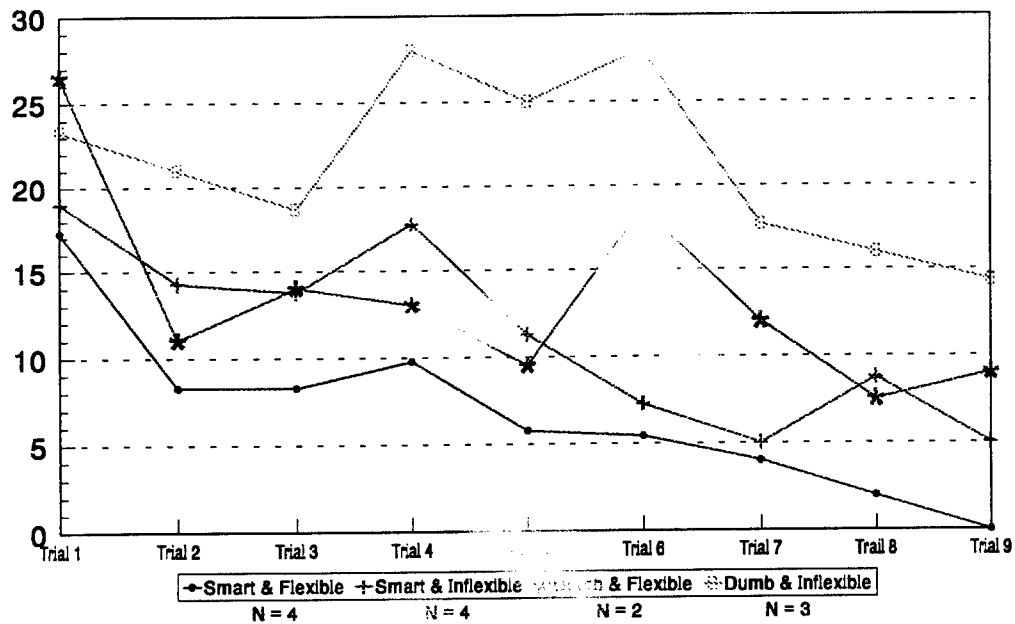


Figure 5: Errors over Trials.

Help over Trials

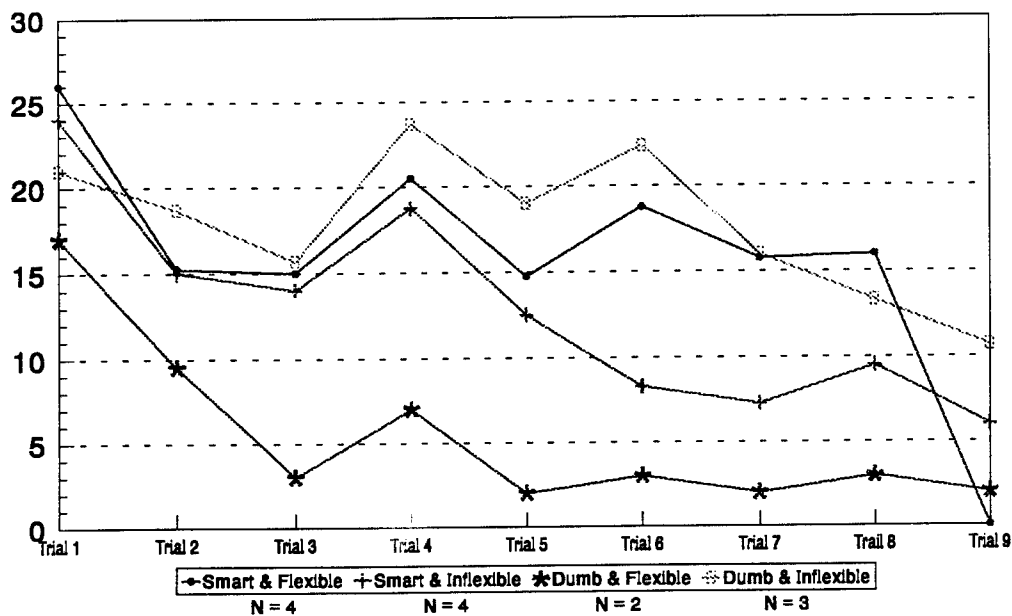
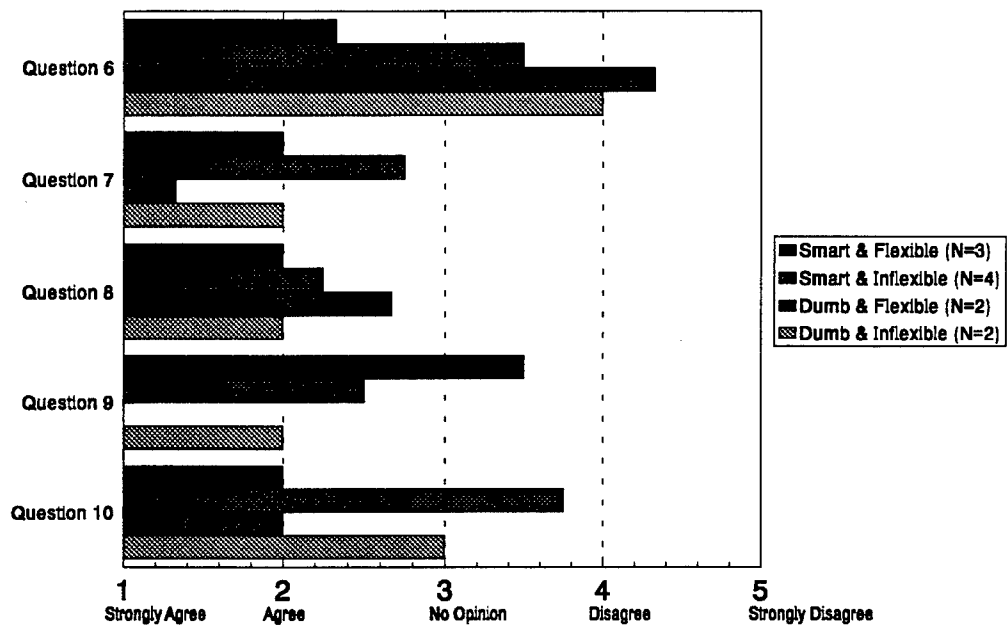
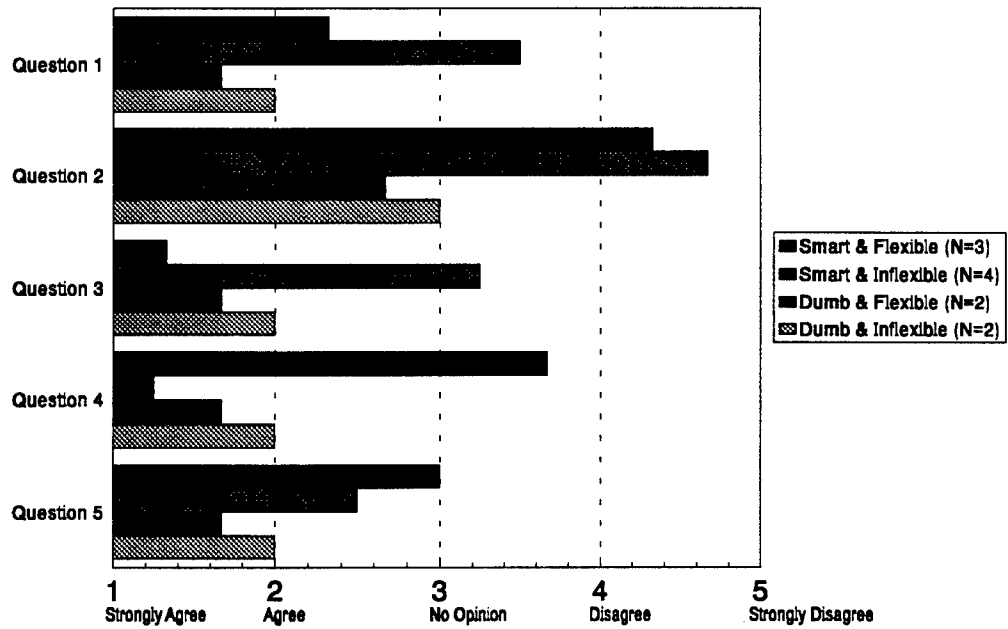
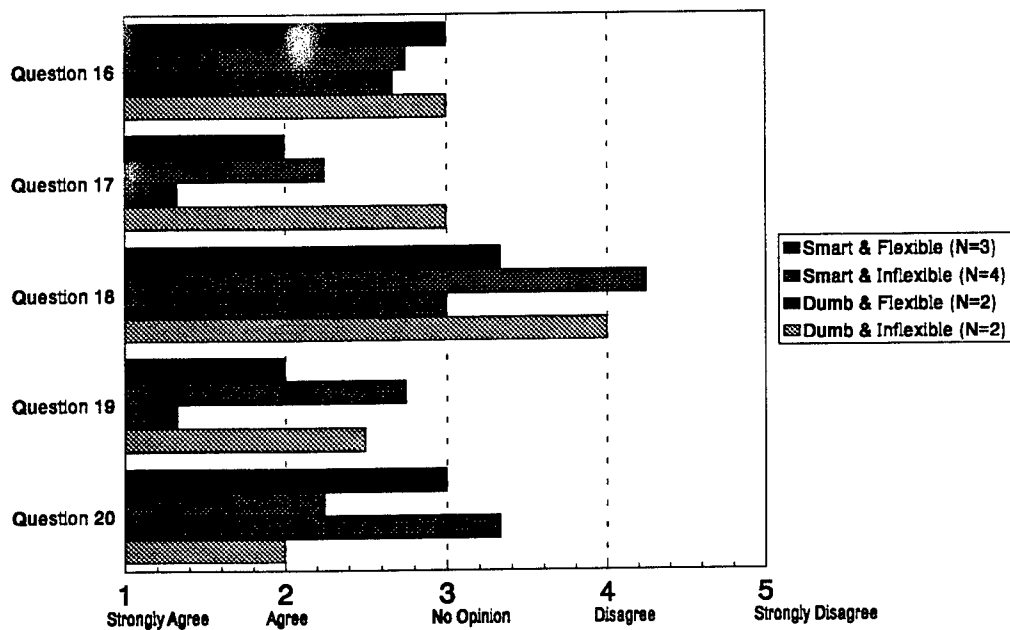
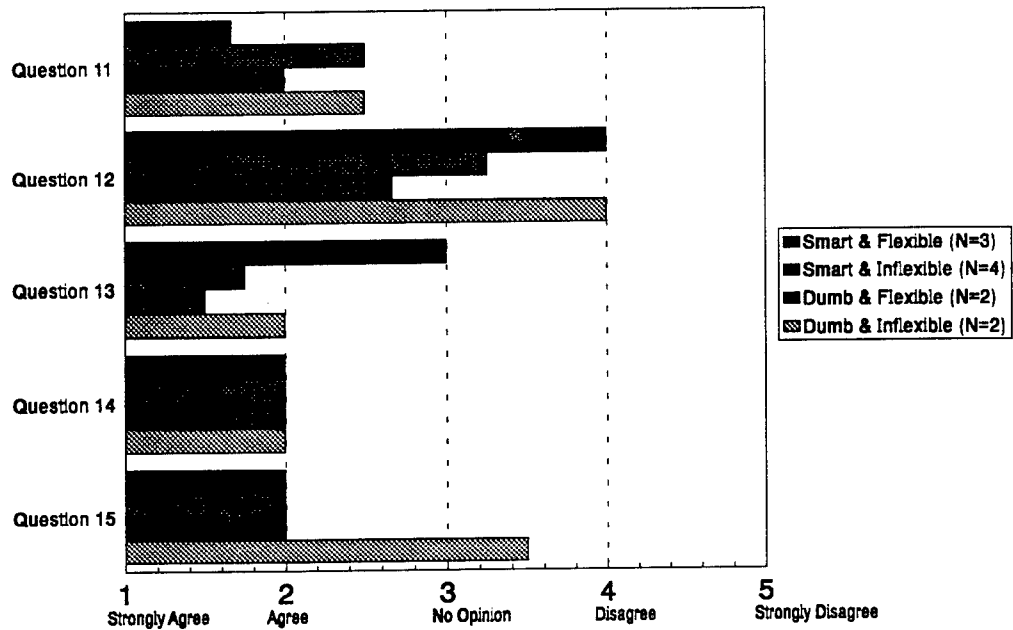
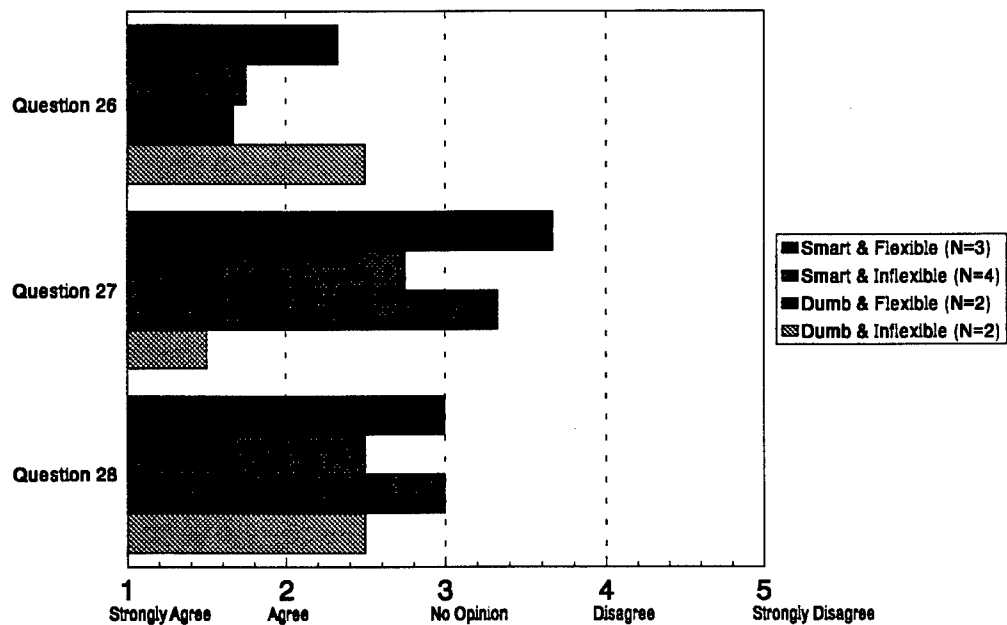
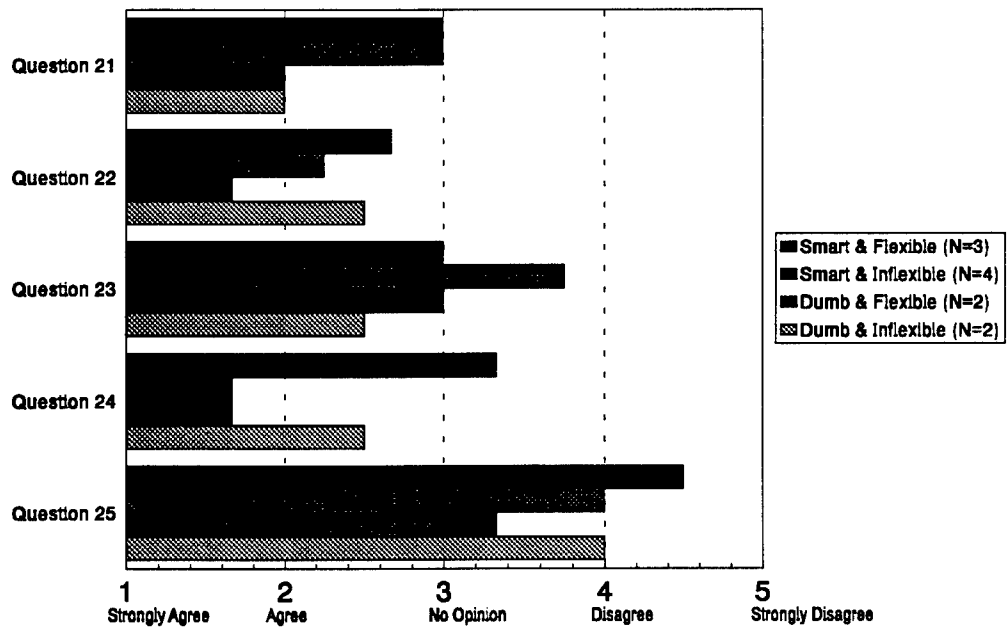


Figure 6: Help over Trials.







**GUIDELINES FOR USING A TERRAIN BOARD WITH
NIGHT VISION GOGGLE TRAINING**

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Final Report for Graduate Student Research Program

Armstrong Laboratory

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Abstract

Standardized Night Vision Goggle (NVG) training is relatively new in the Air Force. One component of the training typically involves exposure to a terrain board. As of this report, no standardized set of objectives or testing materials have been developed for terrain board training. Subject matter experts were interviewed and terrain board demonstrations were attended in order to gather information on terrain board instruction. From this information, objectives and test items were developed for terrain board training. Based on these objectives, an analysis of the terrain board as an instructional medium relative to NVG training requirements was conducted and recommendations were made for enhancements to existing terrain boards.

GUIDELINES FOR USING A TERRAIN BOARD WITH NIGHT VISION GOGGLE TRAINING

Mark J. Gavora

Night vision goggles have been in use for some time. In 1971, the Army began using NVG's (Night Vision Goggles) in aircraft (Verona & Rash, 1989), and other branches of the military quickly followed suit for their aircraft. Today, NVG's are used in every branch of the military including the Reserves and the National Guard. The goggles are used in fighter aircraft, helicopters and transport aircraft, each performing different tasks and having unique requirements. From an Air Force perspective, NVG's are used to enhance night combat effectiveness and safety. The military, however, is not the only user of NVG's. NVG's are also used by police for surveillance (Byrd, 1992) and enhancement of dark environments like subways (George, 1992). The goggles are also advertised in boating magazines (Wales, 1993) for helping boating enthusiasts identify marks or obstacles in the dark.

Development of a standardized NVG training course for the Air Force was accomplished by the Night Vision Program Office, Aircrew Training Research Division of Armstrong Laboratory at Williams AFB in 1992. The developed course was derived, to a large extent, from the Night Imaging and Threat Evaluation Laboratory (Nite Lab) concept developed by the Marine Corp. Part of the Nite Lab concept was a terrain board which was used to dynamically demonstrate NVG effects. By 1991, the first USAF standardized terrain board (different from the one used for course development) was installed at Kirtland AFB. To date, seven have been installed at various locations with plans for more in the future. In addition to these terrain boards, others exist throughout the remaining branches of the military. Although standardized

with respect to design, the Air Force course has not yet standardized procedures for using the terrain board.

From an instructional design perspective, standardized procedures for training and evaluation can be developed using the systems approach (c.f. AFM 50-2, Dick & Carey, 1990). In addition to developing such procedures, an analysis of the terrain board as an instructional medium can take place. What can we use a terrain board to teach? What modifications could be made to the terrain board to enhance its utility? Is another medium likely to be more effective at teaching objectives currently taught using a terrain board? The goals of this research project were to: 1) develop standardized objectives for terrain board based training; 2) conduct a media-analysis of the terrain board as an instructional medium.

The target population, as identified by subject matter experts, for terrain board training will consist of aircrew who have had no significant, if any, experience with NVGs. Instructions will be given regarding how to focus the goggles, adjust the interpupillary distance and turn the NVGs on or off. All students will have a working knowledge of Air Force terminology. No other entry behaviors will be required that have not already been screened for by Armed Forces personnel.

Training Analysis Results

Interviews with terrain board experts and a review of literature relative to NVG training was conducted. Very little has been published regarding terrain board training. From the interviews, only one site had developed training objectives, a U.S. Marine base in Yuma, AZ. These objectives were integrated into the design evaluation chart. Classes typically last about

20 minutes and class sizes typically range from four to eight students. No post instructional tests were developed at any site that was interviewed.

The literature reviewed addressed a number of training content issues. From Verona and Rash (1989), the following issues were identified: 1) identification of roads; 2) lighting effects on NVG performance; 3) detection of powerlines; 4) influence of particulate matter on NVG performance; 5) lack of color information; 6) influence of dirty windows and/or lenses on NVG performance. From Scott (1992), the following training issues were identified: 1) effect of fog on NVG performance; 2) detection of powerlines. From Mason (1990), the following issues were identified: 1) shadowing; 2) moon angle; 3) azimuth. From Oldham (1990), the following issues were identified: 1) depth perception; 2) light level; 3) object contrast; 4) powerlines; 5) lens condensation. From Brickner (1989), the following issues were identified: 1) perceived flatness of terrain; 2) distance estimation; 3) identification of gradients; 4) contrast; 5) light sources; 6) distance estimation; 7) depth perception; 8) low rolling hills, 9) gradients; 10) shadowing. From Berkley (1992), the following issues were identified: 1) depth perception; 2) scene contrast; 3) reflection; 4) snow-covered terrain; 5) identification of size differences; 6) desert terrain; 7) moon position; 8) cultural lighting; 9) distance estimation; 10) shadowing; 11) infrared light sources; 12) slopes; 13) atmospheric conditions; 14) water. One or more objectives was developed for each of these issues and from issues identified through discussions with NVG subject matter experts.

Subject matter experts were also consulted on issues relating to the design of objectives and the overall instructional goal. The resulting instructional goal was developed for terrain board instruction: Students will be able to identify terrain features, atmospheric conditions, light

sources and cultural features distinguishable through night vision goggles. For features that are not distinguishable, students will indicate (by stating or selecting the appropriate option) those features are not identifiable. Consultation with subject matter experts and the literature review were used to develop the following goal analysis: 1) students should be able to identify anomalies that can be seen through the goggles but do not indicate that there are problems with the goggles; 2) students should be able to select those ways in which varying illumination levels can influence what you see through NVGs or how to interpret what is seen through NVGs; 3) students should be able to identify atmospheric conditions; 4) students should be able to identify terrain features; 5) students should be able to identify cultural features.

A subskill analysis was conducted for each element in the goal analysis, then a design evaluation chart was developed containing each subskill with corresponding objectives and test items. Each subskill appears under only one element in the goal analysis regardless of how many elements it could belong to. This was done because students would not be allowed to progress to the next objective until the instructor believed they had mastered the first one. Teaching an already learned objective under a different category would appear repetitive to the students without necessarily increasing their knowledge of the objective's content.

First draft instruction and tests were developed based on the design evaluation chart and formatively evaluated using one on one evaluations ($n=5$). Students in the one on one evaluations had no prior training or expertise with NVGs. They were instructed how to adjust and focus the NVGs. Once preinstructional criteria had been met, students completed the instruction, took a test and were interviewed. The interview consisted of questions regarding the reasons for incorrect responses, overall impressions regarding the instruction including

recommendations for change in the instruction and/or testing materials. Based on performance during the instruction, the tests and interviews, modifications were made to the objectives and test questions in the design evaluation chart. The design evaluation chart was then reviewed again by subject matter experts to ensure its validity and the appropriate changes were made.

An analysis of the terrain board as an instructional medium was developed based on consultation with subject matter experts and results from the one-on-one evaluations for the terrain board at AL/HRA. For terrain boards at other sites, analysis of the medium was based on contour maps, schematics and interviews with terrain board experts at the respective sites. Where a modification to the terrain board could facilitate mastery of an objective, the appropriate recommendations have been made under the change requirements column. In addition to this analysis, the following modifications are recommended for the instructional setting at AL/HRA: 1) have the moon travel across a parabolic rail traversing the X, Y and Z axes so that moon effects can be more easily observed; 2) provide student accessibility to the controls allowing students opportunities to discover how unique combinations of environmental stimuli are seen through the NVGs; 3) remove the top half of the wall separating students from the terrain board or replace top half of wall with glass enabling students to view the terrain through a greater number of angles and altitudes; 4) permanently mount the weather simulator between two plates of glass to eliminate the likelihood of the weather simulator being touched by students and reduce the concavity of the simulator panel; 5) mount weather simulator controls in proximity to other controls to increase the ease of manipulating the weather simulator controls.

The objectives and test items in the design evaluation chart can be used as the basis of instructional content at terrain board sites throughout the Air Force as well as other branches of the military. The test items corresponding to the objectives are not absolute and can be substituted. The design evaluation chart can also be used as a basis for evaluating the terrain board component of existing NVG courses. Course effectiveness can be determined to the extent that the objectives, identified in the design evaluation chart, are learned by students. Once the design evaluation chart and medium analysis are finalized, they will be available by publication as an Armstrong Laboratory Technical Report.

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TWO ISSUES RELATED TO PERSONNEL SELECTION AND PLACEMENT:
PERCEIVED ABILITIES AND BIODATA

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Abstract

The development and improvement of personnel selection and placement measures is a constant concern both in the Air Force and in private industry. There remain many unresolved issues in this area concerning the relationships among such variables as perceived and actual abilities, interests, biodata, and the development of personality. Two simultaneous research programs related to these concerns are described in this paper. Study 1 involved an investigation into subjects' capacities to assess their own knowledge and ability level in a variety of topics. Study 2 examined the correlations among biodata items and present personality structure. Subjects were 273 male Air Force basic trainees. Results from both studies involved low to moderate correlations interpreted as promising, but not sufficiently so as to merit full reliance on such methods in an employment setting. Instead, it is suggested that the two selection techniques under investigation here are perhaps best used as a means of gathering supplementary information on prospective employees.

TWO ISSUES RELATED TO PERSONNEL SELECTION AND PLACEMENT: PERCEIVED ABILITIES AND BIODATA

Kevin A. Gluck

Since roughly the beginning of U. S. involvement in World War I, the military has understood the importance of competent screening and placement of enlisted personnel. As time has passed and the various fields of psychology have matured, it has become apparent that psychologists can indeed play an important role in the creation and improvement of selection and placement measures. At the suggestion of Dr. Raymond Christal, I developed two separate inventories designed to investigate issues related to exactly these types of personnel concerns. Although they were administered back-to-back within the same test session for each subject, the conceptual and theoretical backgrounds of each of the surveys are sufficiently distinct as to merit their being discussed as separate studies.

Study 1

The awareness of having developed an ability in a certain skill area or knowledge of a particular topic, also known as "meta-knowledge" (P. C. Kyllonen, personal communication, June 1993), has been the focus of a fair amount of research in recent decades. Much of the interest in this subject has been generated out of a practical need to increase the efficiency with which employers can find and place personnel. Despite the potential benefits of this selection procedure, it has not often been utilized in actual employment settings (Reilly & Chao, 1982). Levine, Flory, and Ash (1977) propose that this is due to the following three common assumptions: 1) applicants will inflate self-estimates of their own abilities, skills, and knowledge, 2) applicants are incapable of making accurate self-appraisals, and 3) the first two assumptions will result in low validity of the self-ratings.

Research aimed at confirming or debunking those assumptions has resulted in a variety of conclusions concerning self-assessment. Ash (1980), DeNisi and Shaw (1977), and Primoff (1980) all completed studies which compared self-ratings on abilities with objective tests of those abilities. All three obtained low to moderate correlations in roughly the .03 to .59 range. Other researchers have found considerably lower correlations, but their studies typically involved the relationship between self-assessments and something like

supervisory ratings (Ekpo-Ufot, 1979) or job performance (Farley & Mayfield, 1976), which clearly are not objective measures of either ability or knowledge.

It was decided that the disparate results obtained in past research merited an investigation of my own. I developed an inventory meant to measure the correlation between perceived and actual knowledge and ability level in a variety of subject areas and tasks.

Method

Subjects

Subjects were 273 Air Force basic trainees at Lackland Air Force Base in San Antonio, Texas. All subjects were male. Participation in the study was fully voluntary, although it was a scheduled part of the activities of the 21st day of basic training.

Materials

Tests were administered via IBM-compatible Zenith 248 personal computers, and all test items were answered with a mouse.

The Knowledge and Abilities Awareness Inventory (KAAI) was designed to evaluate subjects' awareness of their own abilities and skills, as well as the degree to which they are aware of possessing knowledge about specific topics relevant to Air Force occupations. The accuracy of subject responses on the 55 questions which make up this inventory was to be evaluated in respect to their scores on the Armed Services Vocational Aptitude Battery (ASVAB), which they had completed at least several months prior to their participation in this study. The ASVAB is a test which is administered to over 1 million high school students each year (U. S. Department of Defense, 1987) and which has moderate to high reliability and validity in predicting future job performance in the armed services (Kelso, Holland, & Gottfredson, 1977). Table 1 presents several sample questions from the KAAI. It is important to know that each of these questions was followed on the computer screen by the incomplete sentence, ". . . compared to other individuals of the same sex and approximate age." This gave the subjects a reference group to use in answering each item. Every item in this inventory was answered by clicking the mouse at any location along a 45-point arch scale that ranged

from "Extremely Below Average" (scored as -22) to "Extremely Above Average" (scored as 22). In addition, the fact that this was a computerized test session made it possible to present each of the 55 items in a different random order for each subject.

Table 1
Sample Questions from the Knowledge and Abilities Awareness Inventory (KAAI)

How good is your vocabulary?
How well can you do arithmetic word problems in your head?
How high is your level of mechanical knowledge?
How good are you at video games?
How much do you know about computers?

Procedure

A maximum of 39 subjects were tested during any one session. Subjects entered the testing room as a flight, and stood in front of their assigned computers while listening to the directions. The test proctor explained during the directions that it would be extremely important not to look at anyone else's computer screen in the course of the testing session. This was facilitated by the fact that each computer was located in its own cubicle space, which formed a natural barrier between subjects. After sitting down and making themselves comfortable, subjects entered various vital statistic information (i.e., sex, race, education level), and then received a computerized tutorial on use of the mouse. The mouse was moved to the left side of the computer for left-handed subjects who indicated a desire to have this done.

The entire test battery consisted of eight tests and inventories in the following order: Self-Ratings, Peer Selection, Peer Ratings, KAAI, BALE, VOICE, ABLE, and a Reading Interest Survey. The first three tests were part of an independent probe study created by Dr. Christal as a means of evaluating the validity of self-ratings on personality trait names by comparing them to peer ratings on the same traits. Once they had started the battery, subjects were allowed to work at their own pace. After the subjects had completed BALE, the 5th test in the battery, they were given a 5-minute break, at which time they could leave their seats to get a drink of water or use the bathroom.

Having returned to their terminals, subjects were instructed to begin the second half of the test session. Upon completion of our battery, most subjects started working on a set of tests from a completely separate study. Those who took a bit longer were allowed to stop working and take a nap or read.

Results and Discussion

A Pearson product-moment correlation revealed low to moderate relationships between subjects' perceived abilities and knowledge level in certain areas, as measured by the KAAI, and their actual scores in those subject areas on the ASVAB. Forty of the 55 perceived abilities test items (72.7%) correlated above .20 ($p < .001$) with subjects' scores on the ASVAB. Table 2 lists only those items, however, which correlated above the .30 level with the subtests and composites which make up the ASVAB. The highest correlations with KAAI questions occurred among ASVAB subtests and composites having to do with mechanical, electrical, and mathematical knowledge and ability. Particularly impressive among these are the correlations in the mid- to high-.50's of single items in the Math Knowledge and Auto & Shop Information subtests, as well as the Mechanical and Crafts composite. In addition to the fact that several of the correlations in these areas reach relatively high levels, the nature of the items within each of these groups of correlations makes good common sense. An excellent case in point, but not the only one, is the Math Knowledge subtest. Every item that correlated above .30 with this subtest concerns predicting ability in solving math problems of various sorts or estimating knowledge level in a related area like calculus. This is exactly what the items were designed for, and thus the face validity of these items is strongly supported by the empirical results.

In direct contrast to these promising results is the virtual lack of significant correlations found in such areas as Paragraph Comprehension and Coding Speed. Of course, these are two of the four subtests which make up the Business and Clerical composite, thereby explaining the lackluster correlation found there.

Thus, one notes an apparent trend towards higher correlations in mechanical, electrical, and mathematics areas and lower correlations in tests of verbal ability. How might one explain the dichotomy apparent in these results? First of all, one could hypothesize that the perception of one's ability level in

Table 2

SURTTESTS		
General Science		
.445	How much do you know about science?	.328
.369	How much do you know about outer space?	.304
.367	How much do you know about the kinds of waves that move through the air?	.303
Arithmetic Reasoning		
.453	How well do you solve math problems involving variables?	.556
.443	How good are you at solving algebra problems?	.532
.433	How good are you at solving math problems?	.493
.431	How good are you at solving math problems?	.471
	How well can you do arithmetic word problems in your head?	.354
.355	How much do you know about calculus?	.342
.347	How good are you at multiplication and division?	.309
.331	How familiar are you with the laws of physics?	
.313	How well can you hold numbers in your head while doing math problems?	
Word Knowledge		
.328	How good is your vocabulary?	.408
.325	How much do you know about science?	.381
.300	How well can you figure out the meaning of a word from its use in a sentence?	.377
Paragraph Comprehension (None)		
.321	How good are you at solving algebra problems?	.492
Numerical Operations		
	How good would you be at re-wiring the circuits in a house?	.451
Coding Speed (None)		
.592	How high is your level of mechanical knowledge?	.441
.511	How well can you imagine the movement of different parts of a mechanical device?	.437
.486	How much do you know about working with metal or wood?	.328
.477	How much do you know about the use of power tools?	.325
.473	How much do you know about how your car works?	.305
.465	How good would you be at installing a ceiling fan?	
.465	How familiar are you with tools used in a typical garage?	
.372	How good would you be at re-wiring the circuits in a house?	
.371	How much do you know about electrical circuits?	
Health, Social, and Technology Reasoning, Paragraph Comprehension, Arithmetic Reasoning, Mechanical Comprehension		
.343	How good are you at solving algebra problems?	.562
.343	How well do you solve math problems involving variables?	.521
.315	How well can you do arithmetic word problems in your head?	.418
.314	How familiar are you with the laws of physics?	.413
.309	How much do you know about calculus?	.407
Math Knowledge		
	How good are you at solving algebra problems?	.393
	How well do you solve math problems involving variables?	.393
	How good are you at solving math problems?	.385
	How good are you at solving math problems?	.378
	How well can you hold numbers in your head while doing math problems?	.377
	How well can you do arithmetic word problems in your head?	.324
Mechanical Comprehension		
	How well can you imagine the movement of different parts of a mechanical device?	
	How much do you know about electrical circuits?	
	How high is your level of mechanical knowledge?	
	How much do you know about electricity?	
	How good would you be at re-wiring the circuits in a house?	
Electronics Information		
	How much do you know about electrical circuits?	
	How well can you imagine the movement of different parts of a mechanical device?	
	How good would you be at re-wiring the circuits in a house?	
Auto & Shop Information		
	How high is your level of mechanical knowledge?	
	How good would you be at installing a ceiling fan?	
	How much do you know about how TV's and radios work?	
	How much do you know about the use of power tools?	
COMPOSITES		
Business and Clerical		
(Word Knowledge, Paragraph Comprehension, Mathematics Knowledge, Coding Speed)		
.312	How good are you at solving algebra problems?	
Arithmetic Reasoning, Mathematics Knowledge, Electronics Information, General Science		
.397	How much do you know about calculus?	
.392	How familiar are you with the laws of physics?	
.385	How much do you know about electrical circuits?	
.381	How much do you know about science?	
.373	How good are you at solving algebra problems?	
.373	How well do you solve math problems involving variables?	
.341	How much do you know about electricity?	
.338	How much do you know about the kinds of waves that move through the air?	
.323	How good are you at reading diagrams?	
.301	How familiar are you with geology?	
Mechanical and Crafts		
(Arithmetic Reasoning, Mechanical Comprehension, Auto & Shop Information, Electronics Information)		
	How high is your level of mechanical knowledge?	
	How well can you imagine the movement of different parts of a mechanical device?	
	How much do you know about electrical circuits?	
	How good would you be at installing a ceiling fan?	
	How much do you know about working with metal or wood?	
	How much do you know about electricity?	
	How much do you know about the use of power tools?	
	How familiar are you with tools used in a typical garage?	
	How good would you be at re-wiring the circuits in a house?	
	How much do you know about how your car works?	
	How much do you know about camping and hiking?	

different areas is a function of both the existence and the quality of negative and positive feedback received as a result of past efforts related to those abilities. In other words, if a certain individual consistently received good grades in math classes, that person would probably develop a self-concept of being "good" at math. In the case of math classes, where answers are typically either right or wrong in an objective sense, such a self-concept is most likely accurate. Feedback in Reading and English classes, however, is often much more subjective in nature. It's certainly feasible that a students with a relatively weak grasp of the spelling and grammar rules of the English language could sneak through a large portion of their education with teachers who weren't particularly concerned about such "trivial" matters. These students would theoretically receive average to good grades in a subject in which they were actually performing rather poorly. This situation probably sounds very familiar to those acquainted with the general shortcomings of the educational system or the outrage resulting from the occasional athlete who receives lenient grading in order to be eligible to play in an upcoming competition.

As for the promising results in areas concerning mechanical and electrical aptitude and knowledge, I would argue that these subject areas are specific enough that the average person has an excellent idea whether or not he has been exposed to that topic. Those who grew up around people who worked on cars, tended to do their own repairs on home appliances, or liked to take shop classes in high school would most likely rate themselves as being high in knowledge and ability in these areas. Others, like myself, who shudder at the thought of having to perform even easy tasks like changing a tire, would rate themselves low. It's just that simple.

A critical examination of the test materials themselves may result in further insight into the split in results. Primoff (1980) found that two factors influence the extent of the correlation between self-ratings and other variables: 1) the degree to which both the self-raters and the test developers have the same understanding of the scaled behavior, and 2) the extent to which self-raters have a common base for their ratings (p. 284). Based on his results, it could be the case that different subjects simply vary in their interpretations of what constitutes above or below average performance in areas like Paragraph Comprehension or Vocabulary

Knowledge. In other words, subjects might have had very little exposure to objective measures of items like "How good are you at solving crossword puzzles?", which means that they have difficulty in comparing themselves to others.

Finally, the old assumption that subjects will tend to inflate self-estimates of ability might indeed have negatively impacted the results of this study. The possibility of such an impact prompted an investigation into the matter, which revealed that of the 55 mean ratings of perceived ability, only four turned out to be below average. That indicates that for this sample of 273 Air Force enlistees, above average mean estimates of knowledge and ability were reported in fully 92.7% of the measured items. This result appears a bit suspect and may be indicative of over-confidence on the part of the subjects or even the faking of "above average" responses. Such a conclusion would be supported by certain past research efforts (Ash, 1980; Farley & Mayfield, 1976), which have shown a tendency for subjects to inflate their self-assessments.

Study 2

As part of a simultaneous research project, I began an investigation into the use of biodata information as a predictor of present personality structure. The issue of the degree to which environmental variables interact with genetic factors to affect the development of one's personality, in addition to other aspects of the whole person, has always been central to the "Nature vs. Nurture" debate (Baron, 1989, p. 382). Thanks to the work of some dedicated psychologists, however, few individuals in the scientific community today would argue that one's personality develops wholly irrespective of environmental variables. Earlier life experiences clearly influence present personality characteristics, interests, and abilities. This theoretical perspective is the rationale behind the collection of biographical information (biodata) in an effort to predict some criterion, like job performance. The challenge facing researchers today is to uncover whatever patterns of earlier life behavior might exist that predispose one towards certain personality characteristics.

The reason this is important is that certain relationships have appeared that indicate a connection between one's personality and job performance, success in personal relationships, and the like. For example, Barrick and Mount (1991) demonstrated in a meta-analysis that scores on the personality factor "Conscientiousness" are predictive of subjects' later job performance. This whole issue is closely related to a

project that Christal has been working on involving the measurement of the "Big 5" personality factors (Tupes & Christal, 1992) using individual personality trait names (e.g., Friendly, Envious). The relationship to which I refer develops out of the fact that I used Christal's trait name self-rating survey as the criterion for my study. In so doing, I was able to collect biodata information about subjects' childhood and high school experiences, and then look for correlations with their personality scores, as measured by their self-ratings on the trait names. The motivation behind the study, therefore, was the hope that a strong correlation might be found that would allow one to make inferences about subjects' future behaviors based solely on information about their past. Admittedly, this idea is nothing new to the military, which has been trying for decades to develop a reliable means by which to use biodata as a predictor of attrition (Laurence, 1990). Nevertheless, strong support for continued research in this area can be found in Owens' (1976) chapter on background data, during which he concludes that "biodata is an efficient, robust, and highly valid predictor of a broad spectrum of very practical criteria" (p. 612).

Subjects

The 273 subjects used in Study 2 were exactly the same subjects who had participated in Study 1, since the two inventories were administered consecutively during the same test session.

Materials

The inventory designed for Study 2 was named the Background And Life Experiences (BALE) Inventory. Past research suggests that criterion-related validity coefficients can be improved simply by making the effort to formulate background data items with reference to a particular underlying construct or theoretical framework (Mumford, Uhlman, & Kilcullen, 1992). Keeping this in mind, items for the Background And Life Experiences (BALE) Inventory were generated on the basis of apparent relevance to one or possibly more of the "Big Five" personality factors (Goldberg, 1993; Tupes & Christal, 1992), which include Conscientiousness, Agreeableness, Extraversion, Neuroticism, and Intellect. The BALE Inventory consists of 96 items designed to be answered on a 5-point scale from "Never" to "Always." Subjects responded to the questions by clicking a computer mouse on one of the five boxes offered as options. No numbers were included above the words. The items themselves required subjects to think back to their childhood and high school years in order to indicate

how often they had experienced certain thoughts or done certain things. Table 3 lists a small subset of questions representative of the types of items found throughout the inventory, along with the personality factor used to generate the item.

Table 3
Representative Biodata Items by Personality Factor

Conscientiousness	How often did your guardian(s) reward you for good grades?
	How often were you late for things?
Agreeableness	How often did you get in fist fights during high school?
	How often did you have a "best" friend while you were growing up?
Extraversion	How often did you attend social parties in high school?
	How often did you enjoy being alone?
Neuroticism	How often were you left home alone as a child, without a babysitter?
	How often did you get the feeling no one loved you during your childhood?
Intellect	How often did your guardian(s) read to you when you were younger?
	How often were you tutored because you were having trouble with a certain class?

Christal's self-rating personality survey, which was used as the criterion in Study 2, consists of 30 trait names that have been found to correlate highly and reliably with the Big Five personality factors (Christal, 1993). Therefore, there were six trait names representing each of the five personality factors. Subjects were told to rate each of the 30 trait names as somewhere between "Extremely Characteristic" and "Extremely Uncharacteristic" of themselves on a 45-point arch scale like the one used in the KAAI from Study 1. Ten trait names that served as warm-up items preceded the 30 actually used in the study.

Procedure

Most of the relevant information pertaining to the procedure used in Study 2 has been detailed in the procedure section of Study 1. Suffice it to say that subjects proceeded at their own pace from the KAAI Inventory to the BALE Inventory and then took their five minute break.

Results and Discussion

A factor score for each of the five personality factors was computed for every subject based on their self-ratings of the 30 personality trait names that made up the first test in the battery. A Pearson product-moment correlation of the BALE biodata item responses with these Big Five personality factor scores revealed

moderate relationships between certain past life experiences and present personality structure. Table 4 displays the results of this analysis. Thirty-eight of the 96 items created for the study, or 39.6%, correlated above .20 or below -.20 ($p < .01$) with the personality factors. Only those 38 items are reported in Table 4.

Table 4
Correlations of Biodata Items with the Big Five Personality Factors

<u>CONSCIENTIOUSNESS</u>	
.399	did you pick up and clean your room as you were growing up?
.359	did you proofread an assignment to check for spelling or math errors before turning it in? *
.342	did you make an effort to organize your clothes in drawers and keep them neat?
.326	did you wash your hands after using the bathroom?
.288	did you write an outline before you began an essay or book report for school?
.267	did you take careful notes in school? *
.267	did you save receipts and warranty information from things you bought during high school?
.264	did you help out with chores around the house?
.249	did you save your extra money, either at home or at the bank?
.234	did you get A's in high school?
.226	did you put old homework assignments, projects, or tests in a place for safe-keeping?
.217	were you elected to leadership positions in school organizations?
-.206	did you go more than one day without brushing your teeth?
-.222	did you wonder about the meaning of life?
-.271	did you claim to be sick simply because you didn't want to go to school or work?
-.289	did you forget an important date, like a close relative's birthday?
-.292	did you turn homework in late?
-.312	did you lose things you knew you had earlier, like your car keys, for instance?
<u>EXTRAVERSION</u>	
.397	did you make an effort to meet people you didn't know at parties or other social events?
.380	have you started a conversation with someone in whom you were romantically interested?
.350	were you so confident in your abilities you felt like nothing could stop you? *
.304	did a teacher tell you to stop talking so much in class? *
.236	did you attend social parties in high school?
-.223	did you just feel generally down and "blah" during high school? *
<u>NEUROTICISM</u>	
.394	did you lose your temper with other people in high school?
.312	did you over-react to situations in your life?
.307	did a teacher tell you to stop talking so much in class? *
.259	were you not able to get to sleep at night during high school because you were so worried about something in your life?
.249	did you make an effort to avoid someone you knew because you had no interest in talking to that person?
.246	did you find other people annoying?
.238	did you just feel generally down and "blah" during high school? *
.222	did you get in fist fights during high school?
.213	did you break a rule just for the sake of breaking it?
.203	did you think that you weren't getting enough attention when you were younger?
.201	did you stay in bed for most of the day?
-.215	did you proofread an assignment to check for spelling or math errors before turning it in? *
-.218	did you take careful notes in school? *
<u>AGREEABLENESS</u>	
(no correlations $>$ or $=$.200)	
<u>INTELLECT</u>	
.314	were you so confident in your abilities that you felt like nothing could stop you? *
.311	were you amazed at the variety of life and intricate workings of nature on this planet?
.252	were you interested in current events in high school?
.241	did you read books simply for pleasure and not because you had to for school?
.235	did you think about death and the possibility of an afterlife?
.230	did you feel as if you were interested in going to college while you were in high school?
-.217	did you feel generally down and "blah" during high school? *
* indicates that the item is repeated on another factor	

Using only the biodata items which had correlated earlier above .20 with the Big Five personality factors (those found in Table 4), seven factors were extracted from an oblique rotation of a generalized least squares factor analysis. The seven factor structure was chosen based on the fact that it was the most parsimonious solution for which the Chi-square statistic revealed a non-significant difference between the experimental data and the factor analytic model: $\chi^2(458, N = 273) = 493.95, p > .10$. In other words, seven factors provided the simplest solution for which the fit with the data was acceptable. Nevertheless, some of the

Table 5
Biodata Item Loadings on the Seven Factor Solution

<u>SCHOLASTIC ACHIEVEMENT</u>	
.783	How often did you get A's in high school?
.593	How often did you proofread an assignment to check for spelling or math errors before turning it in?
-.560	How often did you turn homework in late?
.533	How often did you feel as if you were interested in going to college while you were in high school?
.480	How often did you put old homework assignments, projects, or tests in a place for safe-keeping?
.440	How often did you take careful notes in school?
.413	How often did you write an outline before you began an essay or book report for school?
.397	How often were you elected to leadership positions in school organizations?
<u>ORGANIZATION</u>	
.751	How often did you pick up and clean your room as you were growing up?
.723	How often did you make an effort to organize your clothes in drawers and keep them neat?
.589	How often did you save your extra money, either at home or at the bank?
.519	How often did you save receipts and warranty information from things you bought during high school?
.519	How often did you help out with chores around the house?
<u>NEUROTICISM</u>	
.674	How often were you not able to get to sleep at night during high school because you were so worried about something in your life?
.591	How often did you over-react to situations in your life?
.572	How often did you just feel generally down and "blah" during your high school years?
.492	How often did you stay in bed for most of the day?
.474	How often did you lose things you knew you had earlier, like your car keys, for instance?
.439	How often did you think that you weren't getting enough attention when you were younger?
.428	How often did you claim to be sick simply because you didn't want to go to school or work?
.377	How often did you go more than one day without brushing your teeth?
-.360	How often were you so confident in your abilities that you felt like nothing could stop you?
<u>ANTI-SOCIAL BEHAVIOR</u>	
.611	How often did you find other people annoying?
.607	How often did you make an effort to avoid someone you knew because you had no interest in talking to that person?
.513	How often did you break a rule just for the sake of breaking it?
.512	How often did you forget an important date, like a close relative's birthday?
-.494	How often did you wash your hands after using the bathroom?
<u>EXTRAVERSION</u>	
.805	How often did you make an effort to meet people you didn't know at parties or other social events?
.781	How often did you attend social parties in high school?
.504	How often have you started a conversation with someone in whom you were romantically interested?
.419	How often did a teacher tell you to stop talking so much in class?
.360	How often were you interested in current events in high school?
<u>INTELLECT</u>	
.717	How often were you amazed at the variety of life and intricate workings of nature on this planet?
.701	How often did you wonder about the meaning of life?
.675	How often did you think about death and the possibility of an afterlife?
.394	How often did you read books simply for pleasure and not because you had to for school?
<u>AGGRESSION</u>	
.677	How often did you get in fist fights during high school?
.587	How often did you lose your temper with other people in high school?

actual item loadings on the factors created by this analysis presented difficulties in their interpretation. For this reason, a Varimax rotation of a principal components factor analysis was completed. This produced the loadings for the seven factors presented in Table 5. Those seven factors account for a combined total of 48.1% of variance.

Although the correlations are somewhat lower, the results of Study 2 are pleasing and promising for much the same reason as the results of Study 1. Namely, the BALE items that correlate highly with the personality factors make sense and would seem to indicate that we're on to something. A brief look back at Table 4 will support this claim. It seems clear, for instance, that those who have often made an effort to meet people they didn't know at parties would also rate themselves highly on measures of extraversion. Likewise, people of a fairly intellectual vein stereotypically do things like read books for pleasure and were interested in going to college while they were in high school. It makes logical sense.

Opponents of the biodata inventory selection method are quick to point out the potential for faking of "good" responses. It stands to reason that in most cases a person would want to appear as desirable as possible, particularly from an employment standpoint, and is not likely to admit to activities that might be frowned upon by a prospective employer. Certain measures can be taken, however, to reduce the likelihood of such "faking." Schrader and Osburn (1977), for instance, found that warning subjects that a lie detection measure had been built into the biodata inventory significantly reduced the amount of faking that took place. There was no lie detection measure in the BALE Inventory, but an effort was made to eliminate as much motivation as possible that subjects might have felt to fake their answers. Before beginning the battery, subjects were told emphatically that none of their responses to any of the tests would affect their Air Force careers in any possible manner.

Despite this effort, the data indicate that a "social desirability" confound may have influenced the results of the study. The effect, however, is to be found in the self-ratings on the trait names, rather than in the responses to the biodata questions. Subjects consistently rated themselves well above average on traits related to the Agreeableness factor. Six of the top nine highest-rated traits were from the Agreeableness factor. In fact, the trait with the highest mean self-rating is "Friendly" ($M = 17.47$, $SD = 4.9$). The fact that subjects rate

themselves very highly in this factor does not necessarily indicate that they are intentionally attempting to misrepresent themselves in a positive light. It may simply be indicative of a general tendency to consider oneself a nice person (R. E. Christal, personal communication, July 1993). Whatever the case may be, such consistently high and invariable self-ratings may very well explain the lack of correlations above .20 of the Agreeableness factor with items from the BALE Inventory.

Another point that detractors of this selection method might bring up is that the impact of a social desirability confound like the one mentioned above may be compounded by the fact that only nine of the 96 BALE items (less than 10%) are objectively verifiable. This could make it even more likely that a subject would fake good responses. Such concerns can be alleviated to some degree by the results of a study by Shaffer, Saunders, and Owens (1986), in which they found that even moderately subjective items were reported fairly accurately.

Moving back to the more positive side of the results from this study, it is especially promising that more items had high correlations with the Conscientiousness factor than any other. Based on the results of Barrick and Mount's (1991) meta-analysis, one would think that virtually any employer in the world would be very interested in such results. The reason for this is that, theoretically, the more biodata information that correlates with this factor, the easier it becomes to use such information as a means by which to predict the future job performance of applicants for employment positions.

The seven factors extracted from analysis of the answers to the BALE Inventory items seem to support the argument for the use of biodata as predictors of personality development. The fact that more than five factors were extracted might appear on the surface to be an invalidation of the Big Five theory, but a quick examination of the composition of the seven factors in Table 5 will demonstrate that this is not the case. Note that Scholastic Achievement and Organization are essentially subsets of the Conscientiousness factor, while Aggression and Anti-Social Behavior, with only a couple of exceptions, derive their existence from the Neuroticism factor. Thus, we find further evidence for the primacy of the Big Five as a "higher-order" set of personality dimensions. Furthermore, the extraction of such clean and obvious personality factors straight from

responses on biodata items supports the growing argument in favor of the predictive usefulness of biographical information (Fleishman, 1988).

In direct contrast to such optimism, Hough (1992) recently argued that the five-factor taxonomy is not adequately specific for any purpose (e.g., prediction) besides a general description of the structure of personality. This claim, however, is based solely on the measurement of personality through self-ratings and is not at all related to the use of biodata.

General Discussion

Although the results of the two studies are promising and leave open the potential for a great deal of fruitful research in the future, I can not whole-heartedly endorse either the use of perceived abilities in place of actual measured abilities or the use of biodata in place of certain other more valid predictors of future job performance. The reason for this, of course, is that neither measure (perceived abilities or biodata) was correlated directly with later job performance in the present study. Instead, I would suggest that efforts be made to use the two inventories in conjunction with better-established selection procedures, as a means of finding further support for conclusions that can be drawn concerning job applicants' potentials for success in different areas.

Finally, no discussion of the possible relationships among topics like biodata, personality development, or ability measurement can be complete without at least some mention of the possible mediating role of interest. As would be expected, virtually every possible relationship has been investigated over the decades. As would also be expected, different theorists have reached different conclusions. Lowman, Williams, and Leeman (1985), for instance, suggested that while similarly structured, abilities and interests are actually independent systems. Dawis and Sung (1984) included participation in high school activities in their study, and concluded that there is some relationship of activities with abilities and interests, but it is a small one. Azy Barak (1981) reviewed the available research of the time and theorized that the development of interests is a function of a mediating process involving perceived and actual abilities, expected success, and anticipated satisfaction. More recently, Tirre and Dixit (1992; 1993) threw reading interests into the milieu and concluded that they are more highly correlated with ability profiles than with personality profiles.

The potential relationship that each of the studies just mentioned shares with the two reported in this paper should be obvious, but this gets us no where without additional data that is in some way related to the measuring of interests. Recall the last three tests in the battery administered for this study, however, and you immediately see where I'm headed with this. Data has been collected not only on subjects' reading interests, as measured by the same Reading Interest Survey used in the Tirre and Dixit (1993) study, but also data on their career interests, as measured by the VOcational Interest-Career Examination (VOICE). Thus, we have a veritable ocean of data that has already been collected, and which can be used to investigate any number of fascinating issues in this area.¹ Only a relatively small percentage of the analyses that this wealth of data make possible have been completed in the short period of time allotted me this summer. It is my sincere hope that someone will take full advantage of this situation in the near future and begin to investigate more of the possibilities. There are so many unanswered questions.

¹ Initial analyses suggest, for instance, that perceived abilities correlate higher with interests than do actual abilities, particularly in the area of mathematics. Consider the implications for the prediction of future job satisfaction.

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CUE VALIDITY MANIPULATION IN A LOCATION CUEING TASK:
SHARING VERSUS SWITCHING ATTENTION

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Abstract

A location-precuing experiment was conducted that examined accuracy of target discrimination as a function of varying the percentage of valid cues. A central, symbolic cue was used, with validity percentages of 80%, 50%, and 20%. Results indicated that observers allocated attentional resources according to the percentage of valid cues. A model is presented which provides different predictions for (1) switching models in which the choice of a single area to be preferentially processed is varied over trials, and (2) sharing models in which control is exerted over relative amounts of attention allocated to cued and noncued locations. The data for one observer support the sharing model, and the data for another observer do not discriminate between the two models.

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INTRODUCTION

The movement of attention in the visual field, in the absence of or as a precursor to eye movements, is by now well established in the literature. The general procedure in attention experiments is to cue a given location in the visual field, and compare detection or discrimination performance, in terms of reaction time (rt) or accuracy, when targets are presented in cued and noncued locations. The general finding is that performance is better at cued than at noncued locations.

The type of model we prefer for attention is similar to a flexible gradient (LaBerge & Brown, 1989; Shulman, Wilson, & Sheehy, 1985; Egly & Homa, 1984; Podgorny & Shepard; 1983). We recognize that extant gradient models are at present unconstrained and contain many free parameters; however, they emphasize the flexibility of the attentional system to accommodate task demands.

Our gradient-filter concept (Cheal, Lyon, & Gottlob, 1993) has the following characteristics: (1) there is a capacity limitation that can be expressed as an upper bound on the integral or "volume" of attention; (2) movement of attention can be accomplished without traversing intermediate

locations (Miyauchi, Hikosaka, & Shimojo, 1992); (3) concentration of attention is accomplished by growth of the "amount" at the attended location, and as long as the capacity limit is not reached, no corresponding diminishment at unattended locations; (4) when the system is processing at maximum capacity, growth at one location must be accompanied by diminishment at other location(s). As we shall show, this model can fit some of the data in this experiment.

AOC Curves and Sharing vs. Switching

Sperling & Melchner (1978) studied the effect of attending to different parts of the visual field, in a dual-task experiment. They instructed observers in a visual search task to devote different proportions of attention to various areas, and found smooth tradeoffs when the accuracies were mapped onto an AOC (attention operating characteristic) curve. They concluded that a given amount of processing capacity was being allocated over the visual field, and that the mechanism was switching between two states. Possamai and Bonnel (1991) used a dual task paradigm to test the ability of observers to allocate attention to two locations within a single trial. In contrast to Sperling and Melchner, they concluded that their observers were sharing attention between locations.

How Location-Cuing Differs from Dual-Task

The dual-task paradigm uses instructions to split

attention among areas according to a specified ratio, and targets appear at both locations simultaneously. In contrast, we used the location cuing paradigm, in which only a single target appears on each trial.

The location-cuing paradigm consists of trials in which a single location in the visual field is precued, in order to "direct" attention. The target may appear at the cued or the uncued location. The general finding is an rt or accuracy advantage for cued (valid) locations over noncued (invalid) locations.

Why Manipulate Probability?

A main goal of these experiments is to differentiate between switching and sharing models. When cue probability (percentage of valid trials) is manipulated, individual trials are identical across conditions - the factor that varies is the mixture of trials (valid and invalid) within a block. If a difference in response is found, that difference must come from top-down, strategic factors, since there is no difference in stimulus qualities across conditions.

Jonides (1980, Experiment 2) manipulated probability in a discrimination task (R versus L) with 8 possible target locations. The cue was a peripheral arrowhead positioned .7 degrees to the inside of the target location, and had validity levels of 70%, 50%, and 30%. The reaction times for valid locations were lowest at 70%, followed by 50% and then

30%. Invalid reaction times showed the opposite pattern. The conclusions were that observers were either allocating resources unequally within a single trial (sharing) or using a trial mixing (switching) strategy, according to cue probability; however, the results were not determinate primarily because rt was used as the dependent variable.

Shaw and Shaw (1977) also manipulated probability, in a discrimination task, although they did not precue locations; they used a fixed probability distribution of target appearance. Their accuracy data were consistent with unequal allocation of a fixed resource, and also were indeterminate as to switching or sharing mode. Eriksen and Yeh (1985) also manipulated probability, but their results did not differentiate between switching and sharing.

The present experiment will include an analysis to differentiate between sharing and switching models. The properties of the current experiment that allow this analysis are the use of accuracy as the dependent variable, along with the inclusion of three probability conditions of less than 100 percent valid cues.

METHOD

Observers

Two observers, aged 21 and 37, served as observers. The first observer was paid \$7.00 per hour plus performance bonuses to participate. The second observer was the author

of this paper.

Apparatus

Stimuli were displayed on an IBM-XT with an EGA color monitor running at 60 mHz. An adjustable head and chin rest fixed the eye-to-screen distance at approximately 37 cm. Eye movement was monitored with a video camera. Responses were recorded on the numeric keypad of a standard IBM keyboard.

Stimuli

Stimuli were presented as white pixels on a dark gray background, with a total luminance of 80 cd/m^2 . Targets consisted of .75 degree "T"s which could appear in one of two locations six degrees to the right and left of a central fixation point. They appeared in one of four orientations (pointing right, left, up, or down). In order to make detection of target location more difficult, a plus sign which was a composite of all four "T"s was presented at the nontarget location.

Procedure

The order of events in each condition was identical (see Figure 1). A .15 degree fixation bar appeared for 668 msec, followed by a 16.7 msec central arrow cue pointing at one of two locations. After a 300 msec stimulus-onset-asynchrony (SOA), a target appeared at one location, and a plus appeared at the other location. Following the

presentation of the target, a mask was presented which was an outline of all possible targets, superimposed on each of the four locations. The observer's task was to indicate, by pressing the corresponding arrow on the numeric keypad, which direction the target was pointing. The observer was instructed that accuracy, and not speed, was required. There were four blocked conditions which consisted of different percentages (probabilities) of valid cues; observers were informed of what condition was being run. In the 80% condition, the cue indicated the correct location of the target on 80% of the trials. The 50% and 20% conditions used, respectively, 50% and 20% valid cues. The 100% condition was run for one session only, to provide a parameter estimate for the model-fitting.

RESULTS and DISCUSSION

Probability affected the relationship between the valid and invalid accuracies: see Figure 2. The valid and invalid accuracies for each condition (80%, 50%, and 20%) are plotted as an AOC curve. The curves provide evidence that cue probability affects the amount of attention oriented in response to the cue: the greater the cue probability, the greater the attention devoted to the cued location and the less devoted to the noncued locations. This probability effect found with accuracy scores replicates the effects

found by Jonides (1980) and Eriksen and Yeh (1985), with rt.

It appears that control is exerted over some attentional allocation process. The following section will present an analysis to compare predictions of switching and sharing models, and to choose the model that best fits the data.

Switching Models

Various attention allocating processes (under the headings of "switching" versus "sharing") may be postulated to account for the probability effect. The first type of process, which assumes that observers are capable of processing only one target location or small area at a time, would be to switch a "beam" of attention from location to location. The most plausible switching model would hold that observers orient their attention to a cued location on a portion of trials, and the probability effect is explained by differing "mixtures" of correctly and incorrectly oriented trials.

The first between-trials switching strategy to examine is where the observer "pre-oriens" to a noncued location on a proportion of trials related to probability of invalid cue. Thus, on a percentage of trials, the observer would orient to the cued location, and on the other trials, purposely attend to the other (uncued) location. A switching strategy was modeled to investigate effects of a manipulation of the proportion of valid trials.

Some simplifying assumptions in the model are necessary. The switching model here assumes that attention is allocated, after cue onset, as a "spotlight" whose center or "peak" can include just one target location at a time (however, it may span more than one position). If attention is at the target location when the target appears, the target is correctly identified with fixed probability α where α is an expected value over a large number of trials; otherwise if attention is allocated to the other location when the target appears, the target is identified with fixed probability β , where $.25 \leq \beta < \alpha \leq 1$ (.25 indicates pure guessing accuracy in a four-choice discrimination). The parameter α can be called "expected accuracy in the spotlight", and β can be called "expected accuracy outside the spotlight". Note that observed accuracy tends toward expected accuracy as the number of observations increases. Another parameter "c" indicates the proportion of trials that attention is allocated to the cued location.

The same analysis can be applied to the Jonides (1983) or the Eriksen and Yeh (1985) two-process model. In those models, there are two modes of attentional distribution: fully distributed and focused to one location plus distributed to all other locations. The parameter α can apply to expected accuracy when the focus is at the target location at target onset, and β can refer to expected

accuracy in the distributed mode.

For any value of c , valid (V) and invalid (I) accuracies are linear combinations of α and β :

$$V = \alpha(c) + \beta(1-c)$$

$$I = \alpha(1-c) + \beta(c)$$

Parameters α and β are fixed, and c varies across cue probability conditions. In order to maximize overall accuracy, observers should use the cue ($c = 1$) on all trials where the cue has over 50% validity. The above switching model is the special case for two locations; I have also worked out a more general "n" location switching model.

Sharing Model

A model for an attentional system that fits the obtained results may be one that processes all target locations in the display in parallel, with a concentration of resources at the cued location and a lesser amount devoted to noncued locations. Differences in attentional distribution would be accomplished by changing the allocation of processing resources over the display. In terms of the above model, a sharing strategy would consist of control over the values of α and β , while fixing c (at 1 or any other value). Plausible constraints on α and β are that $\alpha > \beta$, and that total capacity (which will be expressed in the next section as a function of accuracy) is fixed.

Model of Capacity

Optimal search theory (Koopman 1956, 1957, Shaw & Shaw, 1977) was developed after World War II to enable searches of large areas with minimal effort (resources) expended. Shaw and Shaw (1977) applied optimal search theory to a discrimination task, and showed that observers allocated a fixed amount of attentional resources optimally in accordance with target probability. The theory provides a mapping between resources expended and expected detection (discrimination) accuracy; conversely, discrimination accuracy can be used to derive total resources or capacity (Φ).

The method used to calculate total capacity Φ is to obtain accuracy at each location, cued and noncued, and derive resources for each location using the relationship $b(x) = 1 - e^{-q}$ (Koopman, 1957; Shaw & Shaw, 1977), where b is accuracy, and q is resources at a single target location. Solving for q ,

$$q(x) = -\{\ln[1-b_c(x)]\}$$

The quantity in the brackets is called the failure rate or survivor function (Townsend & Ashby, 1983). Resources at a given location, q , are equal to the negative of the log failure rate. Using the above equation, values of q are thus derived for cued and noncued locations, and Φ (total resources or capacity) is calculated by summing q for the cued and uncued location. The relationship between resources

and accuracy is best described as a diminishing marginal returns function: as resources increase, accuracy increases, but the derivative (slope) decreases as resource levels increase.

For a given amount of total resource Φ , the tradeoffs between valid (cued location) and invalid (uncued location) accuracies can be described. Figure 3 shows the set of "iso-resource" functions prescribed by different values for Φ . They show that AOC curves conforming to a sharing model with resource conservation are curved. In contrast, AOC curves produced by a switching process would be straight lines (Sperling & Melchner, 1983).

Model Fit

Both switching and sharing models were fit to the data. Both models had 4 free parameters, and the data had 6 values to fit, yielding 2 degrees of freedom. Best fits were found by a parameter space grid search, and the criterion for the best fit was sums-of-squares error (SSE) between model and data.

The switching model takes the following form:

$$V_p = \alpha(c_p) + \beta(1-c_p)$$

$$I_p = \alpha(1-c_p) + \beta(c_p)$$

where the subscript p refers to condition (80%, 50%, or 20%). The parameters α and β refer to accuracy inside and outside the spotlight, and are fixed across condition. The model is

a nested model in that an estimate for α was obtained from accuracy in the 100% condition. Thus, the four free parameters are c_{80} , c_{50} , c_{20} , and β .

The sharing model uses the same formula, but lets different parameters vary across conditions:

$$V_p = \alpha_p(c) + \beta_p(1-c)$$

$$I_p = \alpha_p(1-c) + \beta_p(c)$$

The parameter c is fixed (at 1) across conditions, but α and β are allowed to vary. However, a constraint on α and β is that α and β are yoked. The resource amounts are fixed across all conditions:

$$\Phi = -\ln(1-V_p) - \ln(1-I_p)$$

Therefore this model also has four free parameters: α_{80} , α_{50} , α_{20} , and Φ . The switching and sharing models are both nested in one general model, with different free and fixed parameters.

Figure 4 shows the data and the best fits of each model. For observer LG, the SSE for the sharing model was half that of the switching model, while for CT, the two model fits were equivalent. The data for CT clearly violate both a switching model and a sharing model that conserves overall resources. The data for LG marginally favor the sharing model, although statistical tests of fit remain to be performed.

The next step in this line of research is to produce data with more degrees of freedom. For each additional

condition, the number of points to fit increases by two, while the model free parameters increase by one. Therefore, an experiment with five conditions instead of three may allow a better discrimination between the two models. Also, the analysis may be improved by providing more external parameter estimates, either from data or theory.

CONCLUSION

Two types of model were compared: (1) a switching model, in which the observer attends to either the cued or the noncued location on a given trial, and (2) a sharing model in which the observer controls the allocation to cued and noncued regions within a trial. Based on the fit of the models to the obtained data, the sharing model was supported for one observer, and neither model was better for the other observer. Some suggestions were made to increase the power of the test between the two models: increasing the number of degrees of freedom in the data or decreasing the number of degrees of freedom in the model.

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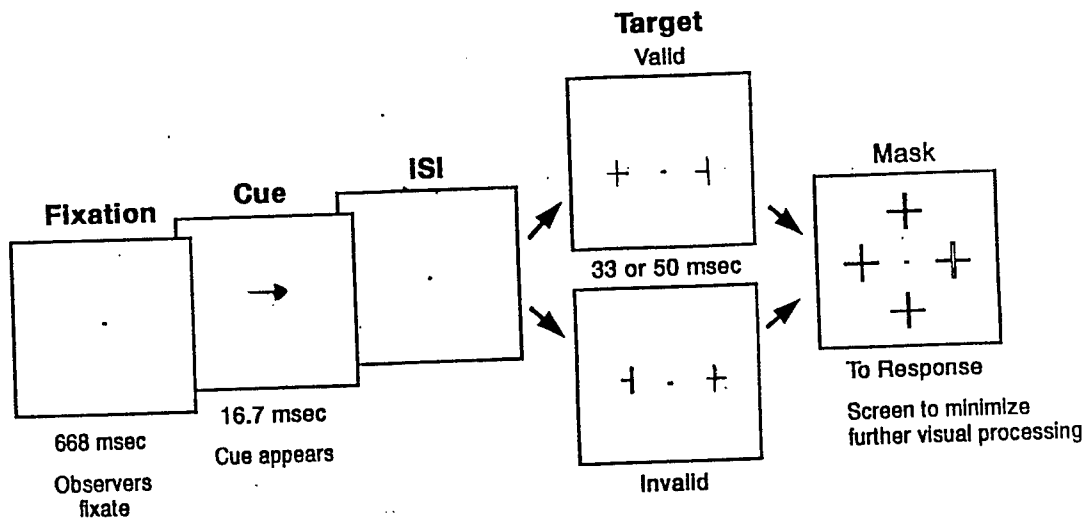


Figure 1. Order of events in a location-cuing trial. Observers fixate on the center bar. The central cue appears, followed by a 300 msec cue-target asynchrony.

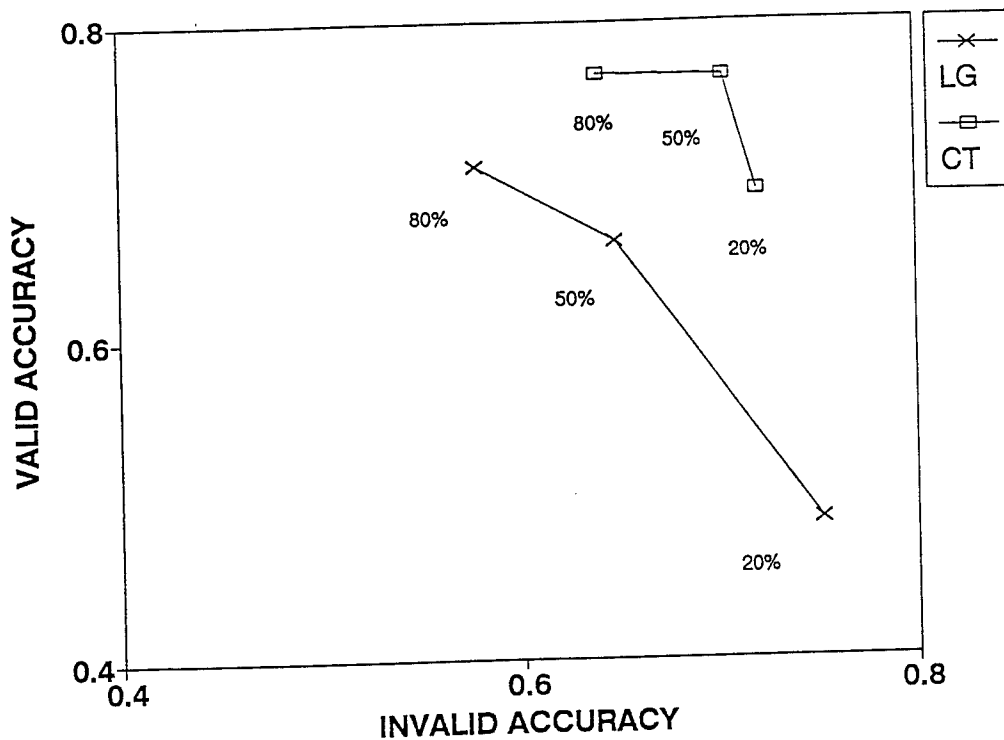


Figure 2. AOC curves produced by the two subjects, CT and LG. The condition (80%, 50%, or 20%) is indicated next to each point.

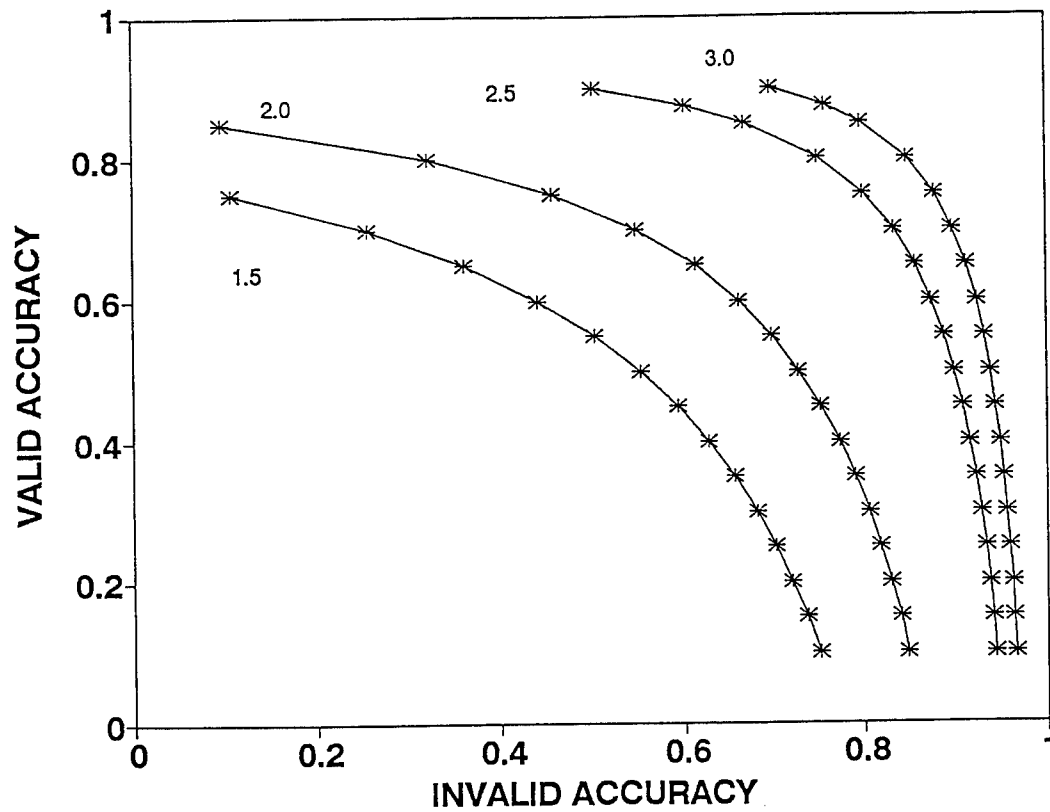


Figure 3. The family of iso-resource curves produced by the sharing model. Each curve is the pattern of valid-invalid tradeoffs for a given value of Φ , total resource.

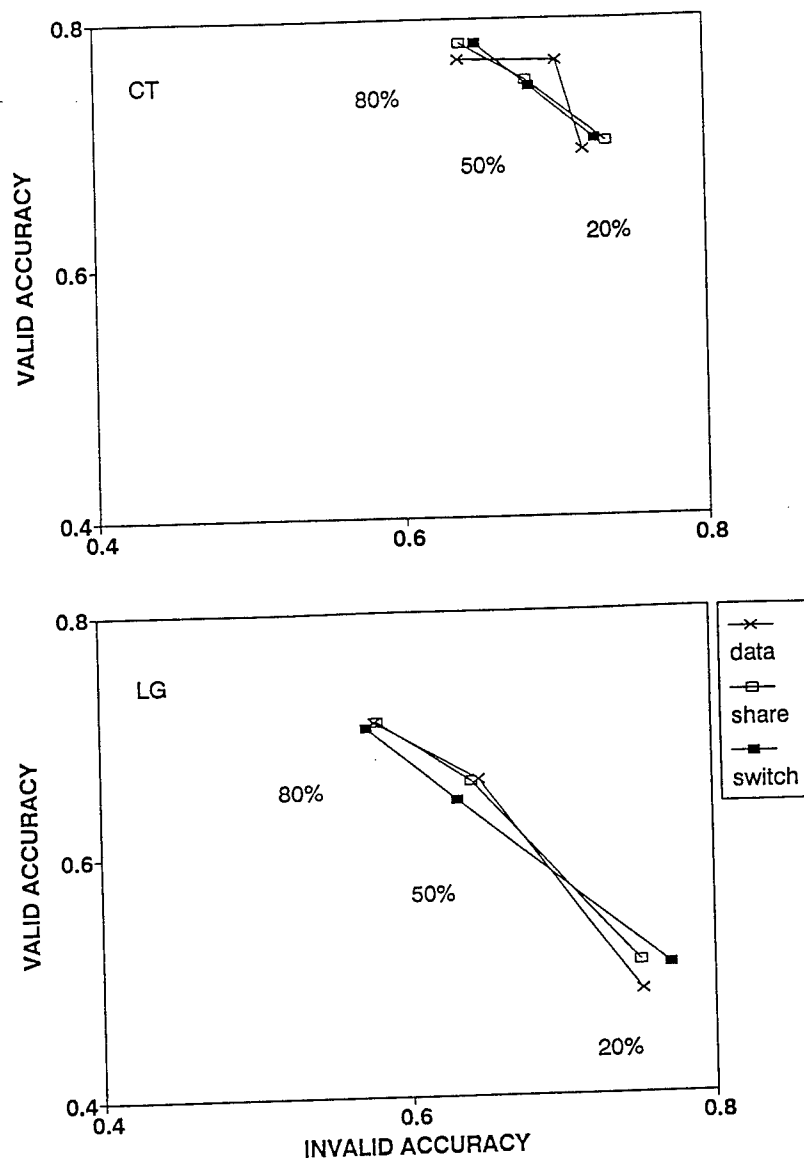


Figure 4. Model fits for both observers. Solid squares indicate sharing model best fit, while open squares indicate switching model best fit.

"FULL" VS. MINIMALIST COMPUTER DOCUMENTATION:
A COMMON-SENSE APPROACH

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**"Full" Vs. Minimalist Computer Documentation:
A Common-Sense Approach**

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ABSTRACT

Creating the user documentation is one of the major tasks involved in any software development project. Notwithstanding, or perhaps because of, the amount of effort that goes into this written documentation, many new users still feel overwhelmed by the large reference manuals. One solution to this problem became apparent during the training session for the high school teachers from across the nation who are taking part in the testing of Armstrong Laboratory's new intelligent reading and writing tutor, *R-WISE (Reading and Writing in a Supportive Environment)*. At the end of the training session, the teachers were surveyed to find out which manual would be most helpful for both themselves and their students, either a full or a condensed version. Reactions were highly mixed, with several teachers recommending that both a long and short version be available. This concept can be further tested with the production of the written documentation for HRT's Science Tutor, which is currently under development.

"Full" Vs. Minimalist Computer Documentation: A Common-Sense Approach

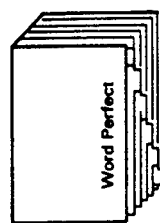
Patricia M. Harn

Introduction

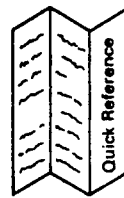
As the wheels of progress in science and engineering roll steadily onward, so does progress in the expanding "sister" discipline of technical communication, which serves to document these changes. Nowhere is an awareness of the need for accurate and easy to use technical documentation more acutely felt than in the field of computer science. In fact, a big share of creating any new software program is writing the documentation, be it an on-line help system, the user interface, or, of course, the set of "hard-copy" user manuals. Many of the new theorists in computer documentation seem to be focused mainly on creating better user interfaces (i.e., computer screens) or on-line help systems in order to some day do away with the need for paper user manuals (Haselkorn, 1988; Horton, 1990). But it is unlikely the general public will be ready to accept book-less documentation any time in the near future. After all, the much-promoted electronic news services of the past five years have not replaced the ordinary daily newspaper, and electronic mail, despite its exponential growth in use, has not yet put the U.S. Post Office out of business.

Thus big software companies like Microsoft and Aldus keep producing an elaborate set of documentation books for each new software package they put out. The cornerstone publication of any of these "documentation suites" is invariably a big reference manual numbering between 300-1000 pages of dense type. Also included in the suite is a separate book describing how to set up the computer hardware itself. Often the suite will include a tutorial booklet that showcases some major features of the program. And now most

software companies include some kind of a "quick card"--a stand-alone glossy cardboard sheet packed with keyboard shortcuts, menu commands, and the like. Due to their highly condensed content and lack of explanatory text, these cards are usually helpful only to experienced users of the program. Below is an example of a typical computer documentation set (Price, 1988).



User's manual



Quick reference card



Installation pamphlet



Disk-based tutorial



Keyboard overlay



Stick-on keyboard code reminder

Purpose and Scope

Despite the painstakingly-created set of paper documents included in most new software packages, the typical new user reaction to the documentation is often a sense of confusion and inadequacy. Therefore, the purpose of my research was to examine and deal with one major reason for this new user confusion: a seeming *overabundance* of information presented in the main reference manual combined with what seems to be an apparent *lack* of information in the quick card. This report will begin with an analysis of the problem,

then will discuss one highly praised solution offered by a leading theorist in the field, and, last, will attempt to synthesize and improve upon these ideas with the results of a recent user survey.

Discussion of the Problem

In August of this year, the Human Resources Training staff, led by Pat Carlson, held a one-week computer training session for high school English teachers involved in the upcoming testing of Armstrong Laboratory's new "intelligent" reading and writing skills tutor for at-risk youths. During the week, the trainers caught more than a glimpse of this new computer user bewilderment with paper documentation. The training session introduced the results of a mammoth three-year software development project called *R-WISE*, which stands for *Reading and Writing in a Supportive Environment*. The program, using some of the latest technology in artificial intelligence, is designed to provide personalized, adaptive tutoring for each student user (Carlson, 1992). All this intelligent tutoring is to occur within the framework of a very user-friendly and often fun computer interface, so that even the most "remedial" of English students will find it helpful.

Over fifty high school teachers from seven schools throughout the United States attended the training session at MacArthur High School in San Antonio, Texas. These teachers represented a cross-section of schools ranging from a high-achieving, upper middle class school in upstate New York to a pair of schools located on an Indian reservation in the desert Southwest. Results of my new user survey given at the end of the training session showed that some teachers classified themselves as "novice" computer users, and some claimed to be "highly experienced," but the majority saw themselves as "occasional users."

Though the trainers "walked the teachers through" every part of the program, the teachers were also encouraged to take advantage of the R-WISE user manual that I designed during my term as a technical documentation writer in the 1993 Graduate Summer Research Program. This manual was an attempt to document in detail almost every facet of the complex program, using the latest graduate level theories and practices in technical communication taught at the University of Washington in Seattle. This school reputedly has one of the best technical writing departments in the nation. The teacher survey that I designed for the end of the training session (See Appendix B) also attempted to garner reaction to the user manual itself. Responses from the teachers who took part in the survey were highly mixed: most considered it to be very clear, organized, and detailed, but about half complained that it was too long. The user manual actually numbers about 75 pages, including innumerable "screen dumps," i.e., pictures of what the computer screen is supposed to look like at a given interval.

Current State of the Art: Minimalism

Of course, such complaints about the length of user manuals have been fielded in the recent past by several leading technical communication theorists. Not the least of these is one John M. Carroll, whose recent work, *The Nurnberg Funnel: Designing Minimalist Instruction for Practical Computer Skill* (MIT Press, 1990) has received much attention for its laudable attempt to deal with what the typical new computer user perceives to be overly long documentation. In this book, Carroll spends the first few chapters analyzing the problems inherent in what he terms the verbose "systems approach" to computer documentation. In Chapter 4 he finally presents his empirically-tested answer to this problem: The Minimalist Manual. Following is an example of his new style of manual, taken from page 148:

DELETING TEXT

USE THE CURSOR KEYS TO MOVE THE CURSOR UNDER THE FIRST *r* IN THE WORD *regular*.

PRESS THE DEL KEY

The DEL key is located up and to the right of the keyboard keys.

Is the Displaywriter prompting you?: Delete what?

► If you make a mistake at this point, use CODE + CANCL and start the deletion again.

USING THE → KEY, MOVE THE CURSOR THROUGH THE MATERIAL TO BE DELETED, THE WORD *regular*.

The word is highlighted: you can see exactly what is going to be deleted before it actually is deleted.

► If the wrong characters are highlighted use CODE + CANCL and start the deletion again.

Figure 28. Incomplete procedures to encourage reasoning

This new kind of user manual should include only the bare bones, Carroll decrees, so the user can follow his or her natural inclination to *use* the program and not just *read* about it. The caption used in the above example offers a concise summary of the minimalist philosophy: "Incomplete procedures to encourage reasoning." Carroll defines this as the "paradox of sense-making People are always trying things out, thinking things through, trying to relate what they already know to what is going on . . ." (74).

Problems with Minimalism

Carroll's vision of the average computer user is perhaps a little too narrowly optimistic. This sparing new kind of manual may work fine for more "adventurous" learners--that is, people who reason *inductively* like a scientist, by trying things out, seeing what happens, forming hypotheses, and, finally, confirming or disproving these hypotheses. But, more than likely, at least half of all computer users, myself included, prefer the tried and true *deductive* approach to learning, meaning we find it more efficient to hear or read about the

general principles first so as to have a framework upon which to hang any relevant, newly-acquired knowledge. Carolyn Plumb, in a thesis report entitled *Cognitive Style and Menu Maps for Computer Information Retrieval Systems: Effects on Performance* (1988), contrasts the inductive versus deductive approach to learning computers under the well-known psychological rubrics of field independence (induction) and field dependence (deduction). Although deductive reasoners are probably every bit as intelligent as inductive reasoners, inductive reasoners may have superior computer ability, Plumb states (31).

Furthermore, John Carroll makes the mistaken assumption that because many of the details provided in "old-fashioned" computer reference manuals are not referred to often, they are not necessary to document at all. But this misses the whole point of creating technical reference manuals in the first place: to document instructions, both broad and detailed, so the information is there just in case it is needed. No writer of a computer manual reasonably expects his or her work to become a best-seller suitable for relaxing bed-time reading. One long-time high school English teacher who took part in my survey made a rather eloquent observation about the seeming excess of detail in the *R-WISE User's Manual*. When asked whether he or she preferred a long and detailed sample of documentation to a condensed version covering the same content, the respondent wrote that the longer was better because "I always prefer to have the ability to obtain more information than I need at present." Given the high cost of answering computer support calls, especially when they are long-distance ones placed during normal business (and school) hours, this teacher's pragmatic rationale on behalf of "full" documentation continues to make good financial sense for software companies.

Methodology

The four-page survey administered at the end of the training session was designed primarily to assess user reactions to the two types of computer manuals previously alluded to, the "full" and the "condensed" or minimalist. (The exact wording of these survey questions can be found in Appendix B; in particular, Sections D and E.) Section D was an attempt to garner both positive and negative feedback on the just-written *R-WISE User's Manual*. Section E measured reactions to a highly condensed--that is, minimalist--version of one segment of the original user manual. To do this, the teachers were given samples of both the original version, which spanned four pages, and the one-page condensed version (See Appendixes C and D). Then they were asked which they preferred.

Of course, before asking the teachers' opinions about the original and condensed versions, we needed to gather some data on the general categories of teaching experience, computer experience, and the teachers' exposure to the user manuals. Such background information helped correlate things like time spent looking over the *R-WISE User's Manual* to ratings of its effectiveness. Indeed, it was difficult for many of the respondents to give an informed opinion about the long manual. The several computer trainers who were physically available for questions all week all but obviated the teachers' self-initiated research into the user manual. With this in mind, specific questions about the content of the manual were avoided. The background information gathered in the early sections of the survey also helped me to compare the user's level of computer experience with his or her opinion of how long the manual should be. Almost without exception, the more experienced computer users wanted a shorter document.

Results of the Survey

Survey responses were about what was expected. Of the 13 people responding, the majority made the written comment that the new user manual was clear and well organized,

but at least as many also said that it was too long. The teachers may not feel so negatively about the length of the document, however, after they go back to their home cities and allow some time to erode the benefits of computer training. In regard to their stated preference for which manual they preferred, the long or the short, opinions were split down the middle. Six said they preferred the condensed version of the manual, six said they preferred the long version, and one was undecided. Though the sample size was a little small, there seems to be no reason to cast these 13 responses aside as unrepresentative of the teacher population involved in the training. Nevertheless, to create more statistical validity, the survey should have contained much larger sample sizes.

Special care was taken in the wording of survey questions to avoid leading the subject into a desired response (Labaw, 1980). For instance, in the enclosed survey, the title of Section E, "Full Vs. Condensed Documentation," was selected rather than "Complete Vs. Minimalist Documentation" because of the more richly-laden verbal connotations hidden within the words "complete" and "minimalist." Another major problem of surveys is that the results are self-reported, meaning that the respondent tends to answer according to how he or she idealizes the world and not necessarily according to how things really are. One way to counteract the negative effects of self-reporting would have been by collecting second-party observations on how well the user fares with either manual. The best way to do this triangulation of the data would be to conduct comparative usability studies to see which subjects learn quicker and produce fewer errors (Redish, Ramey, & Pieratti, 1993). But because of the high labor costs involved in usability testing, only about a half-dozen subjects should be tested.

Conclusions and Recommendations

Results from my new user survey of the high school English teachers point to one reasonable compromise between traditional long reference books and Carroll's minimalist approach: Why not simply abstract a condensed manual from the longer one and present them as two separate books from which the new user may choose? In fact, two of the teachers made similar suggestions--about creating two manuals instead of just one--in their final written comments. One of these teachers was a novice user, and the other classified him- or herself as an occasional user. This occasional user, who also was undecided about whether the long or condensed version was better, put it this way: "It really depends on the student. Some students will want to read in order to understand the process, and others won't want to take the time."

Offering both a short and a long version of the same manual has most likely never been tried or tested, unless one were to classify quick cards as a kind of mini-minimalist manual. However, because the main purpose of a quick card is to serve as a set of reminders for the experienced user, it is of limited practical value for brand new users to a program. Actually, producing a minimalist manual as an alternative to the longer one is a simple, labor-sparing process of deleting many of the details and making a few minor editing adjustments. So the cost of labor should not be a major concern. But some may ask the legitimate question that if the new computer user feels confused and overwhelmed at the sight of, say, a set of four documentation books, would not an additional book merely add to this confusion? The answer to this question is just as legitimate: Use a very straightforward title for each book in the documentation set. That way, the new software user can decide at a glance which book or books are desirable to read. The user friendly naming of user manuals has already occurred in a few isolated instances. For example, the Sony Corporation has seen fit to title the introductory booklet for one of their recently

marketed CD-ROM players as "Read This First." Below the title is a caption stating the intended purpose and audience for the booklet.

This new idea of producing two different versions of the same software user manual can be easily put to the test in the future documentation of Armstrong Laboratory's upcoming Science Tutor. This program, scheduled to be ready for release by January 1994, will be an intelligent tutoring system for high school students needing help with basic science concepts. It will be modeled after *R-WISE*, the reading and writing tutor which is being tested this year. The testing of the two user manuals for the upcoming Science Tutor can proceed in much the same manner as the testing of the computer programs themselves. In other words, there should be a treatment group of teachers and students that receives the benefits of both the short and long user manuals, and there should be a non-treatment group that receives only the standard, longer manual. If my recent survey of the English teachers is a good indicator, the treatment group should not feel put upon by having two user manuals to choose from. The non-treatment group would know nothing of the additional condensed manual. By "blinding" the non-treatment group in this way, the teachers and students would neither feel "deprived" nor tend to under-rate the performance of the single manual they are given to work with.

Toward the end of their semester of using the Science Tutor, both the science teachers and their students can be surveyed regarding the accuracy and completeness of the user manuals they have been given. Then the results of both the treatment and non-treatment groups can be compared, using a standard T-test, to check for significant statistical differences between the groups' ratings of satisfaction.

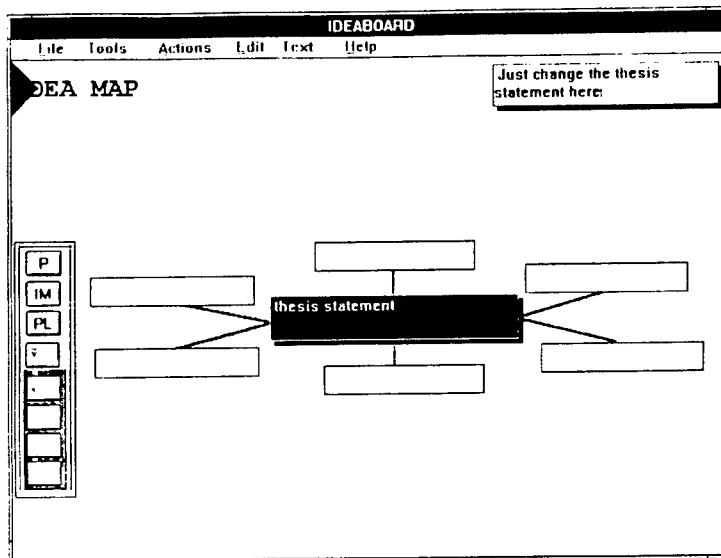
APPENDIX A: REFERENCES

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The Idea Mapping Stage

Now that you have done some planning, it's time to brainstorm and jot down a few points and sub-points for your thesis statement. Because the thesis statement should determine the direction that you are headed on your idea map, make sure all the supporting material you're about to write actually does support the thesis.

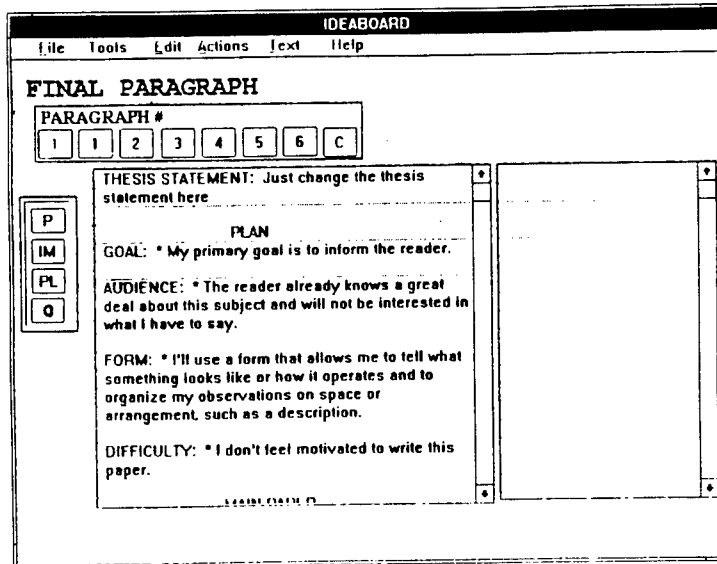
The first screen that appears in the Idea Mapping stage contains a short paragraph of instructions. Once you are finished reading this, click anywhere on the screen to get rid of it. Then you should see the small navy blue thesis "workbox" that was laying underneath the instructions. It has carried over a few key words from the thesis you just typed:



The Screen Layout There are a few terms you need to become familiar with at this point in the program. The navy blue box in the middle is the thesis workbook. The six boxes surrounding it are referred to as topic sentence workboxes, and the box in the upper right-hand corner is best known as the holding workbook.

1. To receive a choice of the following three writing options, Thesis Statement, Thesis Paragraph, and Concluding Paragraph, click on the thesis workbook.

The Thesis Statement option will provide a small workbox in the upper right-hand corner of the screen where you can revise the thesis you have already created. The Introductory Paragraph and Concluding Paragraph options will take you to screens that look very similar to the following, which has a re-cap of your thesis and planning choices on the left side and a workspace on the right for you to draft your entire first or last paragraphs:



The brand new set of buttons just above the thesis statement on this screen is there to help you get around to the other paragraphs that you will soon write. The red-colored letter or number of one of the boxes indicates which paragraph you are currently working in; to change paragraphs, simply click on the desired box. To return to the root screen for the Idea Mapping stage, simply click on the IM (Idea Map) box on the shortened version of your control panel.

2. If you have not already done so, select one of the three options from the navy blue thesis workspace and draft up either a thesis statement, introductory paragraph, or concluding paragraph. Making sure you have a solid thesis statement is the most important option for now.

Some students may prefer to save the drafting of their introductory or concluding paragraphs until after they have finished the rest of the other paragraphs. It's up to you--whatever works best for your writing style.

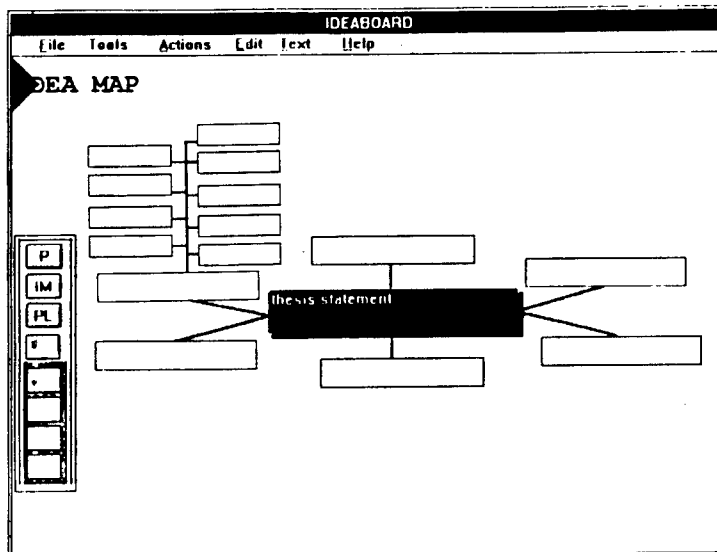
3. To begin jotting down ideas for the other paragraphs, click on one of the small workboxes surrounding the thesis statement. These workboxes are meant to contain key ideas for each of the topic sentences of your supporting paragraphs.

The workbox should turn light turquoise in color and another turquoise workbox should appear in the upper right-hand corner of your screen.

4. Begin typing in key words and phrases for this workbox.

Hitting ENTER will cause the words you have typed in the upper right-hand corner to move into one of the "real" workboxes. These topic sentence ideas may be revised only by using *ACTIONS: CLEAR MAIN IDEA* or *ACTIONS: CHANGE MAIN IDEA*.

A set of nine green "leaves" will suddenly "grow" onto the workbox in which you entered information. Your screen should look something like this:



These nine workboxes are there for you to type in a few key words and phrases to support the main idea of each paragraph. As before, the typing will first appear in the "holding" workbox in the upper right-hand corner of the screen. Hitting ENTER will bring it down where it belongs.

Don't worry if you happened to choose a different topic sentence workbox to get started in than the one shown above; the location of the box you have chosen doesn't matter. It also doesn't matter whether you use up all of the boxes on the screen; use only what you need. And you can take your pick of either filling in all the paragraph topic sentences first--before you fill in any sub-points for your topic sentences--or filling in the sub-points for each paragraph before you move onto the next paragraph's topic sentence. Again, it's a matter of personal work style.

5. To see how it works, fill in the supporting details for the first topic sentence. Then click on another topic sentence workbox to start outlining another paragraph.

NOTE: You must click on the topic sentence workbox before you can start filling in any supporting details.

6. Finish outlining your paragraphs, and then go back and click on the paragraph topic sentence workbox that you want to start writing about first, before any of the others.

Clicking on this topic sentence workbox will set you up properly for the next stage of writing, when you start drafting sentences.

7. To move to the next stage of writing, click on the PL (Paragraph Level) box in the control panel on the left side of your screen.

Congratulations! You have finished the brainstorming and outlining stages of writing--that is, if you have also written your introductory and concluding paragraphs.

The Idea Mapping Stage

It's time to begin outlining your thoughts:

1. To get to the next stage of writing, click on the IM button on the control panel.
2. Read the introductory screen and then click on this screen to get rid of it.
3. To receive a choice of the following three writing options, Thesis Statement, Thesis Paragraph, and Concluding Paragraph, click on the thesis workbook (the box in the center).
4. If you have not already done so, select one of the three options from the thesis workspace and draft up either a thesis statement, introductory paragraph, or concluding paragraph.
5. To begin jotting down key ideas for the other paragraphs, click on one of the small workboxes surrounding the thesis statement.

The workbook should turn light turquoise in color and another workbook should appear in the upper right-hand corner of your screen.

6. Begin typing in key words and phrases for this workbook.

Hitting ENTER will cause the words you have typed in the upper right-hand corner to move into one of the "real" workboxes.

A set of nine green "leaves" will suddenly "grow" onto the workbook in which you entered information.

7. Begin filling in the supporting details for the first topic sentence. Then click on another topic sentence workbook to start outlining another paragraph.

NOTE: You must click on the topic sentence workbook before you can start filling in any supporting details.

8. Finish outlining your paragraphs, and then go back and click on the paragraph topic sentence workbook that you want to start writing about first, before any of the others.

9. To move to the next stage of writing, click on the PL (Paragraph Level) box in the control panel on the left side of your screen.

USING A NEGOTIATION SUPPORT
SYSTEM TO INTEGRATE INTERESTS

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Abstract

The current study used a variable-sum negotiation task to determine the degree to which computer-assisted dyads are better than manually assisted and unassisted dyads at achieving integrative bargaining agreements. Male and female dyads engaged in both a four-issue and an eight-issue negotiation during a single experimental session. While computer assistance did not improve performance for females, computer assisted males obtained a significantly higher proportion of the integrative total on the four-issue task than did unassisted and manually assisted males. In addition, while computer assistance did not appear to improve interest estimation, significant positive correlations were obtained between estimation accuracy and the outcome measure for both tasks.

USING A NEGOTIATION SUPPORT SYSTEM TO INTEGRATE INTERESTS

Kenneth A. Graetz and Jason Seifert

"There is no shortage of disputes."

Howard Raiffa, 1982

The first sentence of Raiffa's (1982) volume, *The Art and Science of Negotiation* remains a humorous understatement. Whether discussing vacation plans or negotiating a contract, social conflict and social interaction often appear synonymous. The current study explores a potential technological solution to conflicts involving *scarce resource competition* (Aubert, 1962; Druckman & Zechmeister, 1973), the allocation of a limited pool of desirable resources (e.g., money, commodities, services, information). *Negotiation* is often viewed as one subtype of scarce resource conflict (Thompson and Hastie, 1990). In labor-management disputes, for example, parties attempt to reallocate tangible rewards (e.g., money and working hours) in a mutually satisfactory fashion. In some cases, the interests of both parties are diametrically opposed or *zero-sum*. On these occasions, the optimal joint outcome is the *distributive* or compromise solution (i.e., the negotiators split the resources in half). While this may be an optimal strategy for maximizing joint profit in zero-sum negotiations, it yields a suboptimal solution when applied to negotiations that include an *integrative* potential. An integrative solution is one that affords both negotiators a higher outcome than the compromise solution. Research in the area of integrative bargaining suggests that such solutions, while frequently present, are not as readily attainable as they might appear. Evidence that negotiators arrive at suboptimal, distributive solutions in negotiations that present an opportunity for interest integration has been found in a variety of case and field studies (Raiffa, 1982; Lax & Sebenius, 1986; Howells & Woodfield 1970; Howells & Brosnan, 1972) as well as in controlled laboratory environments (Pruitt & Rubin, 1986; Bazerman & Neale, 1983).

Why do two negotiators fail to integrate their interests when doing so would result in a better outcome for both? During a typical, face-to-face negotiation, participants are confronted by a milieu of stimuli and tasks that demand their attention. Assuming that interest integration requires more time and cognitive effort than simply settling for the compromise solution, negotiators who are under pressure to reach an agreement may be aware that interest integration is possible, but may not have time to achieve it. Individual negotiators may also be under strict instructions from a constituency or supervisor to *win* the negotiation, either by maximizing the difference between their own outcomes and those of their opponent or by simply obtaining a higher outcome than their opponent.

Another possibility is that one or more of the negotiators may fail to realize the existence of the integrative solution. While a variety of factors are presumed to obstruct and /or delay this insight (Foroughi & Jelassi, 1990), the most pervasive seems to be the erroneous perception that one's own interests and the interests of the other negotiator are diametrically opposed. This has been referred to as the *fixed-pie perception* (FPP) and seems to originate from preconceived beliefs about the social act of negotiation. For example, subjects who were asked to play the role of a negotiator tended to bargain more competitively and obtained lower outcomes than subjects who were not asked to play the role (Enzle, Harvey, & Wright, 1992). One interpretation of these findings suggests the existence of a scripted set of role obligations which place a premium on being a *hard* negotiator and which bias individuals toward competitive behavior. The negotiator who falls victim to this bias may simply opt for the distributive outcome, perhaps after selfishly attempting to obtain a higher outcome at her opponent's expense. The thought of working together to obtain a higher joint outcome is not considered.

It is with these cognitive limitations and biases in mind that the Bargaining Analysis and Resolution Builder (BARB) was designed. This software joins other negotiation support systems (NSS) in an effort to make negotiation problems more manageable and comprehensible for negotiators. BARB resembles existing NSS packages

in its commitment to providing simple tools that do not interfere with the social dynamics of the typical face-to-face negotiation. It is unique in that behavioral prescriptions based on normative decision making models are purposely absent. Also, BARB's session-oriented information displays track and organize negotiator *behavior*, rather than subjective beliefs and/or expectations.

BARB is expected to be a full-featured NSS capable of assisting individual negotiators both in and out of the conference room. The tools targeted for evaluation in the current study are *session-oriented*, that is, designed for use during the face-to-face portion of the negotiation. These tools are part of a database management system (DBMS) written using Borland's Paradox for Windows™ and it's ObjectPal™ object-oriented programming language. The general goals of BARB's session-oriented tools are as follows: (a) to provide information that a human negotiator would normally calculate, (b) to provide information that a human negotiator is incapable of calculating, (c) to generate simple information displays based on the behavior of the negotiators, (d) to minimize the amount of interaction between the negotiator and the computer, and (e) to refrain from providing computer-generated advice based on subjective probability estimates. These tools are designed to be placed on the file server of a local area network. Negotiators in the conference room access the tools using notebook computers. Every attempt is made to minimize the impact of the technology on the social processes at work during the face-to-face portion of the negotiation.

While BARB will eventually be used to handle real negotiation situations, thereby requiring the users to input the negotiation issues, the levels of each issue, and their subjective utilities for each level, the current study presented negotiators with two preloaded variable-sum negotiation tasks (Pruitt & Lewis, 1975; Kelley, 1966; Thompson & Hastie, 1990; Thompson, 1991). While negotiating agreements to these tasks, some individuals had access to a computerized *offer screen*. This screen has three general components: (a) a *pushbutton interface* representing the negotiation problem space, (b)

two *point calculators*, and (c) a *vertical bar chart*. The negotiator is able to view her problem space on the screen, including all negotiation issues, all levels per issue, and her outcome (points) for each level. The negotiator can then pick a level for each issue by clicking the left mouse button (LMB) on the pushbutton representing that level. As the negotiator selects levels with the LMB, the total points per issue are displayed as green, vertical bars on the bar graph. The bars can be compared with existing black bars representing the maximum points the negotiator could win or lose for each issue. A third set of yellow bars are displayed when the negotiator uses the right mouse button (RMB) to select an issue level. In this way, negotiators can simultaneously track two potential offers, a *green offer* and a *yellow offer*. In addition to updating the vertical bar graph, clicking the LMB on an issue level changes the value displayed in the green point total box. This is the total number of points that the negotiator would receive if the final agreement included all of the levels selected using the LMB. Similarly, clicking the RMB on an issue level updates a second yellow point total box. In this way, negotiators receive instantaneous and simultaneous graphical and numerical information comparing the outcomes of two potential offers. Negotiators are trained to track their own offers with the LMB and their opponent's offers with the RMB. It is anticipated that this combination of the interactive problem space with the automatic point calculation and display capabilities will alleviate the problems caused by preconceived beliefs and poor strategy choice. Attentional resources will be redirected away from mathematical calculation of outcomes and toward other tasks. The bar graph will provide the user with a quick visual ranking of each issue's relative importance as well as displaying the issues on which the opponent is approaching (or not approaching) a satisfactory distribution of outcomes.

An experimental lab study was designed to evaluate the degree to which these session-oriented tools help negotiators to integrate their interests. It is hypothesized that computer-assisted dyads will differ from manually assisted and unassisted dyads on a number of measures relevant to bargaining ability. Computer-assisted negotiators will

obtain higher joint outcomes and will achieve higher interest estimation accuracy. Furthermore, these main effects will interact with negotiation complexity, the benefits of computer assistance being more pronounced for highly complex negotiations.

Method

Overview

The current study used a variable-sum negotiation task to determine the degree to which computer-assisted dyads are better than unassisted and/or manually assisted dyads at achieving integrative bargaining agreements. Dyads engaged in both a four-issue and an eight-issue negotiation during a single experimental session. Two between-subjects independent variables were task order and level of assistance. Dependent measures were constructed using the number of points obtained by the dyad for each of the two negotiated agreements as well as each negotiators' estimate of her opponent's interests.

Subjects

Sixty males and 60 females served as subjects in this study. All were students and staff from a midwestern liberal-arts college and were recruited using advertisements placed at various campus locations.

Independent variables

There was one major independent variable in the current study: *type of assistance*. This between-subjects variable had three levels: unassisted, manually assisted, and computer-assisted. The order of presentation of the four-issue and the eight-issue tasks was also manipulated, with the four-issue task occurring first for half of the dyads. Finally, an equal number of male and female dyads participated in the study in order to assess potential gender effects.

Procedure

Subjects arrived in same-sex pairs. During the recruitment phase, experimenters ensured that the dyads were unfamiliar with one another. Upon arrival, one of two experimenters escorted each subject to a private desk to minimize pre-experimental

contact and discussion. After obtaining informed consent, an experimenter introduced the first negotiation task, distributed the payoff schedules, and described the method of payment. Participants were awarded \$10 for their participation in the study. The chance to earn a \$100 *bonus prize* was used to further motivate the participants during the negotiation phase. The experimenter informed each individual that the person who earned the most points would receive the \$100 prize. Subjects were told that the winner would be determined upon completion of the *entire study*. They were also told that the probability of a *tie* was quite high and, in the case of a tie, the recipient would be randomly selected from among the top point earners¹.

During the introduction, the experimenter oriented each subject toward her role in the first negotiation. The current study included two negotiations: *program analyst versus systems engineer* and *salesperson versus buyer*. For each negotiation, an experimenter randomly assigned subjects to one of the two roles. The experimenter then read a role description aloud, while the subject reviewed his payoff schedule. Each protocol also included a short statement describing the opposing negotiator. A *briefing sheet* distributed to each negotiator contained information concerning the negotiation issues and her *supervisor's* interests for each issue. These statements were simply phrased to reflect the point distributions *within* each negotiation issue. No explicit comparisons were made *across* issues at any point during the introduction.

Next, subjects completed a short, paper-and-pencil exercise designed to measure their understanding of their first payoff schedule. The exercise required that the subject determine the number of points he would obtain given a particular negotiated outcome. Subjects in the computer-assisted condition used a pointing device (mouse) to select levels within each negotiation issue from the offer screen. The computer automatically calculated

¹This protocol was used to avoid implying that the negotiators were to compete with one another during the upcoming negotiations.

the points earned by the negotiator given his selections and displayed this total on the screen. The experimenter provided subjects in the manually assisted condition with a simple electronic calculator to use in completing the exercise. Subjects in the unassisted condition were given note paper and a pencil. All subjects were expected to achieve 100% accuracy on the exercise before continuing with the procedure. This pre-negotiation phase of the study (the introduction, role orientation, and exercise) took approximately 20 minutes to complete.

Subjects in the computer-assisted condition then participated in a private, 10-minute training session with an experimenter. This training session was designed to instruct participants on the use of the computer interface. The experimenter described the information displayed on the computer screen, and emphasized the computational method and the basic meaning of each piece of information.

The experimenter then escorted each subject to a conference room where each sat across the table from her opponent. In the unassisted and manual condition, the computers were replaced with cardboard screens of about the same physical size, designed to shield from view any notes taken during the negotiation. Subjects in the unassisted and manual condition received blank sheets of note paper and were allowed to bring their briefing sheets and the payoff schedules into the conference room. Also, those in the manual condition were allowed to use their calculators during the negotiation.

Next, an experimenter introduced the subjects to one another by role (e.g., "This is the program analyst."). The experimenter advised the subjects as to the 15 minute time limit and the consequences of not reaching an agreement. Subjects were told that, if they failed to reach an agreement within 15 minutes, each negotiator would receive zero points for that negotiation. Actually, the experimenters allowed each dyad an extra 5 minutes for negotiation if needed. During each negotiation, dyads were also given a *two minute warning* signal at the 13 minute mark.

The experimenter informed the subjects that, while each had a copy of his own payoff schedule, they were never to show the schedule to their opponent during the negotiation. If this occurred, the experimenter terminated the negotiation and recorded the last offer as the final agreement. Otherwise, there were no limits placed on between-subject communication. Before leaving the room, an experimenter instructed the dyad to knock on the door of the conference room after reaching a final agreement.

The experimenters observed the negotiation from behind a one-way mirror, timing the duration of the negotiation with a stopwatch. Following the final agreement, the participants were escorted back to their private desks and given an *estimation sheet*. The main purpose of this sheet was to obtain subjects' estimates of their opponents' point distributions across issue levels. The estimation sheet was simply a payoff schedule with the point values missing. The issue levels were arranged in a manner identical to the negotiators own payoff schedule. An experimenter asked the subject to fill out the schedule based on her impressions of her opponent's interests.

The second negotiation proceeded much the same as the first. An experimenter oriented each subject to his new role. Subjects received new payoff schedules and completed a second exercise. The negotiators followed the same rules as were used in the first negotiation. After a final decision was reached, the subjects completed a second estimation sheet. An experimenter then paid and debriefed the subjects.

Payoff schedules.

The variable-sum experimental task used in the current study is similar to that used in previous research (Pruitt & Lewis, 1975; Kelley, 1966; Thompson & Hastie, 1990; Thompson, 1991). Each subject is privately presented with a payoff schedule consisting of a number of negotiation issues and a number of potential levels within each issue. The schedule also includes the point values associated with each issue level. Each negotiator is privy to her own payoff schedule only. At no time prior to reaching a negotiated agreement is either negotiator given access to her opponent's schedule.

The eight-issue payoff schedule presented to the program analyst role-player and illustrated in Figure 1, represents a negotiation between two United States Air Force employees: a *program analyst* and a *systems engineer*.

Figure 1

Payoff Schedule - Program Analyst

Number of Maintenance Persons	Scope of Developmental Testing	"Skin" Material Type	Average Time to "Turn"
2 (4000)	2000 hrs (1600)	Type A (2400)	20 min (0)
4 (3000)	4000 hrs (1200)	Type B (1800)	30 min (-600)
6 (2000)	6000 hrs (800)	Type C (1200)	40 min (-1200)
8 (1000)	8000 hrs (400)	Type D (600)	50 min (-1800)
10 (0)	10,000 hrs (0)	Type E (0)	60 min (-2400)

Foreign Interoperability	Average Missions Between Failures	Time to First Delivery	Maximum Speed
13 countries (3200)	10 missions (0)	1 yr (800)	500 mph (0)
10 countries (2400)	8 missions (-1500)	2 yrs (600)	550 mph (-400)
7 countries (1600)	6 missions (-3000)	3 yrs (400)	600 mph (-800)
4 countries (800)	4 missions (-4500)	4 yrs (200)	650 mph (-1200)
1 country (0)	2 missions (-6000)	5 yrs (0)	700 mph (-1600)

Both are assigned to the same program management team and are given the task of determining the desired characteristics of a new unmanned reconnaissance aircraft. The following issues are open for negotiation: (1) *number of maintenance personnel*, (2) *scope of developmental testing*, (3) *skin material type*, (4) *average time to turn*, (5) *foreign interoperability*, (6) *average time between failures*, (7) *time of first delivery*, and (8) *maximum speed*. Each negotiation issue has five levels. Each level is worth a certain

number of points to the negotiator. Each negotiator's total points are calculated by summing across all eight issues following the dyad's final agreement.

In the eight-issue task, two of the issues have identical point distributions for both the program analyst and the systems engineer (*average time to turn* and *foreign interoperability*). Two of the issues have zero-sum or diametrically opposed distributions (*skin material type* and *maximum speed*). The other four are logrolling issues. While the two negotiators have conflicting interests, each issue is less important to one negotiator than it is to the other (i.e., the within-issue point sum is less for one party than it is for the other). *Number of maintenance personnel* and *average time between failures* are logrolling issues that are more important to the program analyst than to the systems engineer. *Scope of developmental testing* and *time to first delivery* are logrolling issues that are more important to the systems engineer than to the program analyst.

The four-issue payoff schedule presented to the buyer and illustrated in Figure 2, represents a negotiation between a United States Air Force *buyer* and a *salesperson* from a large defense contractor.

Figure 2

Payoff Schedule - Buyer

Unit Price	ASAT Defense	Time to First Delivery	Orbits to Change Altitude
7 billion (1600)	All three (0)	1 yr (2400)	1 orbit (4000)
8 billion (1200)	"S" & "T" (-600)	2 yrs (1800)	1.5 orbit (3000)
9 billion (800)	"T" only (-1200)	3 yrs (1200)	2 orbits (2000)
10 billion (400)	"S" & "D" (-1800)	4 yrs (600)	2.5 orbits (1000)
11 billion (0)	"S" only (-2400)	5 yrs (0)	3 orbits (0)

The buyer is interested in purchasing a communications satellite. Four issues are open for negotiation: (1) *unit price*, (2) *anti-satellite defenses*, (3) *time of first delivery*,

and (4) *orbits to change altitude*. Again, each negotiator's total points are calculated by summing across all four issues following the dyad's final agreement.

In the four-issue task, one of the issues has an identical point distribution for both the buyer and the salesperson (*anti-satellite defenses*). One of the issues has a zero-sum or diametrically opposed distribution (*time of first delivery*). The other two are logrolling issues. *Orbits to change altitude* is a logrolling issue that is more important to the buyer than to the salesperson. *Unit price* is a logrolling issue that is more important to the salesperson than to the buyer.

Dependent variables.

There were two major dependent variables in the current study, the proportion of the integrative total obtained by the dyad for each negotiation and the accuracy of the dyad's point estimates. The total number of points obtained by the dyad was divided by the integrative total (10,400 for the four-issue negotiation and 15,200 for the eight-issue negotiation). The estimation-related variable consisted of a measure of the degree to which each member of the dyad realized that the opposing member's points were distributed such that one issue of a logrolling pair was less important than the other. This measure was obtained using the point values from the estimation tasks. For each logrolling pair, a difference score was calculated by subtracting the absolute value of the sum of the estimated point values (across levels) of the less important issue from the absolute value of the sum of the estimated point values of the more important issue. This difference was then divided by the actual difference between the two sums as stated on the opposing player's payoff schedule. For the eight-issue task, a total score for each player was obtained by averaging the scores for the two logrolling pairs. For both the four-issue and the eight-issue negotiation, the score for the dyad was the arithmetic mean of the scores of the two players. Using this measure, a score of zero indicates that both members of the dyad mistakenly estimated the logrolling issues to be of the same importance (i.e., worth the same number of points) to the opposing negotiator. A score of +1.0 indicates that both

members of the dyad correctly guessed the preference ranking and the magnitude of the difference. A score of -1.0 indicates that both members of the dyad correctly guessed the magnitude of the difference but mistakenly reversed the preference order. Scores greater than ± 1.0 are possible using this measure. Such a score would indicate an overestimation of the magnitude of the difference between issues in a logrolling pair.

Units of analysis.

Because the experimental task involved an interaction between two negotiators, the dyad was used as the unit of analysis. Ten male and 10 female dyads were run in each cell of the current 2 (simple task first versus complex task first) x 3 (unassisted versus manually assisted versus computer assisted) design.

Results

Proportion of integrative total

The proportion of the integrative total obtained by dyads in the three different assistance conditions is presented in the first row of Table 1.

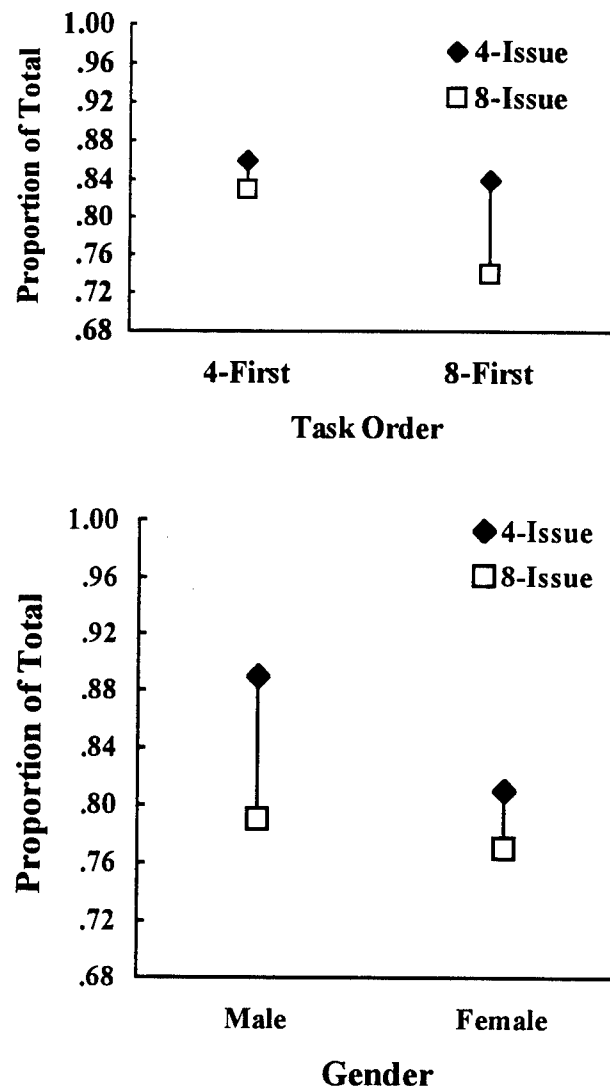
Table 1

Dependent Variable	Level of Assistance					
	Unassisted		Manual		Computer	
	Male	Fem.	Male	Fem.	Male	Fem.
Four-Issue Negotiation						
Prop. of Integrative Total	.85	.85	.88	.80	.95	.78
Estimation Accuracy Index	-.39	-.72	-.23	-.59	-.01	-.67
Eight-Issue Negotiation						
Prop. of Integrative Total	.78	.81	.74	.75	.86	.77
Estimation Accuracy Index	-.69	-.42	-.20	-.65	-.20	-.60

A repeated measures analysis of variance (ANOVA), using the proportions obtained in the four-issue and the eight-issue negotiations as two repeated measures and task order, gender, and level of assistance as independent measures, obtained a significant difference overall, $F(1,48) = 29.56$, $p < .001$. Dyads achieved a significantly higher proportion of the integrative total on the four-issue negotiation than on the eight-issue negotiation. This effect was qualified by significant interactions with task order, $F(1,48) = 7.08$, $p < .05$, and with gender, $F(1,48) = 6.08$, $p < .05$. These interactions are illustrated in Figure 3.

The univariate ANOVA using the proportion of the integrative total obtained in the four-issue task as the dependent measure revealed a significant main effect for gender, $F(1,48) = 11.69$, $p < .01$, qualified by a significant gender by level of assistance interaction, $F(2,48) = 4.37$, $p < .05$. In addition, a significant interaction was obtained between order and level of assistance, $F(2,48) = 4.61$, $p < .05$. The gender by level of assistance interaction is illustrated in Figure 4. While males in the computer condition obtained a significantly greater proportion of the total profit than did males in both the unassisted condition, $t(48) = 2.46$, $p > .01$, and the manual condition, $t(48) = 1.72$, $p <$

Figure 3



.05, female negotiators' performance did not improve with assistance. With respect to task order, negotiators achieved a higher proportion of the integrative total on the four-issue task when this task came first rather than second.

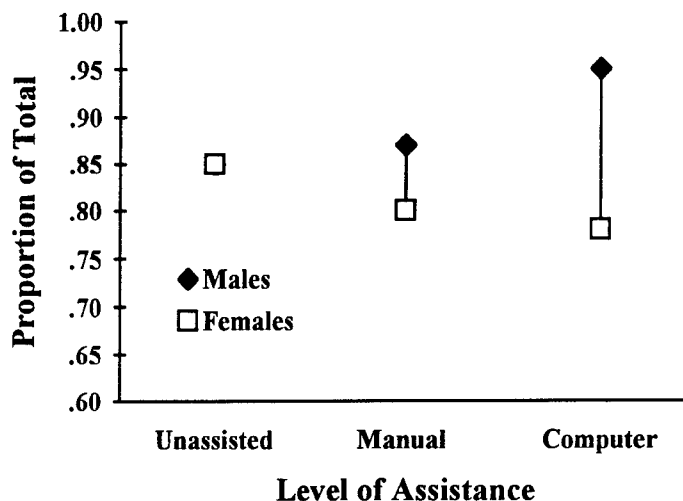
A similar ANOVA was conducted using the proportion of the integrative total obtained in the eight-issue task as the dependent measure. A significant order effect was obtained, $F(1,48) = 15.50$, $p < .001$, with dyads reaching a higher joint profit on the eight-issue task when

this task occurred second in the task order rather than first (means = .83 and .74 respectively). A significant main effect for assistance was also obtained, $F(1,48) = 3.19$, $p < .05$. While not significantly different from each other, the proportion of joint profit in both the unassisted (mean = .80) and the computer assisted (mean = .81) conditions was significantly greater than that obtained in the manually assisted condition (mean = .74).

Interest estimation

The estimation accuracy values obtained by dyads in the three different assistance conditions is presented in the second row of Table 1. A repeated measures analysis of variance (ANOVA), using the values obtained in the four-issue and the eight-issue negotiations as repeated measures obtained no significant repeated measures effects. Separate univariate ANOVAs revealed significant main effects for gender on both the four-issue task, $F(1,41) = 6.84$, $p < .05$, and the eight-issue task, $F(1,41) = 5.93$, $p < .05$. On both the four-issue and the eight-issue negotiation, females received lower accuracy scores than males.

Figure 4



Significant Pearson product-moment correlation coefficients were obtained between the estimation accuracy index and the proportion of the integrative total for both the four-issue, $r(54) = .41$, $p < .01$, and the eight-issue negotiation, $r(56) = .32$, $p < .05$.

Discussion

The results of this first study are mixed. When applied to the four-issue task, computer assistance did not appear to improve females' ability to obtain a higher joint profit and, on the eight-issue task, accomplished little for either gender. In addition, although estimation accuracy correlated with interest integration on both tasks, there was little evidence that this brand of computer assistance improved negotiators' ability to estimate the point values in their opponents' payoff schedules. On the other hand, interest integration by male dyads in the four-issue task was clearly enhanced by computer assistance.

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ABSTRACT

The purpose of this study was to investigate if students learn *better* with on-line *intelligent coaching systems* driven by student modelling than with *passive on-line help systems*?

Most of the research effort in ITS was centered around investigating the implementational issues involved in constructing components and systems. The field has now reached a consensus on the different types of ITS systems, and their respective components. Therefore, it is important to begin evaluating the effectiveness of intelligent coaching systems to alternatives such as passive on-line help systems.

Relevant research is very sparse. Mark and Greer (1991) compared the education impact of several coaching systems to one another. The results indicated that students who learned with intelligent coaching that incorporates student modelling and knowledgeable feedback used fewer steps and had fewer errors when doing their task.

The purpose of this experiment is to investigate if Students learn *better* with on-line *intelligent coaching systems* driven by student modeling than with *intelligent coaching system without student modeling* or *passive on-line help systems*? The experimental study that investigated the question posed by research was conducted at the USAF Armstrong Laboratories. The results demonstrate students who used the coaching system with student

modeling do significantly better than those who used the coaching system without student modeling or passive on-line help.

Evaluation of an Approach to Intelligent Coaching with Student Modeling

Anoosh Shahidi

1. Introduction

1.1 Objective

The purpose of this experiment is to investigate if Students learn *better* with on-line *intelligent coaching systems driven by student modeling* than with *intelligent coaching system without student modeling* or *passive on-line help systems*?

1.2 Significance

This project has made the following contributions:

- The field has now reached a consensus on the different types of ITS systems, and their respective components. Therefore, it is important to begin evaluating the effectiveness of intelligent coaching systems compared to alternatives such as passive on-line help systems. No definitive study has been done to date that answers this question.
- *Extending Goldstien's Genetic Graph*: Goldstein's learning scheme implied a very strong progression from general concepts to specific concepts. Current views of situated learning tend to see initial learning in both directions (meaning also in terms of specifics, from which generalizations are often incomplete). This project extends the genetic graph representation to bring it in line with the current views on learning. This project will evaluate this extension's validity.

- A related issue that was addressed during this research is what are the limits on passive on-line help and intelligent coaching systems. Are there situations where passive on-line help fails to help the student? Are there categories of bugs that are better remediated through intelligent coaching with student modeling?

2 Background

It is important to point out that there is no generic answer to the question posed by this proposal that makes a universal statement regarding intelligent coaching systems. The following sections will discuss variety of coaching systems, passive on-line systems, measurement criteria, type of errors, and domain. A choice had to be made with regard to each. Therefore, the result of this study is limited in scope and will have to be expanded in subsequent projects. It is also important to mention at this time that the domain chosen for this project is spreadsheet programming.

2.1 Intelligent Coaching

Intelligent coaching systems provide unobtrusive assistance while the student is involved in an independent learning activity. Students can get stuck on "plateaus" of proficiency. Making them aware of further possibilities is the task of the coach. However, the art of coaching is subtle: interventions must be effective in preventing plateaus without destroying interest in the activity. Coaching differs from other tutoring situations such as mixed initiative dialogues in that the student is always in complete control of the activity. There are different types of coaching systems with different degrees of "intelligence". I will describe each type of coaching system and justify the one that I intend to implement for this project.

- The "Minimal" Coaching Strategy: One type of coaching system offers support for developing solution-enabling mental representations and strategies within each student by helping the student to think about the situations. In a well structured software environment such as spreadsheets, there is no evidence that such an approach is more beneficial than just seeing error messages for their incorrect actions.
- The Self-Reflective Coaching Strategy: Another approach to implementing coaching systems attempts to foster learning by utilizing another student learning strategy called self-explanation. As students learn new material, they spontaneously explain it to themselves, by relating the new material to previously learned material. This class of coaching systems does not teach a subject by direct exposition, but leads the student by successive questions to formulate general principles on the basis of individual cases (i.e., a Socratic dialogue), to examine the validity of one's own hypothesis, to discover contradictions, and finally to extract correct inferences from the facts one already knows. Socratic tutors contain rules which seem to embody the local decisions made by the tutor in conducting their dialog with the student. Global tutorial goals such as the correction of a pervasive misconception are often pursued by human tutors, but are ignored in the local applicability conditions of the rules.
- Traditional Coaching Systems: This type of coaching system uses concepts that have become traditional in intelligent coaching system research. The coaching system is divided into four distinct modules: domain expertise, model of the student, diagnosis process, and pedagogical strategies. Traditional coaching systems claim that their communication process is organized around learning: that

is, around the successful integration of new material and new experiences into the student's existing body of knowledge. This requires pieces of information that are specifically used for pedagogical purposes, although by nature they belong to the domain. For instance, prerequisite relations and measures of relative difficulty are crucial to flexibility in assembling instructional sequences. Other pedagogically oriented pieces of domain knowledge include rationals for explanations in terms of goals and causes, as well as conceptual or taxonomic relations between pieces of knowledge that facilitate the use of analogies and abstraction. *Therefore, generalization of knowledge and discrimination of relevant similarities and differences can be fostered explicitly.* It was the goal of this project to evaluate the educational impact of this type of coaching system compared to passive on-line help systems.

2.2 Passive Help Systems

On-line help systems have even more of a variety than intelligent coaching systems. They provide a range of assistance from simple command assistance to elaborate and detailed tutoring. Specifically, the types of assistance offered include command, help and error assistance, prompting, on-line tutors, and on-line documentation. By definition, passive help system dialogues must be initiated by the student. Command and help assistance are available on most interactive systems. On-line tutors are available on systems where the primary user is not familiar with computer technology (e.g., wordprocessors). Error assistance, prompting, and on-line documentation are still less available because software developers are not convinced of their benefits. The kind of help that the user will be provided in this experiment will be command and help assistance.

2.3 What do I mean by "BETTER": Evaluation of Effectiveness

The question that is being asked could be rephrased as "what is the educational impact of an intelligent coaching system when compared to passive on-line help." The criteria that educators have found useful in assessing educational impact include *transfer*, *retention*, *learning time*, and *completion rate and number of errors*. The appropriate measures chosen for this study are completion rate and number of errors.

3 Methodology

Three applications were developed for this study:

- Spreadsheet environment with just passive on-line help.
- Spreadsheet coach without student modeling (rudimentary coach). The student was only told what is wrong, and it is only up to him/her to further explore the environment using and on-line help to find a solution.
- Spreadsheet coach with student modeling (intelligent coach with knowledgeable feedback) where the feedback to the student explicitly fostered generalization of knowledge and discrimination of relevant similarities.

The following steps were taken to develop the three systems. First a pilot study was conducted to determine the right target student population and the right level of tutoring. Then the spreadsheet environment with passive on-line help was developed. Next the two coaches were developed. The following is a more detailed description of these steps.

- *Pilot Study:* The instructor interviews and one-on-one student testing was carried out in the University of Pittsburgh Computer Learning Center (CLC). Since the expert instructors at CLC had had anywhere from 4 to 9 years of experience teaching spreadsheets, they had a fund of knowledge about typical errors that students encounter in working with

spreadsheets. The result of this step was a catalog of spreadsheet bugs that will be used by the diagnostic process of the intelligent coach. Most novice errors are behavioral.

Intermediate users are more interesting because in addition to behavioral errors they also demonstrate a certain amount of strategic knowledge as a result of having their own goals that can result in intentional errors. As a result of instructor interviews and one-on-one testing it was determined that students who complete the first four sessions of the CLC course on EXCEL will be the target population. One-on-one student testing was used to validate the error taxonomy and identify areas where on-line help is of limited use.

The following describes the implementation of various software modules for the intelligent coaching system with student modeling:

- 1) Expert Model: The expert model for this project has knowledge of structure of spreadsheets (e.g. where to label cells, where to enter values, correct use of formulas, etc.).

- 2) Learning Model: Goldstein (1982) employs an overlay approach to a genetic graph in which the student's knowledge is described in terms of the nodes of the graph, learning behavior in terms of the edges, and progress in terms of the paths in the graph. The nodes of the genetic graph represent the facts, rules or procedures that describe a piece of expert knowledge (a skill, a subskill, or a concept) as well as deviations thereof. The links are the evolutionary relationships between the nodes. Goldstein defines these links as analogies, generalizations, specializations, refinements, simplifications, deviations, and corrections.

In a departure from the original use hierarchy proposed by Goldstein (1982), I have developed an equivalent representation of the genetic graph for spreadsheets, but using an abstraction hierarchy as opposed to the original use hierarchy. There

are two kinds of inheritance links. (1) Refinement links, where the child procedures manipulate a subset of the data manipulated by the parent procedure. This relation represents the evolution of a procedure to take account of a finer set of distinctions. For example distinguishing between the two methods of formula entry procedures - the function method and the cell addition method. (2) Generalization/specialization links where the child procedure is obtained from a parent procedure by quantifying over some constant. For example, cell referencing via absolute addressing as opposed to relative addressing can be generalized as the *addressing procedures* used within the *function method*.

3) The diagnostic and Remediation Processes: At this stage the purpose is to discover both which knowledge, correct or incorrect, has been used to produce behavior, and which relevant knowledge has been overlooked. While the former require the use of *model tracing* (Corbett and Anderson 1992), the latter requires the use of *differential modeling*, whereby the knowledge used by the student can be compared to that which the expert would have used (e.g., WEST, GUIDON). This comparison yields a list of issues the student failed to apply. These issues can then be used in remediating the student.

For the rudimentary coach, the diagnostic module was disconnected from the student modeling module. So feedback to the student was only limited to what was wrong with no further elaboration. In case the intelligent coach has a large education impact when compared to passive on-line help, the rudimentary tutor allows us to see if that is due to the student knowing what was wrong, or the context sensitive information that the intelligent coach provides.

4 Experimental study

This section describes the experimental study that will be carried out as a part of this research to test the validity of the theoretical questions being asked. As described in the previous sections, the research question that drives this research is whether intelligent coaching supports learning better than on-line help or the rudimentary coach in the spreadsheet domain. The answer to this question can be obtained by carrying out an experimental study to examine the relationship between the three approaches and learning. If the intelligent coaching with student modeling approach promotes greater learning, then the question is answered positively.

4.1 Subjects

The subjects for this experiment were between the ages of 18-30 with a high-school diploma with no previous experience in spreadsheet programming. All of them received 3 hours of training on spreadsheet basics. The sample was chosen randomly, so individual differences such as motivation to learn was equally distributed between groups. This minimizes extraneous factors influencing the performance of the treatment groups and one control. There were two treatment groups: one group was assigned to the intelligent coaching system with student modeling, the other group was assigned to the rudimentary tutor, and a control group which only interacted with the passive on-line help group. There were 30 subjects per group.

4.2 Procedure

4.2.1 Training

All subjects received 3 hours of instruction. During this phase, students did practice problems very similar to that of typical Excel training session. The on-line help system was

taught to all students as a part of this segment. The students were encouraged to interact with the environment and on-line help.

4.2.2 Learning phase.

The students were divided into three groups: the on-line help group, the intelligent coaching group, and the rudimentary coach group. Each group solved trial problems. The duration of this phase was recorded for each student. The control group's training time was at least as long as the two treatment groups. The intelligent coaching group and the rudimentary coaching group were briefly instructed on how to use the coach at the beginning of this phase. The training problems were chosen to represent a fixed set of tasks that is familiar to anyone with a high-school diploma (e.g., a income/expense report, or home budget)

4.2.3 Post-test

During this phase, the two groups were given a test problem to solve. The duration and the student's interaction with the system was recorded. The specific variables that will be measured are explained in sections below. The tasks that the students will be solving was similar to those in the learning phase.

4.3 Independent Variables

The independent variables in this research were, broadly speaking, the control group and the two kinds of treatment: on-line help, intelligent coaching, and rudimentary coaching. It is important to consider what should constitute training with the system. The main issue that must be addressed in this section is the desired period of training for the two different treatments and the control group. Based on the observation of pilot students, a set training period consisting of two practice problems in a single session is chosen. The

control group spent as much time on task during the training phase as the two treatment groups. Since research shows that feedback and tutoring generally slows down a subject (Elkerton, 1988), the control group was given an additional problem. This would ensure that the control group would spend at least as much time on task as the two treatment groups during the training phase.

4.4 Dependent Variables

The criteria that were identified as the most important for this evaluation were the number of intentional errors during post-test, and the final score on the post-test spreadsheet.

4.5 Hypotheses

The null hypotheses for the experiment are as follows:

H₀₁- Teaching procedural knowledge by means of a coaching system using student modeling, coaching system without student modeling, and. no instruction (on-line help only) will have no difference in student achievement as measured by dependent variable of the number intentional errors committed during post-test..

H₀₂- Teaching procedural knowledge by means of a coaching system using student modeling,. coaching system without student modeling, and no instruction (on-line help only) will have no difference in student achievement as measured by dependent variable of the final score on the post-test problem (number of task goals achieved).

4.6 Results

This section discusses the results of the experimental study and discusses them in terms of their relevance to the theoretical questions being examined. The major findings of the study relate to statistical comparisons of dependent variables.

4.6.1 Achievement and achievement-related measures

The first hypothesis for this study concerns the total number of intentional errors committed during post-test. It is important to describe the difference between intentional errors and behavioral errors. A behavioral errors is what programmers call a syntactical error which results in an error message (the result of the formula does not compute into a number). A behavioral error is what programmers call a logical error which does not produce an error message (the result of the formula does compute into a number).

Intentional errors include efficiency errors. Some examples are:

- Adding the wrong column or row of numbers.
- Using numbers rather than cell addresses.
- Using absolute addressing in formulas across several columns or rows rather than relative addressing.

Table 1 shows means and standard deviations for each group on the dependent variable of number of intentional errors. Students who completed the task with fewer intentional errors performed better than those who had more errors. Table 1 also shows the F-ratio and significance levels for a one-way analysis of variance of the dependent variable. Subjects who used the coaching system with student modeling will be referred to as treatment 1. Subjects who used the rudimentary tutor will be referred to as treatment 2.

Table 1. ANOVA results for the number of intentional errors

	Control (n=30)	Treatment 1 (n=30)	Treatment 2 (n=30)
Mean	27.33	8.43	20.53
St.D.	13.9	6.42	15.67
F crit	3.101		
F	17.16		
Probability	0.08		

As can be seen the coaching system with student modeling had a significant effect on the number of intentional errors. The poorest performance occurs in treatment 2 where the user is only told what is wrong with no knowledgeable feedback. Table 2 shoes the F-ratio and significance levels for a one-way analysis of variance between treatment 2 and control.

Table 2. ANOVA results for the number of intentional errors between control and

Treatment 2

	Control (n=30)	Treatment 2 (n=30)
Mean	27.33	20.53
St.D.	13.9	15.67
F crit	4.007	
F	3.157	
Probability	0.08	

The results from Table 2 shows that there is no significant difference between control and treatment 2. Table 3 shows means and standard deviations for each group on the dependent variable of final score on the post-test problem. Since there were 25 task goals to be achieved, maximum possible score was 25.

Table 3. ANOVA results for the final post-test scores

Control (n=30) Treatment 1 (n=30) Treatment 2 (n=30)

Mean	14.89	22.41	14.4
St.D.	6.32	3.16	8.16
F crit	3.101		
F	15.4		
Probability	0.0000018		

Results from Table 3 shows that there is a significant improvement in the number of task goals achieved for subjects who used the coaching system with knowledgeable feedback. Table 4 shows means and standard deviations for each group on the dependent variable of final score on the post-test problem for just the control and treatment 2 subjects.

Table 4. ANOVA results for the final post-test scores for control and treatment 2

	Control (n=30)	Treatment 2 (n=30)
Mean	14.89	14.4
St.D.	6.32	8.16
F crit	4.006	
F	0.066	
Probability	0.79	

Results from Table 4 shows that there is no significant difference between the control group and treatment 2. Table 5 also shows the F-ratio and significance levels for a one-way analysis of variance of the post-test performance times.

Table 5. ANOVA results for the final post-test performance times

	Control (n=30)	Treatment 1 (n=30)	Treatment 2 (n=30)
Mean	16.5	16.7	20.74
St.D.	5.92	8.5	9.14
F crit	3.101		
F	2.71		
Probability	0.072		

Table 5 shows that there is no significant difference in the post-test performance times.

5. Discussion and Conclusion

It can be assumed from the data in table 2 and 4 that just telling the student what is wrong during the training phase is not enough. The student still has to search through the on-line help system to find a solution. Since students have the solution print-outs during the training phase, most of the time they are able to tell "there is something wrong", or "there must be an easier way". Control subject are able to go directly into the help system and look for solutions, but treatment 2 students are distracted by the feedback and cannot form their own hypothesis. It seems that just telling the student what is wrong without knowledgeable feedback produces a cognitive load that impedes learning.

Table 5 shows that there are no differences in performance times between groups. This mainly due to the fact that during the training time the subjects in control or treatment 2 groups can adopt sub-optimal methods which yield results just as quickly as the optimal methods. This is a unique feature of the domain of spreadsheets and the results can differ in other domains.

Of the three instructional versions of the spreadsheet tutor, only the coaching system with student modeling can be considered intelligent, since it incorporates both individualized student modeling and knowledgeable feedback. The other tutors do not incorporate AI techniques. The knowledgeable tutor was much more demanding in terms of implementation than the other versions. Additional modeling , programming, and performance enhancement costs are the norm in developing intelligent tutoring systems: the cost of I in the ITS. By indicating that knowledgeable coaching is preferable in this

procedural domain and showing that it can be provided by an intelligent coaching system utilizing individualized student modeling, this research suggests that the investment is worthwhile.

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A NOTE ON THE EXISTENCE OF RACIAL BIAS
IN SUPERVISORY RATINGS OF JOB PERFORMANCE

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ABSTRACT

Recent research on the effects of race on ratings of job performance has focused on the question of whether or not Black and White supervisors rate members of their own race more favorably than members of another race. In general, these studies have found that both Black and White supervisors tend to rate Whites higher than Blacks. While this finding is notable, it fails to adequately address more fundamental questions regarding the existence and nature of racial bias in supervisory ratings. A reexamination of the data from these studies revealed that (a) both Black and White raters reported a mean group performance difference favoring Whites, (b) Black and White supervisors did not agree on the magnitude of this difference, and (c) the difference between Black and White supervisory ratings of White ratees appeared negligible, while the difference between Black and White supervisory ratings of Black ratees was quite large, suggesting that racial bias occurred in the rating of Black job performance. Implications of these findings for differential validity and prediction is discussed.

A NOTE ON THE EXISTENCE OF RACIAL BIAS IN SUPERVISORY RATINGS OF JOB PERFORMANCE

INTRODUCTION

Background

In a meta-analysis of rater-ratee race effects, Kraiger and Ford (1985) found that White raters gave White ratees higher ratings than they did Black ratees. Conversely, Black raters were found to give Black ratees higher ratings than to White ratees. Such a pattern has serious implications for both the practitioner and the researcher. This sort of bias, if it in fact existed, would impair the ability of performance ratings to provide accurate information for the wide variety of organizational functions that rely on it. Of further interest to researchers is the effect such a bias would have on validity studies employing performance ratings as criterion measures. For example, reconciling differences in mean group performance on ability measures depends on finding true mean group performance differences on the job (see, e.g., Schmidt, 1988). The presence of racial bias in performance ratings would make such a reconciliation difficult.

Subsequent studies challenged the Kraiger and Ford (1985) finding that raters favor members of their own race (Oppler, Campbell, Pulakos, & Borman, 1992; Pulakos, White, Oppler, & Borman, 1989; Sackett & DuBois, 1991). Each of these has pointed out a fundamental shortcoming in the Kraiger and Ford (1985) meta-analysis. The majority of studies in the meta-analysis from which effect coefficients were drawn employed a between groups design where each subject was evaluated by a single rater. Very few of the studies used both

a Black and a White rater to evaluate the same individual; therefore, the observed differences in mean ratings for Blacks and Whites could have been due to true mean performance differences.¹

To overcome this limitation, these researchers conducted repeated measures (within groups) analyses. The repeated measures consisted of performance scores of individuals rated by both a Black rater and a White rater and were obtained from the U.S. Army's Project A (Oppler et al., 1992; Pulakos et al., 1989; Sackett & DuBois, 1991) and the U.S. Employment Service (USES) (Sackett & DuBois, 1991). The repeated measures analysis controls for true performance differences by using the same rates for both the Black rater treatment and the White rater treatment. The results from these studies using repeated measures analyses differed substantially from those of the meta-analysis. All three studies found that, on average, both Black and White supervisors gave Whites higher ratings than Blacks. This finding supported previous assertions that a true difference exists in mean group job performance between Blacks and Whites (Schmidt, 1988).

While such a finding is important, it does not directly bear on the larger question of whether or not racial bias exists. If there were a true mean performance difference between Blacks and Whites, and there existed no racial bias in the performance ratings, Black and White raters should not only agree on the direction of the difference in mean job performance, they should also agree on the magnitude of the difference.

¹Kraiger and Ford recognized this limitation and noted it twice in their article (pp. 58 & 62-63).

Meaning of Race Effects

Table 1 shows the typical rater-ratee race effects study design. There are four cells corresponding to the four possible rating combinations: White rating White, Black rating White, White rating Black, and Black rating Black. There are three effects associated with this design.

The difference between the row marginal means, $M_{W.} - M_{B.}$, defines the ratee race, or between groups, effect. This is the only effect which is not considered evidence of racial bias. The ratee race effect reflects the mean difference in job performance between Blacks and Whites as judged by all raters, Black and White.

Table 1. Typical Rater by Ratee Race Effects Study Design

	White Rater	Black Rater	
White Ratee	M_{WW}	M_{WB}	$M_{W.}$
Black Ratee	M_{BW}	M_{BB}	$M_{B.}$
	$M_{.W}$	$M_{.B}$	

Note. The first letter of the subscript denotes ratee race; the second letter denotes rater race. Marginal values are indicated with the dot.

The rater race effect, unlike the ratee race effect, reflects a source of bias and is defined by $M_{.W} - M_{.B}$, the difference between the column marginal means. In the between groups design, where each rater treatment (Black and White) rates a separate sample of ratees, the ratee race effect is also a between groups effect. The difference $M_{.W} - M_{.B}$ reflects the

mean performance difference between the group (sample) of Black and White ratees rated by Whites and the group of Black and White ratees rated by Blacks. Such a difference could confound multiple effects.

Repeated measures designs, on the other hand, use identical ratee samples for both the Black and White rater treatments, making $M_{.W} - M_{.B}$ a within groups (i.e., ratee group) effect with an expected value of zero. The presence of a nonzero rater race effect means that one group of raters (Black or White) systematically rates everyone (both Black and White ratees), on average, higher than does the other group of raters. Even though this type of ratings bias does not actually alter the ratee race (between groups) effect, it could prove inequitable in the workplace and troublesome in most research settings where it is rare that an individual receives performance ratings from both a Black supervisor and a White supervisor.

Even though the rater race effect is technically a biasing effect, it is the interaction effect that defines what is typically considered racial bias. A significant interaction suggests that the two groups of raters (Black and White) do not agree on the magnitude of any performance difference between the two groups of ratees. This disagreement can take a variety of forms, but in graphical terms, the ratee or rater race effects are nonparallel. In the Kraiger and Ford (1985) meta-analysis, for example, the finding was that both groups of raters found a mean performance difference, but it was reversed. The Black raters saw the difference in favor of the Black ratee group; the White raters saw the difference in favor of the White ratee group. Graphically, the effects were not only nonparallel, but they crossed. Again, however, it must be pointed out that the suboptimal design found in the majority of the studies used in the meta-analysis made any clear interpretations of interaction problematic.

A repeated measures design is better suited to properly detect this interaction. In such a design, we know the true performance difference between the repeated measures to be zero and the true difference between group performance means to be equal from one rater treatment to the other. Even with differences in leniency between Black and White raters (i.e., an actual rater race effect), the two simple within groups effects should be parallel. A significant interaction would, therefore, indicate a systematic distortion of true performance relationships by one or both rater groups.

METHOD

Results from two studies employing repeated measures designs were reexamined for evidence of interaction. The results from the between groups analyses presented in these two studies were not examined for reasons stated above. The first study, Pulakos et al. (1989), used a sample of 1,820 U.S. Army soldiers--561 Black, 1259 White--each rated by a Black and a White supervisor. Although Pulakos et al. (1989) presented results of three performance dimensions, the present study limited its reexamination to the technical skill and job effort dimension, since the other two dimensions, personal discipline and military bearing, are not comparable to performance dimensions found in civilian jobs (Sackett & DuBois, 1991, p. 875). The second study, Sackett and DuBois (1991), in addition to the U.S. Army sample studied in Pulakos et al. (1989), incorporated a sample of 331 Black and 286 White civilian ratees.

Three forms of evidence were examined. The first derived from the analyses of variance (ANOVAs) presented in the original studies. The second and third forms of

evidence were obtained by computing standardized difference statistics (d values) from the repeated measures designs. Sackett and DuBois (1991) presented d values for ratee race effects from the between groups analyses of both studies. It would be more useful to examine the d values from the repeated measures analyses, particularly for rater race effects, since the expected value of these effects is known to be zero. Fortunately, Sackett and DuBois (1991) presented standardized means of supervisory ratings of job performance from which both ratee race and rater race d values for the repeated measures designs could be computed (p. 875). (Recall that in the repeated measures design, ratee race is the between groups effect and rater race is the within groups effect.) Hence, the second form of evidence derived from comparing the d values for Black raters with those for White raters to determine whether or not the between groups effects were parallel. The third form derived from comparing the d values for the Black ratee group with those of the White ratee group to assess differences in the within groups effects.

RESULTS

The reexamination revealed clear evidence of rater-ratee race interaction. The most immediate evidence lies in the results of statistical significance tests for interaction conducted for the repeated measures ANOVAs. For the military data, a significant interaction effect was found with $F(1,1820)=11.84$, $p<.001$ (Pulakos et al., 1989, p. 775). Likewise, a significant interaction was found in the civilian data, $F(1,615)=14.17$, $p<.001$ (Sackett & DuBois, 1991, p. 875).

The between groups and within groups d values are presented in Tables 2 and 3 respectively. Table 2 shows that, for both the civilian data and the military data, between groups d values were positive for both the White rater and the Black rater treatments. This indicated that both Black and White raters reported higher mean group performance for Whites than they did Blacks. There were, however, sizable differences between these d values, suggesting an interaction effect. In the civilian data, the d values for the White rater treatment were precisely nine times larger than those for the Black rater treatment. This disparity was smaller in the military data but still quite large (nearly seven times). For the civilian data, the difference for the White rater treatment was .333 (uncorrected for unreliability), with a difference of .037 for the Black rater treatment. Similarly, for the military data, the standardized difference for the White rater treatment was .300, compared to .044 for the Black rater treatment.

A clearer picture emerged when within groups d values were examined. These are presented in Table 3 and represented graphically in Figures 1 and 2. Since the true performance difference between these repeated measures is known to be zero, a nonzero value suggests systematic differences in the way Blacks and Whites rate members of that particular group of ratees. This was the case for all ratee groups (Black and White, military and civilian), although they might appear negligible for the White ratee groups.

Table 2. Standardized Ratee Race (Between Groups) Effects Listed By Rater Race

Data Set	White Rater		Black Rater	
	Uncor- rected <i>d</i>	Corrected <i>d</i> ^a	Uncor- rected <i>d</i>	Corrected <i>d</i>
Civilian White (N=286) Black (N=331)	.333	.398	.037	.044
Military White (N=1259) Black (N=561)	.300	.359	.044	.053

Note. Positive values mean that White ratees received higher ratings than Black ratees from that group of raters.
^aCorrected for attenuation using $r_{yy}=.70$. See Pulakos et al. (1989) and Sackett and DuBois (1991).

Table 3. Standardized Rater Race (Within Groups) Effects Listed By Ratee Race

Data Set	White Ratees		Black Ratees	
	Uncor- rected <i>d</i>	Corrected <i>d</i> ^a	Uncor- rected <i>d</i>	Corrected <i>d</i>
Civilian White (N=286) Black (N=331)	.029	.035	-.267	-.319
Military White (N=1259) Black (N=561)	-.034	-.041	-.290	-.347

Note. Negative values mean that Black raters assigned higher ratings than White raters for that group of ratees.
^aCorrected for attenuation using $r_{yy}=.70$. See Pulakos et al. (1989) and Sackett and DuBois (1991).

In the civilian data set, $d=.029$ (uncorrected) for the White ratee group, indicating that White raters rated Whites slightly higher than did Black raters. An opposite and much larger effect was found for the Black ratee group ($d=-.267$). Black raters rated Blacks higher than did White raters. In the military data, Black raters rated both Whites and Blacks higher than did White raters. Again, the contrast was much larger for Black ratees than for White ratees ($-.290$ versus $-.034$).

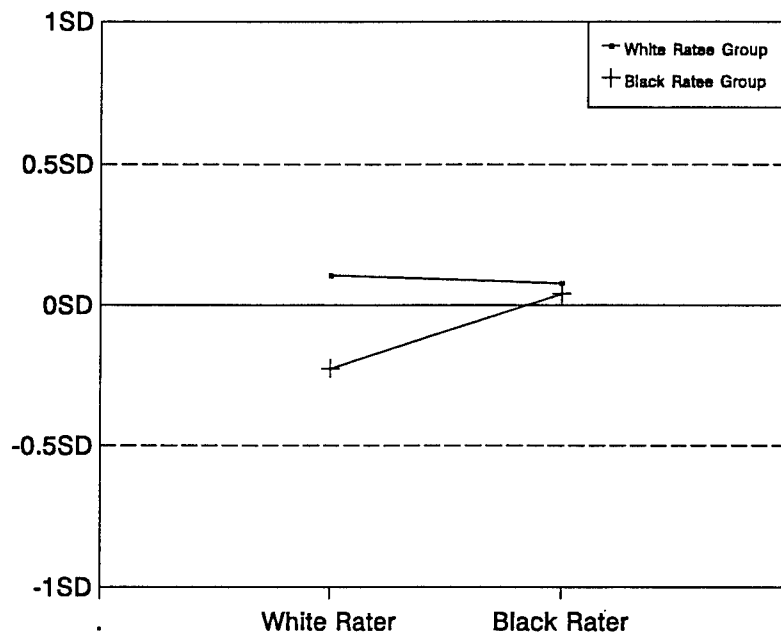


Figure 1. Standardized Means and Effects Within Ratee Groups (USES Data)

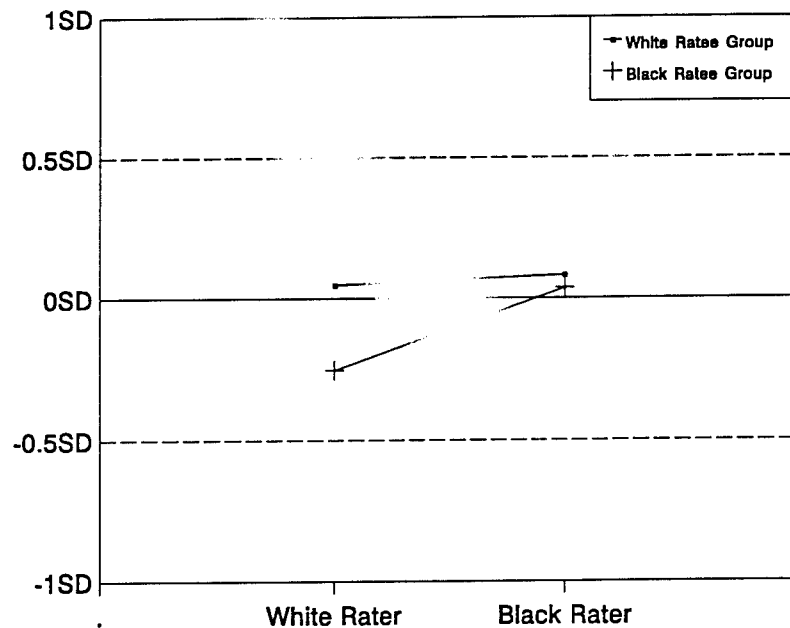


Figure 2. Standardized Means and Effects Within Ratee Groups (U.S. Army Data)

DISCUSSION

These within groups d values, whose expectations are known to be zero, the large differences in between groups d values, and associated significance tests provide clear evidence for rater-ratee race interaction. Given an identical sample of Black and White ratees, White supervisors gave significantly higher mean ratings to White ratees relative to Black ratees than did Black supervisors. It is hardly disputable that racial bias exists in these supervisory ratings of job performance.

Both Black and White raters reported a mean group performance difference favoring Whites (see Table 2). They did not, however, agree on the size of this difference. The perhaps trivial difference between Black and White raters when assessing Whites, coupled with the large difference between these two groups of raters when assessing Blacks, suggests that the problem lies in the assessment of Black job performance. It is impossible to discern from these data whether the bias resulted from White raters understating Black performance, Black raters overstating it, or both.

This reanalysis is an important step in recognizing that racial bias can exist in supervisory ratings of job performance, something Kraiger and Ford (1990) suggest we have been reluctant to do. It is not sufficient to demonstrate agreement among Black and White raters that Whites perform at a higher level than Blacks, as this line of research has tended to do. We must recognize what systematic bias does exist, try to understand how and why it occurs, and develop strategies toward eliminating it. Because organizations and researchers rely extensively on supervisory ratings of job performance, our future research efforts should be directed at better understanding the nature of the disparity between raters of different races, particularly the disparity between Black and White supervisors when assessing Black job performance.

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THE EFFECTS OF PREGNANE STEROIDS ON TRICHLOROETHYLENE
METABOLISM IN RAT BRAIN MICROSOMES

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METABOLISM IN RAT BRAIN MICROSOMES

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Abstract

The effect of chloroform and pregnan steroids on the metabolism of Trichloroethylene (TCE) in rat brain microsomes was assessed. Gas chromatography and a modified vial-equilibrium technique were used to assay TCE removal from headspace. Pregnane steroids are synthesized during pregnancy via P450-mediated metabolism and display anesthetic properties in the vertebrate central nervous system. Trichloroethylene is also metabolized by cytochrome P450-mediated activity and has been used as an anesthetic. Rat brain microsomes contain 0.07 nmol P450/mg of protein. TCE metabolism by rat brain microsomes at a TCE headspace concentration of 1000 ppm was 45 nm/min/mg of protein at 3 minutes at 37°C. Chloroform, a widely used volatile anesthetic potentiated TCE metabolism to 146 nm/min/mg of protein representing a 321% increase in metabolism. In the presence of the following pregnane steroids at 10^{-6} M: isopregnanolone, pregnanolone and epipregnanolone inhibited chloroform enhancement of TCE uptake by 32%, 27% and 18% respectively. TCE and chloroform were identified using gas chromatography. Temporal resolution and retention time of both compounds were affected by an increase in oven temperature (120°C) thus reducing data acquisition time by 60%. The present study suggests that brain metabolism of TCE is enhanced by chloroform and reduced by pregnane steroids.

THE EFFECTS OF PREGNANE STEROIDS ON TRICHLOROETHYLENE METABOLISM IN RAT BRAIN MICROSOMES

Joseph L. Curtis

INTRODUCTION

Trichloroethylene (TCE) is primarily used as a solvent in industry, and was used in the past as an anesthetic in medicine. Its industrial uses include, a cleanser for textiles, a degreasing agent for metal parts, a solvent for adhesives, and a lubricant (Bruckner et al. 1989). In humans, TCE is used as a general anesthetic in surgical, dental, and obstetrical procedures, and as an analgesic in the treatment of trigeminal neuralgia (Kimbrough et al. 1985). Various progesterone metabolites also display considerable hypnotic activity in the central nervous system. Pregnane steroids are 5α reduction products of progesterone and possess hypnotic activity. Pregnane steroids potentiate the effects of GABA at discrete binding sites on GABA_A receptors (Morrow et al. 1989). Trichloroethanol (TCEtOH), an active metabolite of TCE also potentiates GABA_A receptor function producing anesthesia in the central nervous system (Lovinger et al. 1992).

The brain is a site of extensive steroid biosynthesis and metabolism. The cholesterol side-chain cleavage enzyme, cytochrome P450_{sc}, catalyzes the formation of pregnenolone, the major intermediate in neurosteroid synthesis. In vitro experiments using rat brain microsomes have identified two major routes of steroid metabolism that recognize pregnenolone as a key substrate. 5α -reductase and steroid acyl transferase represent two enzymes systems responsible for neurosteroid

metabolism (Vourc'h et al. 1992; Akwa et al. 1991). Pregnanolone, a pregnane steroid derived from pregnenolone is a 5 α -reduction product with anesthetic actions. During estrus and pregnancy, 5 α -reduction products are particularly elevated in the brains of female rats, thus the designation pregnane steroids (Paul and Purdy, 1993). Pregnenolone, a δ^5 -3 β -hydroxysteroid is esterified into its lipoidal derivative (L) PREG-L by δ^5 -3 β -hydroxysteroid acyl transferase. PREG-L is thought to function in myelin synthesis during early brain development (Vourc'h et al. 1992). Rat brain microsomes also metabolize pregnenolone into a 7 α -hydroxylated polar metabolite 7 α -OH-Pregnenolone. This polar form of pregnenolone represents an alternative metabolic form that may be important in controlling the accumulation of neural active steroids in the brain (Akwa et al. 1992). Thus, rat brain microsomes contain the steroidogenic and metabolic enzymes for hypnotic steroid metabolism.

Cytochrome P-450 (P-450) represents a family of hemoproteins involved in the metabolism of xenobiotics (e.g. drugs, and foreign chemicals) and endobiotics (steroids, fatty acids, and cholesterol) (Anandatheerthavarada et al. 1992). The cytochrome P-450 content in the rat brain is typically one-tenth of the hepatic levels, and it exists in multiple forms which are selectively induced by phenobarbital, ethanol and 3-methylcholanthrene (Anandatheerthavarada et al. 1990). Phenobarbital is a potent GABA_A receptor agonist responsible for hypnotic actions in the central nervous system. This barbiturate selectively induces the cerebral P-450 (188% induction) and reductase (270%) activities associated with the mono-oxygenase activities mediated by P-450IIB1/IIB2 isoenzymes (Anandatheerthavarada et al. 1992). P-450IIE1

an ethanol-inducible isoenzyme, is expressed constitutively in untreated rat brain microsomes. The chronic administration of ethanol induces the cerebral P-450 (144%) and reductase (170%) activities associated with the mono-oxygenase activities mediated by the P-450IIE1 isoenzyme (Anandatheerthavarada et al. 1993).

In rat liver, two forms of TCE metabolizing P-450 isoenzymes have been induced by pretreatment with phenobarbital and ethanol. Phenobarbital induces P-450IIB expression, and ethanol induces cytochrome P-450IIE1 (Nakajima et al. 1990). These isoenzymes differ in molecular weight, spectral properties, electrophoretic mobility, immunological properties, and substrate and product regiospecificity (Ryan et al. 1985). Phenobarbital specifically induces P-450IIB which is a high- K_m type isoenzyme with low affinity for TCE. P-450IIE1, the ethanol-inducible enzyme is catalytically identical to a low- K_m type isoenzyme that exists in liver microsomes from untreated rats. The low- K_m type isoenzyme, P-450IIE1 is of special interest because it displays a high affinity for TCE and operates at low substrate concentrations (Nakajima et al. 1990).

Immunocytochemical localization studies using rat liver antisera have demonstrated the co-occurrence of P-450 with NADPH cytochrome P-450 reductase in the neuronal soma of brain glial cells (Ravindranath et al. 1990). These findings are highly suggestive and support a possible role for P-450 mediated metabolism in the pharmacological modulation of drugs acting on the central nervous system (Bhamre et al. 1993).

Because the brain is a site for steroid metabolism, and a target for the pharmacological and toxicological effects of a wide variety of environmental chemicals and drugs, the potential importance of the brain P-450 system is of special interest. In the present study, I examined the ability of rat brain microsomes to metabolize TCE in the presence of pregnane steroids using a vial-equilibrium method to evaluate P-450 enzyme activity for volatile chlorinated halocarbons.

MATERIALS AND METHODS

Animals. Male Fischer-344 rats of 8 to 10 weeks of age were used throughout. They were housed two per cage in an animal room (20°C). The animals were given water and fed a commercial pellet diet of Purina Lab Chow ad libitum.

Chemicals. Trichloroethylene (TCE)(99+%, epoxide stabilizer free), chloroform (99+%, epoxide stabilizer free), were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Steroids and all reagents used to prepare rat brain microsome were purchased from Sigma Chemical Co., Inc. (ST Louis, MO).

Preparation of microsomes. Whole brains from male Fischer-344 rats (200-250g) were homogenized gently (6 strokes of a teflon homogenizer) in 9 vols. of ice-cold buffer consisting of 10 mM Tris-HCl and 154 mM KCl (pH 7.4). The homogenate was centrifuged at 17,000 x g for 20 min. at 5°C. The supernatant was then centrifuged at 105,000 x g for 1 hour at 5°C. The microsomal pellet obtained was resuspended gently in one ml of homogenization buffer per gram original tissue

weight. Microsomes were divided into aliquots at a concentration of 7-8.5 mg of microsomal protein/ml and frozen at -70°C.

Microsomal protein and cytochrome P450. Brain microsomal protein content was measured according to the method of Lowry et al. (1951) and brain cytochrome P450 content was measured according to the spectrophotometric method of Omura and Sato (1964). Rat brain microsomes were screened for the presence of P-450IIE1 by immunoblot analysis by the method of Tobin et al. (1979).

Immunodetection of P-450IIE1. Rat brain microsomes were compared to rat liver microsomes for the presence of P-450IIE1 using rat liver antisera to P-450IIE1. Rat liver microsomes were used as a protein standard ranging from 0.1 µg to 2.0 µg.

TCE metabolism in vitro. Rat brain metabolism of TCE was assessed by measuring the rate of substrate disappearance according to a modified vial-equilibration method of Sato and Nakajima (1979): the final volume of the reaction mixture was adjusted to 1.0 ml containing 0.7 mg microsomal protein, 1.0 mM NADP, 20 mM glucose 6-phosphate, 2 U glucose-6-phosphate dehydrogenase, 0.1 M K-phosphate buffer (pH 7.4) and TCE at 1000 ppm.

TCE analysis. 780 µl of rat brain microsomes were pre-incubated for 15 minutes at 37°C in the presence of 1000 ppm TCE. After the pre-incubation period, NADPH regenerating system and 20 µl of steroids (100 µM) were added and gas quantitation of TCE remaining in headspace was accomplished. 1000 µl of gas from the head space volume was analyzed with a Hewlett Packard 5890 Series II gas chromatography instrument using flame ionization detection. A 6ft x 1/8 in. Supelco

column packed with 0.1% SPTM 1000 on 80-to 100-mesh CARBOPACKTM C was maintained at 120°C with an Air/Hydrogen (309:25 v/v) carrier gas flow of 29 ml/min. Injector temperature was 175°C and flame ionization detector temperature was 250°C. TCE metabolism and partition coefficient was calculated by area under the peaks and recorded using a Hewlett Packard 3392A integrator.

RESULTS

Microsomal Protein and Cytochrome P450

Rat brains contained 0.07 nmol of P-450/mg of microsomal protein as measured by the spectrophotometric method. Antibody to P-450IIE1 reacted specifically with the rat liver microsomes at all concentrations. The experimental samples containing rat brain microsomes also reacted positively with rat liver antisera to P-450IIE1 from 1.0 µg to 20 µg of microsomal protein.

TCE Uptake

The partition coefficient of TCE into control rat brain microsomes at 0.7 mg of protein was determined to be 2.79 at 37°C. TCE metabolism was assessed at 1000 ppm which corresponds to a concentration of 40.7 µM in the headspace volume. The microsomal concentration of TCE under these conditions was 114 µM/L of solution. Metabolism was assessed by measuring the rate of substrate disappearance from the headspace volume. The highest quantity of TCE taken up by control rat brain microsomes was 55.14 nmole at 3 minutes (Fig 2). The quantity of TCE taken up after 3 minutes quickly declined to 29 Nm and remained stable. The rate of TCE

uptake at 3 minutes was 25.12 nmol/min/mg of protein. This rate rapidly declined over a 10 minute period.

TCE And Steroid Metabolism

TCE metabolism was additionally measured after a 3 minute incubation in the presence of pregnane steroids (Fig. 4). Microsomes exposed to TCE in the presence of the NADPH regenerating system increased metabolism by 67%. This increase demonstrates that NADPH is necessary for TCE metabolism. Chloroform, an alkyl halide and central nervous system depressant was used as a vehicle for the delivery of pregnane steroids. Chloroform potentiated the metabolism of TCE in rat brain microsomes at a rate of 146.3 nmoles/min/mg of protein (Table 1). This rate is 321% greater than the control rate of TCE uptake at 45.55 nmoles/min/mg of protein. This suggests that chloroform enhances the actions of P-450 isoenzymes responsible for TCE metabolism.

Three pregnane steroids at 10^{-6} M were tested for their ability to effect TCE metabolism (Fig 4). Isopregnanolone, pregnanolone and epipregnanolone inhibited chloroform enhancement of TCE metabolism by 32.4%, 27.5% and 18.7% respectively. Isopregnanolone, a 5α reduction product that is β -hydroxylated at the 3 position of the steroid's A ring nucleus produced the greatest reduction in TCE uptake. Pregnanolone, a 5β reduction product is α -hydroxylated at the 3 position also inhibited TCE uptake. Epipregnanolone, a 5β reduction product which is β -hydroxylated at the three position inhibited TCE metabolism the least. This result suggests that the 5α structure is important to the isoenzyme(s) responsible for TCE metabolism. It also suggests that

β -hydroxylation at the 3 position contributes to the inhibition of TCE metabolizing P450 isoenzymes.

Identification of TCE and Chloroform

TCE and chloroform were separated using gas chromatography (fig 5). Both alkyl halides were identified by their specific retention times (table 2). The behavior of these chlorinated hydrocarbons was observed over a thirty degree temperature range from 90°C to 120°C. At 90°C, chloroform displayed a retention time of 2.19 minutes and TCE displayed a retention time of 7.23 min. At 120°C, chloroform displayed a retention time of 1.12 minutes, whereas TCE displayed a retention time of 2.99 minutes. By increasing the temperature, the retention time of chloroform was reduced by a little more than 1 minute, whereas the retention time of TCE was reduced by 3 min. This difference indicates that the behavior of TCE is affected by temperature to a greater degree as compared to chloroform.

Run time was greatly affected by increasing oven temperature when identifying the chlorinated hydrocarbons TCE and chloroform. At 90°C, the run time was 9 minutes, while increasing the temperature to 120°C reduced run time to 3.5 minutes. This suggests that the identification of these chemicals is temperature sensitive.

Discussion

Various steroid metabolizing enzymes coexist with constitutive P-450 and reductase enzymes involved in regulating brain metabolism and xenobiotic detoxification (Vourc'h et al. 1992; Akwa et al. 1991; Anandatheerthavarada et al. 1993; Nakajima et al. 1990). Rat brain microsomes contained 0.07 nmol of P-450/mg of protein, which strongly agrees with previously reported levels of P-450 in normal animals at 0.08 nmol of P-450/mg of protein (Anandatheerthavarada et al. 1993). Recently, immunocytochemical evidence for the constitutive expression of P-450IIE1 in normal rat brain has been presented (Hansson et al. 1990). P-450IIE1 is an ethanol-inducible isoenzyme that mediates the bioactivation of ethanol, TCE and the environmental carcinogen dimethyl nitrosamine (Tu et al. 1985; Nakajima et al. 1988; Anandatheerthavarada et al. 1993). In this study, the content of P-450IIE1 in rat brain microsomes was found to be 10-fold lower than that of rat liver.

TCE at 1000 ppm was quickly taken up by control rat brain microsomes. Within 3 minutes, 55.14 Nm of TCE was taken up by 0.7 mg of rat brain microsomes at a rate of 25.12 nmol/min/mg-of-protein. In the presence of the NADPH regenerating system, rat brain microsomes metabolized TCE at a rate of 45.55 Nm/min/mg-of-protein. Considering the small amount of P-450 contained in rat brain tissue, this rate of TCE metabolism is significant.

In the presence of chloroform, TCE was metabolized at a rate of 146.3 Nm/min/mg of protein representing a 321% increase in metabolism. Chloroform, also known as trichloromethane is metabolized by P-450 to trichloromethanol (Abou-Donia.1992). In a similar manner, TCE is

metabolized to trichloroethanol (Nakajima et al 1990). Interestingly, these chlorinated hydrocarbons are both converted to halogenated alcohols by the activities of P-450 and alcohol dehydrogenase (Nakajima et al 1989). This similarity suggests that chloroform may act as a substrate for the same P-450 isoenzymes involved in TCE metabolism. Recent studies, suggests that halocarbon anesthetics such as chloroform and TCE produce their effects by displaceable binding to specific low affinity (high K_d) protein sites (El-Maghrabi et al. 1992). These finding suggests that chloroform enhances TCE metabolism by binding to specific P-450 sites causing changes in enzyme structure and function.

Pregnane steroids inhibited TCE uptake in the presence of chloroform. Three pregnanolone analogues were used to determine if a structural-functional relationship exists between the P-450 isoenzymes responsible for steroid metabolism and halocarbon detoxification. Isopregnanolone produced the greatest level of inhibition at 32.4% which could be attributable to its α -reduction at the 5 position. Pregnanolone, a steroid with hypnotic properties inhibited TCE metabolism by 25.5%. This result is particularly interesting because pregnanolone possess a 5 β reduction moiety, and can also occur in brain tissue as a 5 α analogue (Corpechot et al. 1993). The 3 α -hydroxyl moiety was also maintained to preserve the pregnane configuration which contributes to it hypnotic effects (Im et al. 1989). During pregnancy, elevated plasma levels of progesterone directly correlates to increased concentrations of pregnanolone and 3 α -hydroxy-5 α -pregnan-20-one in the brain (Corpechot et al. 1993). This suggests that the P-450 isoenzyme responsible for TCE metabolism may also recognize pregnane steroids as

substrates based on their structure. This conclusion is also supported by the finding that epipregnanolone a 5 β 3 β analogue of pregnanolone displayed the least amount of inhibition. Epipregnanolone inhibited TCE metabolism by 18.7% respectively. One explanation for this could be found by examining steroid structure. The 5 β structure of epipregnanolone changes the position of the A-B ring junction to the cis form repositioning the 3 β -hydroxyl group below the plane of the A ring. The same can be said for pregnanolone in the 5 β configuration. The difference in activity between these two steroids surrounds the position of the 3-hydroxyl group. In pregnanolone, the 3 α -hydroxy moiety is positioned adjacent to the plane of the A ring conferring a different stereochemistry to this steroid molecule. Possibly, the combination of 5 α -reduction and hydroxylation at the 3 position of the A ring make pregnane steroids suitable substrates for P-450 activity, and confer them the ability to effect halocarbon metabolism.

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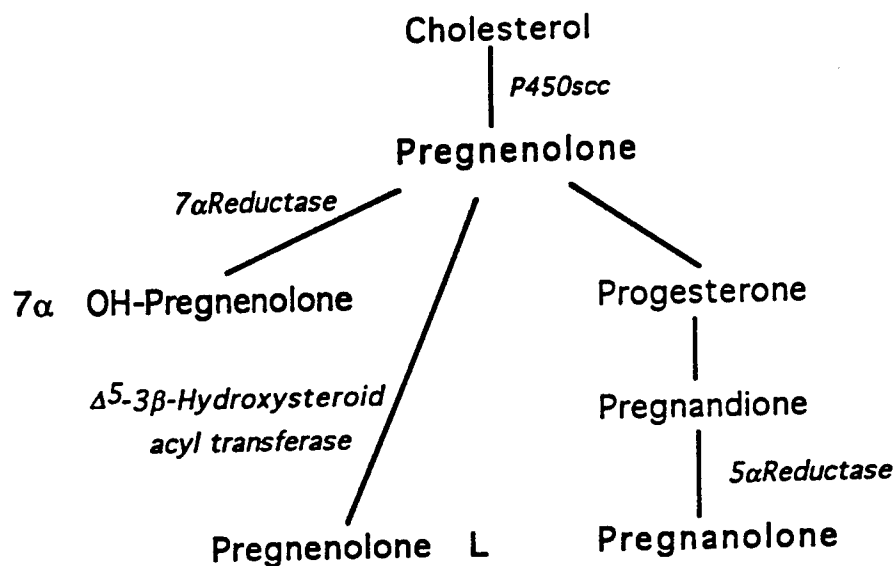


Figure 1 Neurosteroid metabolic pathways in Rat brain microsomes. Pregnanolone is synthesized via 5 α reductase through the pregnane steroid pathway. Pregnenolone L is synthesized through the acyl transferase pathway. The polar steroid metabolite 7 α OH-pregnenolone is synthesized through the 7 α reductase pathway.

UPTAKE OF 1,000 ppm TRICHLOROETHYLENE FROM HEADSPACE
BY CONTROL RAT BRAIN MICROSOMES (0.7 MG)

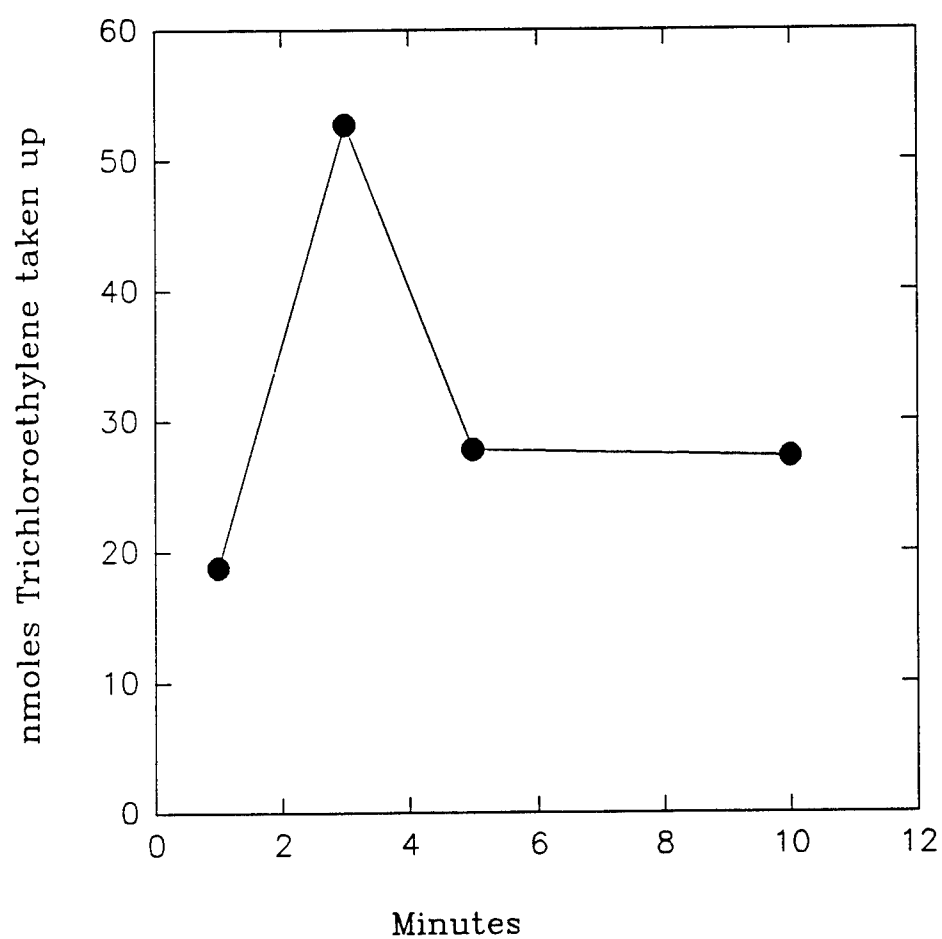


Figure 2

Data are presented as mean of two experiments.

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UPTAKE OF 1,000 ppm TRICHLOROETHYLENE FROM HEADSPACE
BY CONTROL RAT BRAIN MICROSOMES (0.7 MG)

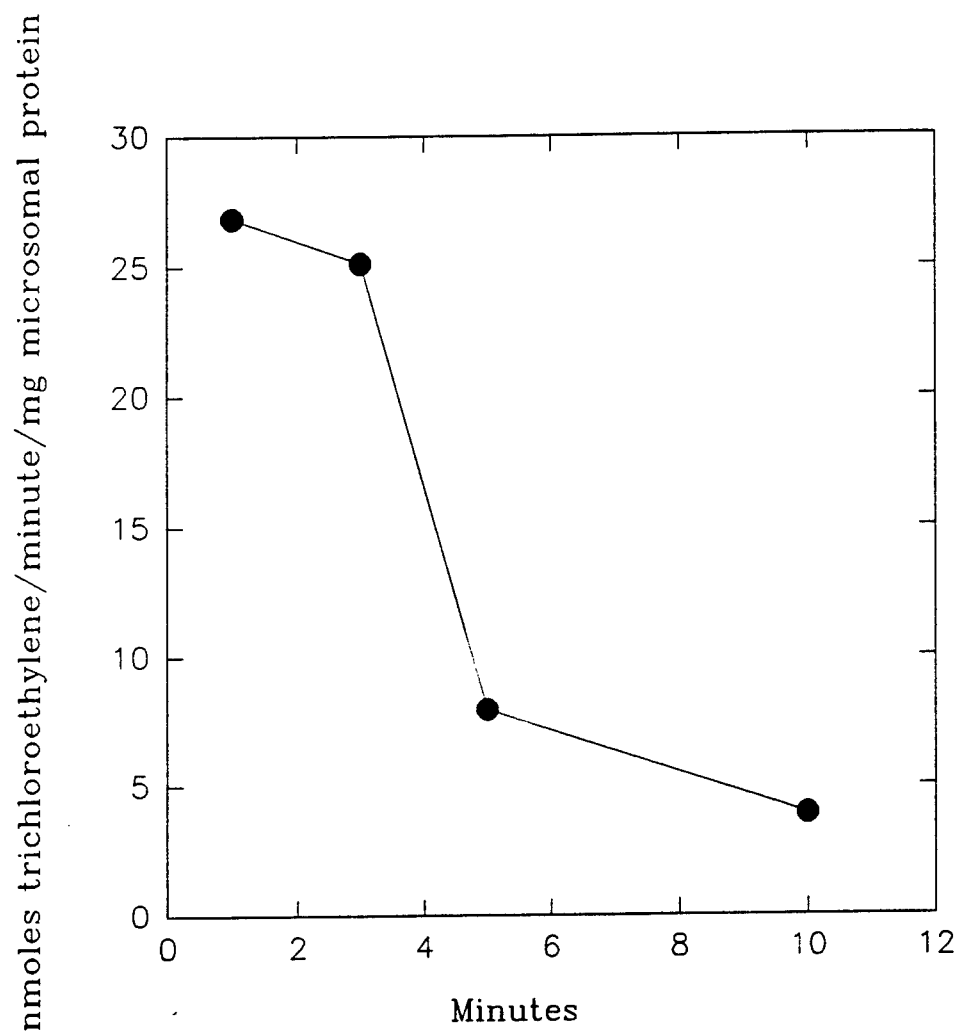


Figure 3
Data are presented as mean of two experiments.

TCE Uptake in Rat Brain Microsomes in the Presence of Chloroform and PregnaneSteroids

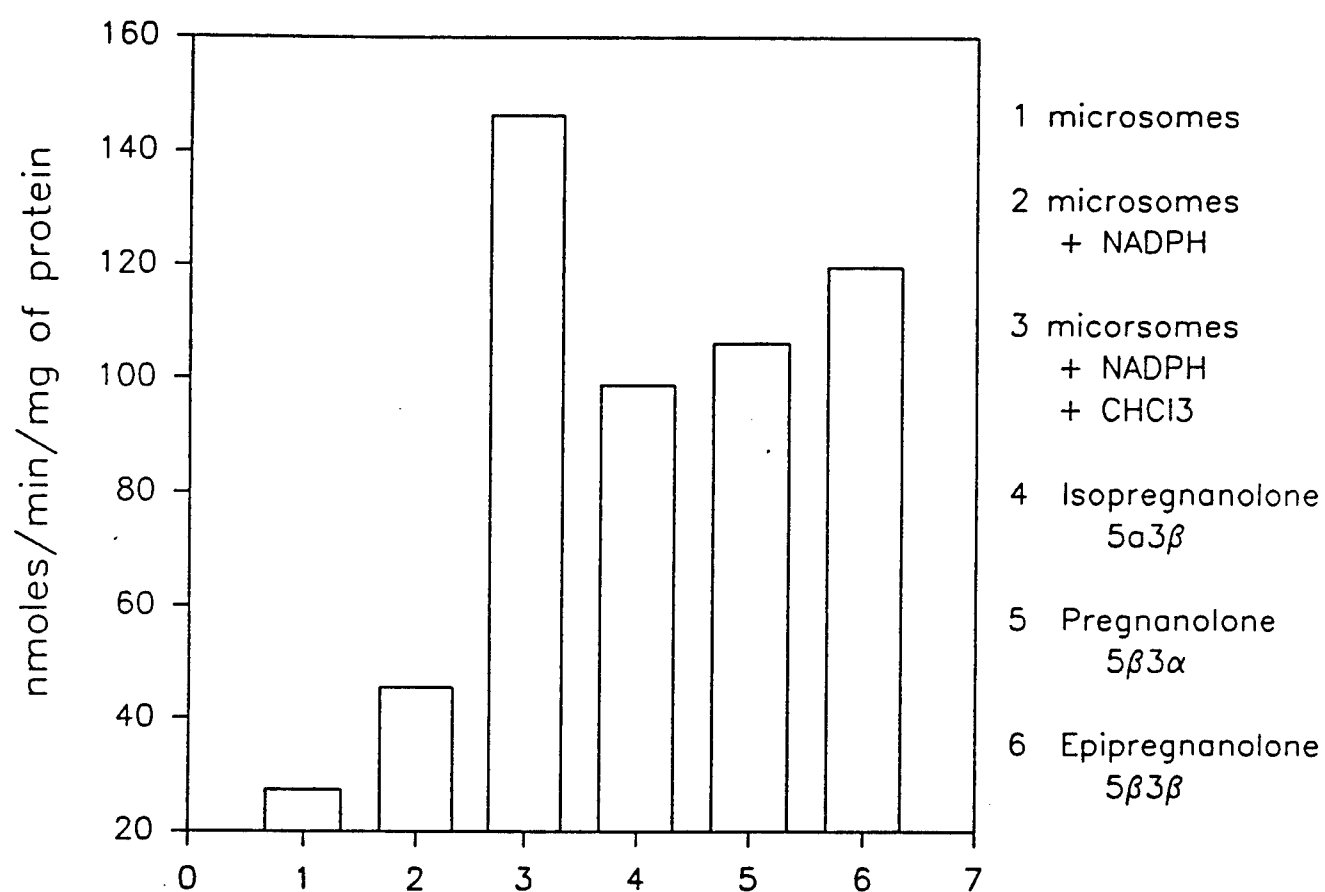


Figure 4 TCE and Steroid Metabolism.

Uptake of TCE by Rat Brain Microsomes From Headspace

	nmoles / minute	nmoles/min/mg protein
Buffer alone	25.77	—
Buffer + CHCl ₃ *	65.17	—
microsomes alone	19.11	27.3
microsomes + NADPH	31.89	45.55
microsomes + NADPH+ CHCl ₃	102.41	146.3
<i>Steroids **</i>		
isopregnanolone	69.16	98.8
Pregnanolone	74.23	106.04
Epipregnanolone	83.65	119.5

Table 1

Note: TCE was present in headspace at 1,000 ppm, which partitioned into the microsomal solution to give a TCE concentration of 114 μ M/liter of solution.

Brain microsomes were coincubated with TCE and steroids for 15 min at 37 C.

* CHCl₃ used as a vehicle to deliver steroids at a final concentration of 10mM.

** Steroids were present in a final concentration of 100 μ M in CHCl₃.

Identification of TCE and Chloroform using Gas Chromatography

Temperature (oC)	Retention Time		Run Time (min)
	TCE	Chloroform	
90	7.23	2.19	9
95	6.15	1.94	7
98	5.6	1.8	6.5
100	5.26	1.71	6
115	3.43	1.24	4
120	2.99	1.12	3.5

Table 2

Temporal Resolution of TCE and Chloroform
Using Gas Chromatography

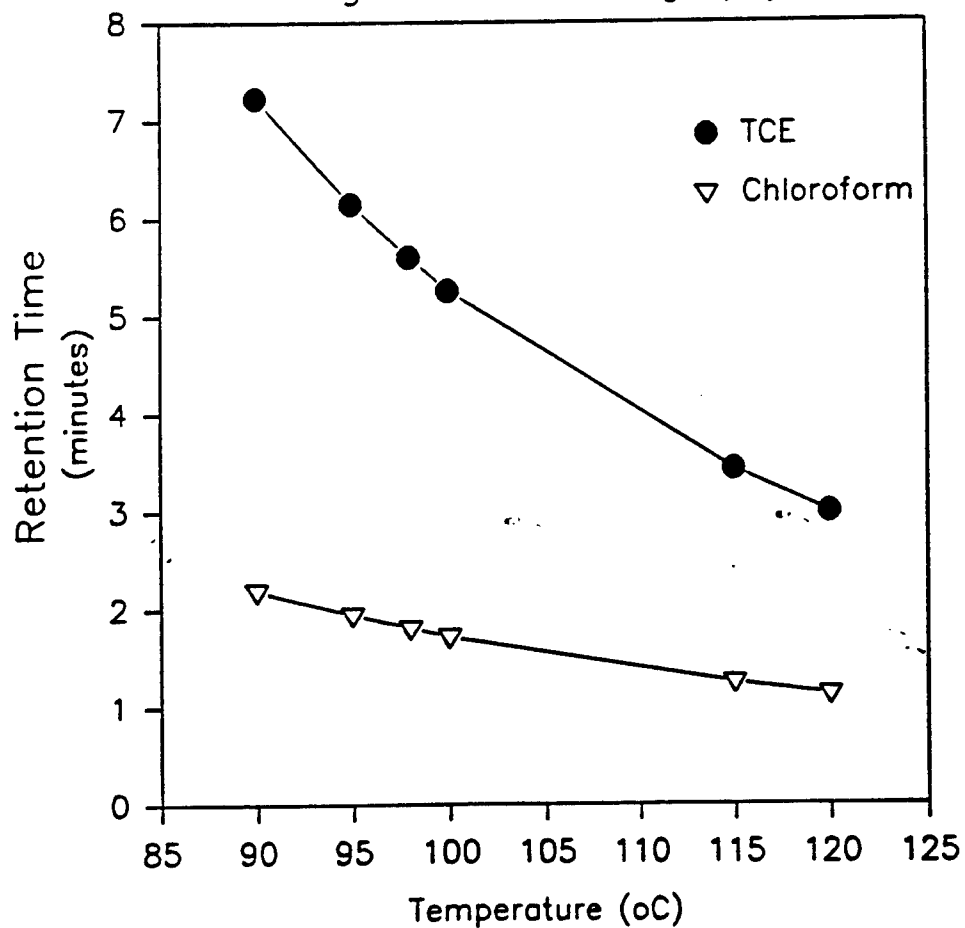


Figure 5

A STUDY OF NEURAL GRAFTS ON MEMORY

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Final Report for:
Graduate Student Research Program

Armstrong Laboratory

Sponsored by:
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August, 1993

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Allen Jefferson
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Armstrong Laboratory
United States Air Force

ABSTRACT

The feasibility of neural transplantation used to enhance memory was studied. In this paper, only the staining and slicing techniques of the experiment were detailed. A microtome was used to slice the paraffin-embedded brain and the Luxol-fast Blue stains were used.

A STUDY OF NEURAL GRAFTS ON MEMORY

Ellen Jefferson

INTRODUCTION

I served as co-investigator on an experiment conducted in the Radiofrequency Radiation Division of Armstrong Laboratory. The purpose of the experiment, was to determine the feasibility of using neural transplantation techniques to transfer memory from a trained fetus to a naive adult rat. It is an important study in furthering our knowledge about how the brain encodes memory and where in the brain the neural basis of memory is located. The study was of particular interest to the Air Force because the use of cognitive enhancers could significantly facilitate learning and memory and improve performance of Air Force pilots and other operators. The development of cognitive enhancers would be stimulated through analyses of the neural components present in brain tissue that have been shown empirically to contain a precisely defined "memory" (operationally defined by this study). The outcome of this experiment could be used to test other hypotheses about the neurophysiological substrate of memory and will also be useful in determining the feasibility of using neural grafts to treat nervous system damage. In the following pages, the only part of the experiment that will be explained will be the slicing and staining of the brains that have neural grafts in them.

METHODOLOGY

1. The method of slicing paraffin-embedded rat brains used was a microtome that was set at a three micron thickness for each slice. The brain was hydrated in ice water for between thirty minutes and two hours before slicing was begun (the brain should not be hydrated overnight because it will dissolve!).

2. A hot water bath was set up well in advance at about fifty degrees celsius. About a fourth of a teaspoon of plain non-flavored gelatin was added to the hot water bath and allowed to dissolve. The gelatin allows the brain to adhere to the glass slide and is used to mold the section. Remove air bubbles from the sides and bottom of the hot water bath by stirring.

3. Next, the brain was mounted on the microtome and a razor blade was "faced" against it. The process of facing is described in the following apparatus section. Once an accurate slice was made, it was transferred to the hot water bath and allowed to sit until all visible wrinkles in the brain were smoothed. Since several sections were desired, it was usually easiest to cut a ribbon of four or five slices, making the slice in the middle the one intended to be kept.

4. While the slices were sitting in the bath, the slides were numbered in pencil on the frosted end with the number of the rat (ie. T275) and with the number of the slice (the first section was one, the next one to be kept was two, and so on). Only every tenth slice after the first was kept and the slice was mounted on the side of the slide that had the numbering. When a slice that was supposed to be kept was ruined in some way or did not look as good as the slices around it, the next good slice was kept instead of the intended one but the slide was marked with a plus or minus how many slices away the new slice was from the original slice intended for keeping (ie. +3 or -2). If this procedure needed to be used, it was very important to remember that the slice after the one that was not correct should have been numbered according to the intended slice before it, NOT the actual slice that was used. For example, if a slice is intended to be number six on a slide but is ruined so that two slices behind that slice needs to be used, the slide should be labeled T---, 6 -2 and the next slice that is to be kept will be twelve slices in front of the last slice kept but will be numbered T---, 7. After every four slices that were kept, the brain was rehydrated in the ice water for twenty more minutes.

5. When detaching the wanted slice from the ribbon, the corner of a slide was used to cut along the perforation between slices. This prevented crumbling of the slice.

6. The slices intended for keeping were all mounted on the slide in the same orientation and in generally the same place on the slide.

7. Once the brains were sliced and mounted on slides, the slides were

transferred to an oven for melting of the paraffin in which the brain was embedded. They sat in the oven for about ten minutes under 105 degrees Fahrenheit heat.

8. When the paraffin was visibly melted, the slides were removed and placed in Baxter SIP Brand Americlear using a slide holder (usually holds 25 slides). The slides were allowed to sit for as little (minutes) or as long (days) as needed in the Americlear, until the technician was ready to stain the sections, but weren't left out of any solution for more than a few minutes until they were coverslipped.

Staining the sections involved use of the Luxol-fast Blue and cresyl violet stains, the creating of which will be explained in the Apparatus section.

9. Before beginning the staining procedure, the Dubnoff Metabolic Shaking Incubator was filled with water up to a point where the water would not spill when the mixer was turned on. Slide holding cups were filled with 200 mls water and placed in the mixing tray along with the cups holding the stain so that none of the cups could turn over due to the mixing motion. Usually one cup of each stain was enough to complete the staining procedure. The heater on the mixer was turned on in advance so that the temperature reading was fifty to seventy degrees Celsius. Whenever the Luxol-fast Blue stain was not in use, it was covered so as to prevent evaporation of the solution.

10. To begin the procedure, the slides were transferred from the Americlear to 2-Propanol, in which they sat for a couple of minutes.

11. The slides were then placed in the Luxol-fast Blue cup that was in the mixer. The mixer was in motion when the slides were in the tray so that the solutions were not allowed to separate and thus differentially stain the slides.

12. After thirty minutes of sitting in the Luxol-fast Blue stain, the slides were placed in a Lithium Carbonate solution. There was no set time for how long the slides should sit here but they were removed when (after a few minutes) the color seemed to drain from the brain and streak.

13. Once this happened, the slides were placed in a solution of seventy percent Ethyl Alcohol and were allowed to remain there until there was virtually no blue color left in the slide, that is, there was blue in only areas rich in axons such as the corpus callosum. The brain looked "ghostly".

14. After this effect was reached, the slides were placed in the cresyl violet stain and again, the mixer was turned on. The slides sat there for about seven minutes until the brains were very, very dark.

15. At this stage, they were removed and placed in the first of three solutions of ninety-five percent Ethyl Alcohol for about two or three minutes. The slides were then looked at for fading; the brains were still relatively dark. They were then placed in the second ninety-five percent EtOH solution, basically just to rinse the slides and then they were moved to the last cup of ninety-five percent EtOH. The slides were removed from the third solution when they were lighter in violet color than when they were first pulled out of the cresyl violet stain but they weren't lavender (the best way to describe the wanted color was purple with a tinge of red). The brains weren't so dark, though, that the blue on the corpus callosum and such areas couldn't at least be faintly seen with the naked eye.

16. Once the desired stain was reached, the slides were placed in 2-Propanol again for a few more minutes and then moved on to an Americlear solution for rinsing.

17. After about one minute in Americlear the slides were placed in another can of Americlear until they were ready to be coverslipped.

18. Coverslipping involved Permunt glue. The slides were taken out of the Americlear one by one and two drops of glue were placed on the side of the slide with the writing, directly onto the brain slice. A coverslip was then mounted on top of the glue and pressed from one side to the other with a finger to get rid of air bubbles. The slide was then dunked for a second in the Americlear and set aside to dry (preferably leaning on something). After all the slides were coverslipped in this manner and allowed to dry, the technician went back to the slides and cleaned them up. This was accomplished

by using a Q-tip dipped in Americlear to swab off the sides and front and back of the slide to rid it of excess glue. The slides were allowed to dry again and then the cleaning process was repeated. After the slides were dry, they were placed in a slide case that had their correct rat number on the front and were shelved for later viewing.

APPARATUS

"Facing" involved moving the brain closer and closer to the blade via turning the crank on the right side (the left-hand crank moved the brain ten times the distance the right-hand crank did and so to avoid accidental overcutting it was only used when the brain was very far from the blade or when the mount needed to be retracted because of overextension) until the blade cut a slice of the brain that included the whole area of the wax and the brain. A clockwise turn of the right-hand crank brought the brain three microns (the set thickness) closer to the blade and therefore sliced a three micron section of the brain. If the blade was crooked in relation to the brain, the brain-mount was adjusted by turning the knobs around the mount. Tightening those knobs took the respective side or corner of the brain further away from the brain whereas loosening the knob brought that area closer to the blade.

Making up the stains of Luxol-fast Blue and Cresyl Violet was adapted from the book, Histological Processing for the Neural Sciences by Eileen LaBossiere. The Luxol-fast Blue was made by adding 1g of Luxol-fast Blue to 1000 ml of 95% EtOH. When that was dissolved, 5 ml of 10% glacial acetic acid was added to the solution. The 10% glacial acetic acid was made by adding 1 ml pure glacial acetic acid to 9 ml distilled water. One should wear gloves and eye protection and always add acid to water. The purpose of the Luxol-fast Blue stain was to stain the myelin sheaths in the brain, which are concentrated around axons. The Cresyl Violet stain was made by adding 1 g of Cresyl Violet to 1000 ml of distilled water. After this was mixed, 150 drops of 10% glacial acetic acid was added from an eye dropper to the staining solution. This was stirred thoroughly and then vacuum filtered. The purpose

of the Cresyl Violet was to act as a counter stain to the Luxol-fast Blue and stain the neural cell bodies. The Lithium Carbonate was created by adding 0.5 g Lithium Carbonate to 1000 ml of distilled water and stirred thoroughly. The 70% EtOH was made by adding 300 ml distilled water to 700 ml pure EtOH and 95% EtOH was made by adding 50 ml distilled water to 950 ml pure EtOH. The Lithium Carbonate and the EtOH was used to destain the brains after they were stained and counterstained, to obtain the correct contrast, by making the cell membranes more permeable and thus allowing some of the stain to seep out. Therefore, the longer the stained brains were left in the Lithium Carbonate or EtOH, the lighter in color the staining became.

References

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AN ERGONOMIC STUDY
OF
AIRCRAFT SHEETMETAL WORK

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ABSTRACT

Ergonomic risk factors of aircraft sheetmetal tasks were studied at Kelly Air Force Base in San Antonio, Texas. It was observed that most of the tasks performed by the workers involved fairly high risk of developing cumulative trauma disorders (CTDs) of the upper limbs. An ergonomic screening questionnaire filled out by workers indicated a prevalence of mild forms of CTDs among 77% of the workers. Control measures were recommended to reduce progress and/or development of CTDs; a training program in basic Ergonomics was also suggested as a means to make workers aware of CTD and increase adherence to recommended improvements in work methods. When implemented, the recommended control measures will have a positive effect on sheetmetal worker health and help to reduce future compensation claims associated with CTDs.

An Ergonomic Study
of
Aircraft Sheetmetal Work

Paul S. Ray
and
Gina Masterson

INTRODUCTION:

Currently forty percent (40%) of all occupational illnesses reported to Armstrong Laboratory is related to CTDs. A large number of these CTDs occur in sheetmetal shops throughout the Air Force. In recent years, the Air Force has seen an increase in incidence of cumulative trauma disorders (CTDs) in the sheetmetal shop at Kelly Air Force Base, reported through the Air Force occupational illness data registry. This shop involves extensive manual operations using powered and non-powered hand tools and presents a great potential for causing cumulative trauma disorders.

The objectives of this study were to identify the ergonomic risk factors and determine ways to minimize the incidence of musculoskeletal injuries resulting from the sheetmetal tasks required for aircraft maintenance.

EXPERIMENTAL SETTING:

The sheetmetal workshop is located in building #375 of the Aircraft Directorate at Kelly Air Force Base. It is a large hanger accommodating the maintenance, inspection, and repair of B-52 and C-5 aircraft. The hanger is divided into two main sections: the "big hanger" houses a number of aircraft in various stages of maintenance, and the "backshop" section houses more than twenty-five different work areas. About one thousand military and civilian

employees work in this hanger. The day shift starts at 6:00 A.M. and ends at 4:45 P.M. There is a lunch break of forty-five minutes and two ten minute breaks, resulting in a net work time of five hundred and eighty minutes. A second shift, comprised mostly of volunteer workers, begins at 4:00 P.M. and ends at 2:00 A.M. The schedule has a four-day work week, starting on Monday and ending on Thursday. The work at the shop is self-paced.

DEMOGRAPHIC COMPOSITION OF SHEETMETAL WORKERS:

There are one hundred and forty-four civilian workers in the shop. Out of these, twenty-two workers are assigned on loan to other departments. Seventeen workers are assigned to the second shift. The allocation of workers varies each week to accommodate the variation in workload at the base. Fifteen percent of the workers are female. Eighty-three percent of the workers belong to the age group of thirty to forty nine years and sixty-five percent of them have over ten years of experience at the shop. The workers belong to four ethnic groups, as shown in Table 1.

Table 1: Ethnic Origin Of Workers	
ETHNIC GROUP	PERCENTAGE
a. American Indian	1.5
b. Black	4.5
c. Caucasian	18.2
d. Hispanic	75.8
Total	100

METHODOLOGY:

Evaluation of The Baseline Status

The current level of occurrence of cumulative trauma disorders

among the sheetmetal workers was assessed from:

- 1) historical records, and 2) a survey using an ergonomic disorder screening questionnaire.

Historical Records

A) Safety Office Record: This record is generated from worker complaints evaluated by a military physician and classified as injury cases. The mishap records from October 1991 through April 1993, were reviewed. This report presents cumulative trauma injury cases for six body parts: neck, back, arm, hand including wrist, fingers, and trunk. The incidence rate was found to be comparatively high in recent years as given in Table 2. Most of these reported cases involved back and arm injuries due to overexertion.

Table 2: Incidence Rate of Injury	
YEAR	INCIDENCE RATE/100 WORKERS
1991	7.7
1992	7.7
1993	6.9

B) Occupational Illness/Injury Report (AF Form 190): This record is generated from worker complaints evaluated by a military physician, and classified by Military Public Health as occupational illness cases. A review of the last five years' records of AF Form 190 revealed the following facts:

- 1) The total number of CTD cases reported for all Air Force bases during the last five years (1987-92) is 1,119.
- 2) The number of sheetmetal workers represent 13% of the total

CTD cases.

- 3) The numbers of CTD cases reported during 1987-92 by selected (former) logistic bases are as shown in Table 3.

Table 3: Number of CTD Cases Reported During 1987-1992					
BASE	Kelly	Tinker	Hill	McClellan	Robins
NO. OF CASES	12	44	44	27	13

Reporting of CTD cases from other (former) logistics bases is significantly larger than that from Kelly. This fact might be an indication of lack of ergonomics awareness at Kelly.

- 4) The average number of days on limited duty per CTD case for sheetmetal workers during 1987-92 is given in Table 4.

Table 4: Average Number of Days On Limited Duty 1987-1992				
BASE	Kelly	Tinker	Hill	McClellan
NO. OF DAYS	119	8	15	42

The number of days of limited duty at Kelly might be an indication of much worse disease conditions at this base. Increased awareness and worker training could facilitate identification of the disease and reduce the need of long periods on limited duty.

- 5) CTD cases classified by type for selected (former) logistics bases during 1987-92 are given in Table 5.

Table 5: CTD Cases by Types for Kelly and Hill AFB

CTD TYPE	BASE		TOTAL	PERCENTAGE
	Kelly	Hill		
CTS	8	26	34	60.7
Tendinitis	4	18	22	39.3
Totals	12	44	56	100

The incidence of carpal tunnel syndrome (CTS) was found to be the most frequent disease, followed by tendinitis of the upper limbs. This fact indicated the worst problem to be frequent deviation of the wrist while performing the sheetmetal tasks, followed by hyperextension of the arms and shoulder.

Disorder Screening Questionnaire

A survey was conducted to assess the existing cases of CTD among the sheetmetal workers using a disorder screening questionnaire developed by the Air Force. A total of sixty-six people responded to the survey and returned the completed questionnaires. Seventy-seven percent (77%) of workers reported suffering from some kind of cumulative trauma disorder (CTD). Fifty-six percent (56%) of them reported suffering from multiple disorders. The most frequently cited CTD was for the wrists (44%), closely followed by cases of hand disorders (42%), and low back pain cases (36%). Fifty-seven percent (57%) of the workers had introductory training in ergonomics and seventy-four percent (74%) in using back belts.

The survey indicates that the prevalence of CTDs among the sheetmetal workers was very high (77%). However, it appeared that severity of the disease was still within bearable limits and as such had not been reported to the authorities.

The details of CTD cases at Kelly reported during the last five years (AF Form 190) are given in Table 6. The following inferences can be drawn from this table:

- 1) Twenty-five percent of the total CTD cases reported are females. This level is high in comparison to the percentage of female workers (15%) in the group.
- 2) There is no incidence of lost days indicating the severity of the cases is within bearable limits.
- 3) The workers in the age group above 30 years account for most (75%) of the CTD cases. This fact probably indicates that these employees had adequate time to develop CTDs.

Table 6: CTD Cases Among The Sheetmetal Workers at Kelly			
Case No.	Gender	Age	Limited Duty Days
1	F	56	2
2	M	49	0
3	M	44	7
4	M	51	30
5	M	37	0
6	M	42	56
7	M	42	273
8	M	34	0
9	M	27	21
10	M	40	62
11	F	34	973
12	F	33	0

IDENTIFICATION OF ERGONOMIC RISK FACTORS:

The approach to identify the ergonomic hazards consisted of taking observations on the shop floor to record the details of typical sheetmetal tasks, and analyzing the details with a focus on ergonomic risk factors.

Most of the sheetmetal tasks were found to consist of drilling, riveting, and countersinking operations using pneumatically operated hand guns. The major sheetmetal tasks observed consisted of repairing the following: 1) B-52 nose cowls, 2) remove skin - wrap panel B-52, 3) install skin - lower wrap panel B-52, 4) install skin - upper wrap panel B-52, 5) align fittings - cowl panel B-52, 6) flight controls C-5, 7) replace web - side cowls C-5, 8) align fittings - side cowls C-5, 9) underside pylon C-5, and 10) inside pylon C-5. Based on this study, six groups of risk factors identified were as follows:

1) Posture: The workers in this shop were found to work in awkward postures during most of their work time, resulting in bent neck, hyperextension of shoulder, hyperextension of arms, and extreme deviations of hand and wrists. These are illustrated in Figure 1.

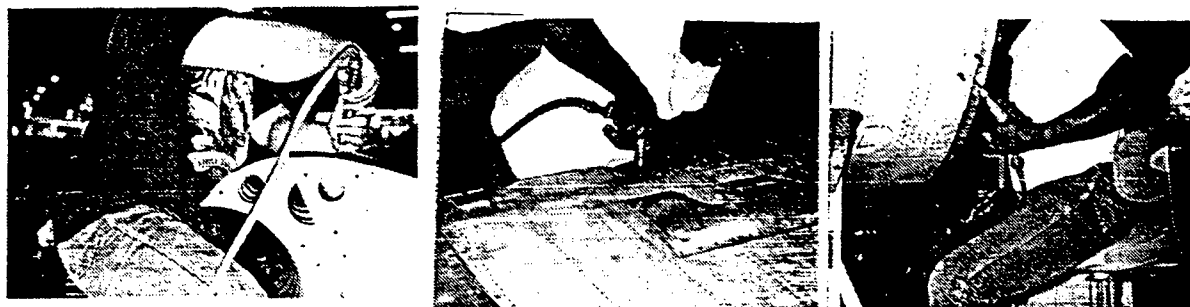


Figure 1: Tasks Performed With Deviated Limbs

Repair work of C-5 pylons required workers to perform overhead work for long periods of time (Figure 2), while other tasks required them to lie down inside the pylon and work in confined space with extremely deviated hands.

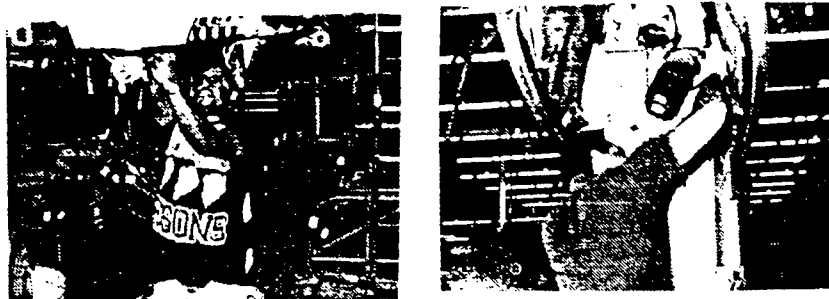


Figure 2: Overhead Tasks at Pylon

2) Force: The sheetmetal tasks required extensive use of pneumatically operated hand guns. These operations required considerable manual force to keep them at specific locations on metal surfaces. The situation was aggravated due to the fact that the hand guns, weighing from two to fourteen pounds, had to be carried manually during operations. Several areas used rivets made of alloy steel (MONEL) which were very hard. Replacing these required drilling through the rivets using significantly heavy force. Most of the pneumatic guns were operated by one finger, either the index finger or the middle finger.

3) Vibration: Another frequent operation, driving rivets, required the use of pneumatic rivet guns. These guns made too much vibration. Only a few workers were found to use hand gloves. Many of the workers had discontinued their use because the gloves supplied did not fit well, or were not useful, while others did not get replacements of worn out ones. There appeared to be no concerted program for selection, usage, and replacement of hand gloves. However, hand gloves alone were not adequate to eliminate the injurious effects of vibration. The guns that we observed in the shop were not provided antivibration type handles and therefore offered no protection from tool vibration. Ear plugs and ear muffs were provided to the workers for protecting their ears from noise exposure.

4) Tool Design: The repair work in the sheetmetal shop required an extensive use of pneumatic tools. The pneumatic tools which are currently in use are operated by only one finger, either the index finger or the middle finger. In addition to this, most of the pneumatically operated tools were not balanced. The center of gravity of the tools should be near their handles. Otherwise, the unbalanced weight of the tools tires the hand and arm muscles quickly. This is particularly true when the arms are extended outward as illustrated in Figure 3. The handles of the existing pneumatic tools consisted of bare metal and exposed hands to skin compression and abrasion from contact with tool surfaces.

5) Mechanical Stress: Manual trimming of metal sheet to size using a large scissor required significant force to be applied by hand and fingers. The scissor handles were not covered with pliant rubber and thus caused significant stress in fingers (Figure 4).



Figure 3: Tasks with Extended Arms

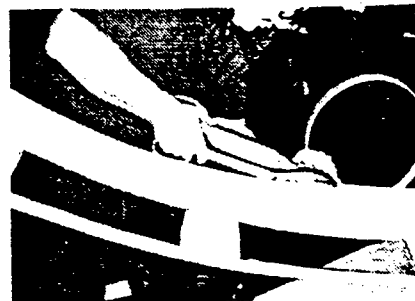


Figure 4: Using Scissor

6) Repetitiveness: This shop practiced self-paced work, but it was found that the workers stopped only when they felt pain, when the task was complete, or when they had to make a tool change. They were not taking micro-rest pauses consciously to relieve the musculoskeletal stress. Drilling or riveting, at a rate of 10-15 seconds per cycle, continuously for half of an hour or more, if practiced daily is likely to induce CTD.

RISK FACTOR SCORES FOR TASKS OBSERVED

Evaluation of the ergonomic severity of the ten representative sheetmetal tasks was done using the a checklist similar to one developed by Armstrong and Lifshitz (1987). The summary of the analysis is given in Table 7.

Table 7: CTD Risk Factor Scores										
CTD Risk Factors	replace o/skin B-52 nose cowl	remove skin B-52 wrap panel	install skin B-52 lower wrap panel	install skin B-52 upper wrap panel	align fitting B-52 cowl panel	flight control c-5	replace veb c-5 side cowl	align fitting c-5 side cowl	under-side c-5 pylon	inside c-5 pylon
Physical Stress	0	0	0	0	67	0	0	67	0	0
Force	50	50	50	50	0	50	50	0	50	50
Posture	25	25	0	25	25	25	0	25	25	0
Work Station	33	67	33	33	33	33	67	33	33	33
Repetitiveness	0	0	0	0	0	100	0	0	0	0
Tool Design	60	60	60	60	20	60	60	20	60	60
overall score	33	39	28	33	31	39	33	28	33	28
<u>Legend</u> Max Job Stress= 0; Min. Job Stress= 100										

The overall score of the risk factors of sheetmetal tasks carried a higher than average risk of developing CTDs. Repetitiveness, work station design, and posture of the workers were judged to carry high risk. The nature of jobs resulted in repetitive motions. The quality of work station design affects the posture of workers during the work. As such, these two factors are

related. Other factors were judged to have an average level of CTD risk. The repair of the C-5 pylon underside involving overhead work was judged to carry very high risk of developing a CTD of all the upper body parts including the neck. While working inside a pylon, the workers were subjected to considerable thermal stress. During the summer months, the temperature inside the pylon was found to be greater than 90 degrees fahrenheit. The workers were required to use coveralls as protection from contaminants (cleaning chemicals, grease, etc.) while working inside the pylons. As such, the workers were subjected to temperature ranges exceeding 100 degrees fahrenheit. In addition, no provision of ventilation was found to exist inside the pylons.

CONTROL MEASURES:

The overall control measures consist of: a) training the workers in the basics of ergonomics, b) improving the design of work stations to facilitate less stressful postures, c) using ergonomically designed hand tools, and d) implementing administrative procedures that support the prevention of cumulative trauma disease among the workers.

Control measures appropriate for a number of operations observed on the shop floor are detailed below.

REPETITIVE MOTIONS

1) Rest Pauses: Train the workers to take micro-rest pauses and not to continue drilling or riveting for more than five minutes at a stretch. A rest pause of a few seconds will help to reduce musculoskeletal stress significantly. The job is self-paced and small rest pauses are not expected to reduce productivity at all.

2) Mix Work Locations: Revise work method by shifting the work area during drilling and riveting from lower to upper level and left hand to right hand side, and vice-versa of the work piece every five minutes. This step will shift work stress from one group of muscles to another and thus further limit the musculoskel-

etal stress.

WORK STATION AND POSTURE

In general, provision of adjustable work tables and chairs is required to improve postures during work. In addition, some specific measures are suggested for the ten components observed.

3) B-52 Nose Cowl: Provide step-stool (Figure 5) with height adjustable from twenty to thirty-two inches for sheetmetal repair work of the B-52 nose cowls. Currently, the nose cowl is positioned on a thirty inch high wooden stand. The repair work is to be performed sitting on the step-stool for the lower areas and standing on the step-stool for the higher areas.

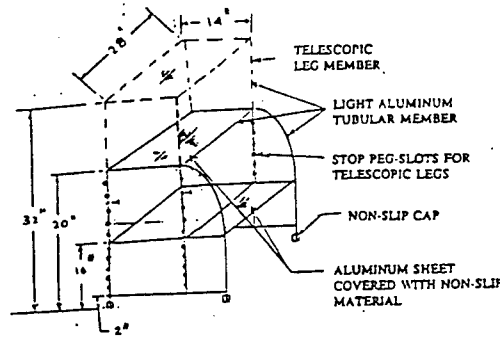


Figure 5: Step-stool

4) B-52 Nose Cowl: Mechanize the operation of cleaning the nose cowl. The suggested fixture should have the ability to rotate the cowl in any direction and jolt it to shake out any loose material left inside the cowl from the repair work.

5) B-52 Lower Wrap Panel: Modify the assembly fixture (F/S 56000) so that the wrap panel on the fixture can be rotated to bring all work areas within easy reach of the workers.

6) B-52 Upper Wrap Panel: Provide step-stools (Figure 5) with height adjustable from twenty to thirty-three inches for sheetmetal repair work of wrap panels on the fixture (#MBPSB/46511). For low level work areas, perform the work sitting on the stool and for high level work areas, perform the repair standing on the step-stool.

7) B-52 Upper Wrap Panel: Use special riveting tool bit with inclined stem for low and high level work area, if required, to bring the wrist into a neutral position (Figure 6).

SPECIAL RIVETING TOOL BITS

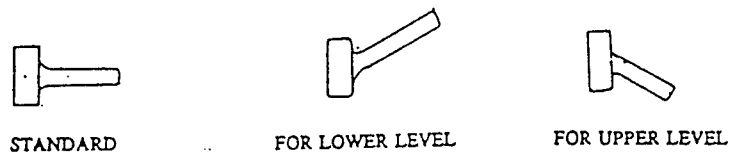


Figure 6: Special Riveting Tool Bits

8) C-5 Flight Controls: Provide adjustable height dollies for the flight control component repair work as shown in Table 8.

Table 8: Suggested Dolly Heights

COMPONENT	WORK-AREA HEIGHT	DOLLY HEIGHT	WORK POSITION
outer flap	21-19 in	21* in	sit
lower rudder	36-46 in	18-28 in	sit/stand
elevator	36-46 in	18-28 in	sit/stand
aileron	36-46 in	18-28 in	sit/stand
outboard ele- vator	21-31 in	9-19 in	sit

9) C-5 Side Cowl: Modify the assembly fixture (C/N 02688A) so that the cowl mounted on the fixture can be rotated through 180 degrees to bring all of the work areas within easy reach of workers.

10) B-52 Side Cowl: Provide an ergonomic chair (Figure 7) for

the overhead repair work done under the side cowl positioned on the sawhorse (work stand) with the convex side up (Figure 8).

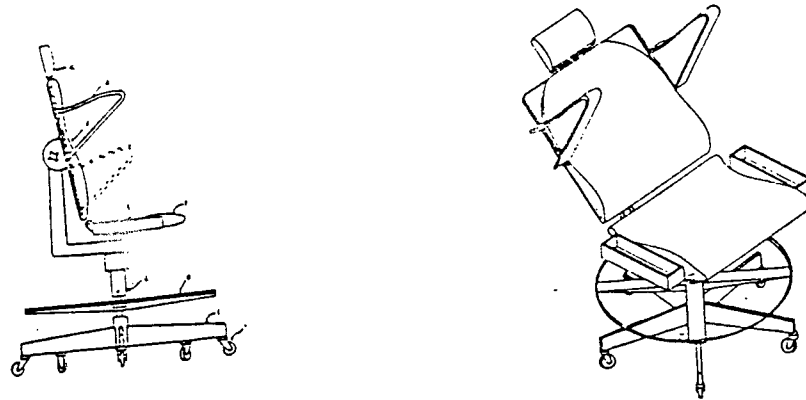


Figure 7: Ergonomic Chair

11) C-5 Pylon: Provide ergonomic work chair (Figure 7) for maintenance and sheetmetal work done under the C-5 pylon. This device will provide the needed support for head, neck, shoulder, and arms at appropriate heights and thus reduce the high risk of developing CTD among the workers.

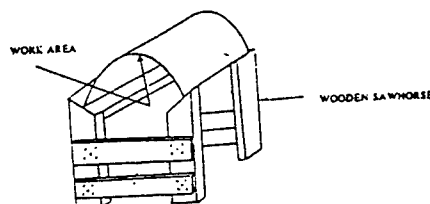


Figure 8: Side Cowl on Sawhorse

12) C-5 Pylon: Provide piped cool air flow to the workers inside the pylon. A good arrangement consisting of an air conditioner unit near the pylon work area supplying piped cool air inside each pylon will alleviate the thermal stress of workers

inside the pylon.

FORCE

13) All Departments: Provide overhead balancing devices to carry the weight of pneumatic tools. These devices will eliminate the static load of carrying the tools during sixty to seventy percent of the task time. See Figure 9.

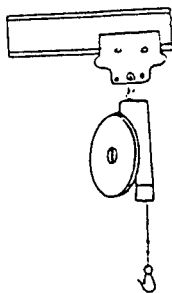


Figure 9: Balancing Device

14) All Departments: Mechanize the manual trimming of sheets to size. A pneumatically operated saw will be adequate. The present method of trimming with hand scissors is stressful to fingers, wrist, and hand.

TOOL DESIGN AND VIBRATION HAZARD

15) All Departments: Use heavier tools to reduce vibration, provided they are suspended on overhead balancing devices.

16) All Departments: Provide antivibration type hand tools and handles.

17) All Departments: Select new pneumatic tools having the following features:

- a) Larger triggers suitable for operation by two or more fingers instead of only one finger.
- b) Tool handles should be located under the tool's

center of gravity as shown in Figure 10.

- c) The tool handles should be of antivibration type and covered with non-slip pliant material.

TYPES OF RIVET GUNS

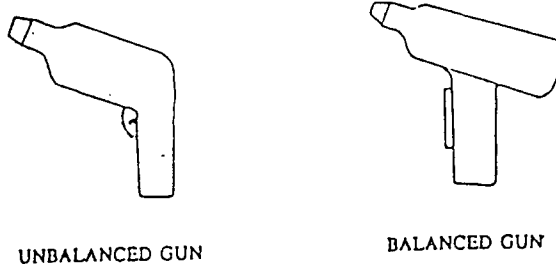


Figure 10: Handle Location of Balanced Guns

MECHANICAL STRESS

18) All Departments: Use hand gloves when handling sheetmetal having sharp edges. Bare hands must not be used during these operations.

19) Riveting with the bucking operation is one of the most hazardous tasks in the sheetmetal shop. An effort should be made to design a special riveting gun to eliminate the need for bucking. It will be worthwhile to investigate the possibility of developing special blind rivets that can be used in all areas of aircraft without need of bucking.

PROTECTIVE CLOTHING

20) All Departments: Provide hand gloves to reduce the effect of vibration during riveting, impact strain during bucking, and contact strain with hard surfaces. The hand glove should be suitable for the vibration frequencies of the tools used in the shop and have the following characteristics:

- a) padding at palm side and all areas of hand coming into contact with the tool,
- b) wrist support band, and
- c) covered fingers (finger tips may be exposed for some tasks).

OTHER CONTROL STEPS

21) Organize a training program on Ergonomics for the workers and shop supervisors. Video tapes are available from The National Institute of Occupational Safety and Health and are a good training method.

22) Install safety posters at suitable shop locations to keep the workers aware of the ergonomic hazards in the shop.

23) Install periodical review for the tool maintenance program to ensure availability of tools with proper sharpness. This step will help to prevent development of excessive tool vibration.

24) Plan to rotate workers from high risk tasks to low risk tasks periodically.

25) Include screening for ergonomic disorders in occupational clinical exam program for workers of this shop to identify the onset of CTDs, so that corrective steps may be taken at a much lower cost and avoid compensation claims in the future.

DISCUSSION:

The awareness of ergonomic risk factors at the workplace has increased significantly during recent years. The manifold increase in the number of CTD cases reported reflects this awareness among workers. This trend is likely to continue in the near future and is likely to result in significant compensation claims. As such, it will be economically advantageous to implement an ergonomic intervention program to prevent progression of CTD among workers and thus reduce the risk of exposure to compensation claims in the future.

As sheetmetal work for all aircraft maintenance work is very similar in nature, the current study may be applicable to other Air Force bases as well. As such, the potential benefits of this study are much larger and need not be limited to Kelly only. The ergonomic chair proposed for the C-5 pylon maintenance can be beneficially used in similar maintenance jobs of other aircraft and is expected to have universal applicability.

CONCLUSION:

Most of the sheetmetal repair tasks at Kelly carry an average to high level risk of developing CTDs. Twenty-five control steps have been suggested to minimize the ergonomic risk factors from the current work methods followed on the shop floor. A large percentage of the workers at present are suffering from some sort of cumulative trauma disorder, affecting various body parts. The reporting of such incidence has been low, so far, probably due to very little awareness of these job-related diseases. Implementation of a concerted ergonomic intervention program now will be timely, to prevent the progression of CTDs among the workers and help in reducing the potential for significant compensation claims in the near future.

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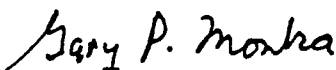
Taboun, S. M., and Dutta, S. P., 1993. Ergonomic Analysis and Evaluation of Electronic Assembly Operation. In: Advances in Industrial Ergonomics and Safety V. Edited by Nielsen, R. and Jorgensen, K., Taylor & Francis, London. pp 29-36.

Using the Scanning Electron Microscopy for Fiber Analysis

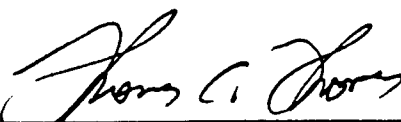
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Final Report for:
Summer Research Program
Armstrong Laboratory

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July 28, 1993

Using Scanning Electron Microscopy for Fiber Analysis

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Abstract

The air asbestos analysis laboratory at Brooks Air Force Base requires a method for identifying the composition of incoming fibrous material. Using morphology and the elemental composition of the sample obtained utilizing an Amray Electron Microscopy 1820 equipped with a Tracor Northern X-ray Analyzer II, it is possible to determine whether an air sample contains asbestos, fibrous non-asbestos or other minerals. The standards and unknown data was obtained by the author using SEM-EDXA technique to establish a reference library for the identification programs and check the analyses process under real conditions. In all, over ninety minerals have been analyzed by the SEM-EDXA as standards and statistical processed to determine the mean and first deviation. To enhance the productivity of the asbestos laboratory, computer programs has been written by the author's mentor. To rapidly accomplish this work, the programs allows the identification of asbestos, high calcium containing minerals, high silicon containing minerals and miscellaneous materials. Analyses of unknown fibers were nearly 100% during the preliminaries review of the procedure.

Introduction

Asbestos is well known for the many medical problems it can cause, ranging from lung cancer to asbestosis.(1) Therefore, human exposure in the environment is of special concern. This concern requires proper analyses of samples to determine the extent of the health hazard pose by fibrous material in the environment air.(2)

Currently the Occupational and Environmental Directorate of Armstrong Laboratory at Brook Air Force Base uses the NIOSH method 7400 to count fibers. This method counts fibers on air filters but is unable to distinguish the composition, nature of the material on the filter, or the level of health hazard.

However, using the NIOSH method 7400 along with SEM-EDXA creates a powerful tool that can be used to determine the composition of samples and help in evaluating the health hazard level in the environment where the samples were obtained.

Instrumentation

Standard minerals were pre-mounted on aluminum studs offer precoat with two coatings of carbon paint. A third coat was applied and the minerals were then placed on the studs before the third coat was allowed to dried. This provides adequate adherence between the minerals and the studs and prevents background

interference from aluminum. The studs were then coated with a gold-palladium alloy using the Anatech LTD Hummer VI. Each stud to be coated is placed in the coating chamber and the chamber is evacuated to 25 millitorr. Argon is introduced to purge the chamber and the chamber is again evacuated to 25 millitorr or less. The chamber is filled a second time with argon to approximately 75 millitorr. The current is controlled at 10 milliamperes and the sample is coated with 10 nanometers of the gold-palladium alloy.

After coating with the metal alloy, the sample is placed in the Cary Model 1820 Scanning Electron Microscopy equipped with a Tracor Northern Series II X-ray Analyzer (TN-II) for analyzing. Based on the previous work performed at AL/OEA, instrument parameters were established to achieve maximum performance. The minerals samples were processed at a X-ray takeoff angle of 38.5 degrees, a detector slide position of 45 millimeters, a sample probe distance of 12 millimeters with an electron beam of 20 kiloelectron volts and 20 microamperes current using a 7.5 micrometers beryllium detector window.

The sample is placed in the vacuum chamber and the allowed to pump down. The sample is bombarded with electrons and the X-ray emission is analyzed for each element with the Tracor Northern II X-ray Analyzer. Data acquisition for the standard minerals were 200 seconds. The latter analyzed a number of different elements, the basic ones were Silicon, Magnesium, Iron, Calcium, Manganese, Sodium, Aluminum, Sulfur, Phosphorus,

Chlorine, and Potassium; some addition element data was achieved according to the known composition of the mineral.(see table)
The percent elemental composition for each mineral is listed in a typical printout sheet,Appendix-1, along with spectrum, all data is stored in a binder in DEA for future reference.

The TN-II, processed the raw data, using ZAF corrections for which corrects for atomic number(Z), absorption(A), and fluorescence(F).

A computer program written for these calculates was processed with the mean percentage composition for each element in the mineral along with the standard deviation.

Results and Discussion

This research project explored the use of the Amray 1820 Scanning Electron Microscope equipped with the Tracor Northern X-ray Analyzer as a tool for analyzing unknown fibrous material for their composition. This method now allows the air asbestos lab to make a qualified decision as to the exact fibers in the air sample.

For the minerals, Rhodonite and Celestite, two distinct sets of composition were obtained, obviously the samples were a mixture and until the correct composition can be determined, both set of data are listed.

Three computer programs were written this summer by Dr. Larry R. Sherman, he used the data in Table-I as a reference

library. In all, sixty minerals were analyzed by the author and added to thirty four standards obtained in the previous summer fellowship.(3)

Acknowledgements

The author wishes to thank L.R. Sherman for all his assistance and encouragements in this project and throughout this summer. The author also wishes to thank all the personnel at Brooks, especially K.T. Roberson, T.C.Thomas and R.Anderson for their warm hospitality. They made the summer a rewarding and enriching experience, I also like to thank the Air Force Office of Scientific Research and RDL for granting me this fellowship. And a very special thanks goes to my family and girlfriend, Dawn, who helped me more then they could ever image. Thanks to everyone.

Table 1
Minerals and majors element components

Number	Mineral	Element	%composition	Stand.dev
801	Quartz	Si	97.34 ±	0.67
		Fe	1.80 ±	1.03
802	Calcite	Ca	95.92 ±	1.45
803	Orthoclase	Si	59.69 ±	1.32
		Al	19.20 ±	2.00
		K	13.34 ±	5.38
		Na	8.77 ±	3.13
		Si	60.52 ±	1.15
804	Microline	K	21.04 ±	0.60
		Al	16.75 ±	0.35
		Si	62.84 ±	4.31
805	Albite	Al	18.17 ±	0.94
		Na	14.15 ±	2.77
		Si	47.90 ±	5.93
806	Labradorite	Al	21.92 ±	4.90
		Ca	13.37 ±	4.86
		Na	7.30 ±	1.61
		Si	50.13 ±	0.70
		Mg	19.33 ±	1.03
807	Hornblende	Na	9.93 ±	2.94
		Fe	7.98 ±	0.76
		Ca	7.57 ±	1.54
		Al	3.40 ±	0.91
		K	1.85 ±	0.29
		Si	57.16 ±	2.16
808	Tremolite (non-fibrous)	Mg	24.09 ±	1.06
		Ca	13.11 ±	1.01
		Mn	3.25 ±	1.64
		Na	1.51 ±	0.41
		Si	63.63 ±	3.76
	(fibrous)	Mg	29.81 ±	1.77
		Si	47.96 ±	2.55
809	Augite	Ca	23.64 ±	4.75
		Mg	13.63 ±	2.65
		Fe	11.44 ±	1.50
		Al	1.91 ±	0.49
		Ca	67.34 ±	4.50
810	Apatite	P	31.42 ±	6.25
811	Tourmaline	Si	40.79 ±	4.84
		Al	33.91 ±	2.89
		Fe	19.06 ±	8.66
		Na	4.92 ±	1.48
		Mg	3.21 ±	1.74
812	Titanite	Ca	59.31 ±	10.46
		Si	37.13 ±	12.66
		Fe	4.19 ±	1.79
		Al	2.75 ±	1.23

Table-1(cont.)

Number	Mineral	Element	%composition		Stand.dev
813	Olivine	Mg	43.94	±	2.69
		Si	42.73	±	2.79
		Fe	11.85	±	3.34
814	Nephelite	Si	64.96	±	0.87
		Al	17.95	±	0.44
		Na	15.97	±	0.95
815	Sodalite	Si	33.04	±	0.96
		Na	28.87	±	2.14
		Al	26.50	±	0.34
		Cl	11.08	±	1.81
816	Lepidolite	Si	58.40	±	1.44
		Al	23.92	±	0.80
		K	15.98	±	0.62
817	Garnet (Almandite)	Fe	33.70	±	4.50
		Si	32.15	±	2.78
		Al	18.70	±	1.85
		Mg	8.91	±	1.38
		Ca	5.69	±	1.42
818	Siderite	Fe	89.21	±	3.34
		Mg	4.86	±	3.25
		Mn	4.66	±	1.10
		Na	2.79	±	1.96
819	Beryl	Si	78.43	±	0.66
		Al	19.28	±	0.29
820	Corundum	Al	95.42	±	2.41
		Fe	2.65	±	2.14
821	Corundum, Var.Emery	Al	63.78	±	38.20
		Si	57.79	±	32.62
		Fe	4.48	±	2.86
822	Aragonite	Ca	97.33	±	3.58
823	Monazite	P	74.45	±	7.89
		Ca	10.70	±	4.03
		Si	7.07	±	1.65
824	Topaz	Al	53.26	±	0.26
		Si	45.46	±	0.34
825	Rutile	Ti	96.40	±	1.12
827	Zircon	P	60.75	±	2.17
		Si	37.43	±	1.59
828	Vesuvianite (Californite)	Ca	41.40	±	2.59
		Si	35.50	±	1.89
		Al	16.40	±	0.81
		Mg	4.64	±	0.45
		Fe	1.08	±	0.73

Table-1(cont.)

Number	Mineral	Element	%composition	Stand.dev
829	Rhodonite (Fowlerite) (Data Set I)	Mn	56.74 ±	2.24
		Si	37.40 ±	2.61
		Ca	2.65 ±	2.17
		Na	2.11 ±	0.65
		Mg	1.89 ±	1.25
	(Data Set II)	Mn	93.07 ±	5.33
		Si	4.85 ±	3.50
		Ca	2.07 ±	1.55
830	Biotite	Fe	22.04 ±	3.57
		K	12.52 ±	1.69
		Al	11.24 ±	1.22
		Na	3.33 ±	1.94
		Ti	2.18 ±	0.40
		Mn	1.24 ±	0.25
831	Thaumasite	Ca	55.94 ±	0.45
		Si	42.73 ±	0.76
832	Prehnite	Si	39.31 ±	9.46
		Ca	37.52 ±	9.74
		Al	19.01 ±	4.71
		Fe	9.95 ±	5.43
833	Pectolite	Si	45.56 ±	1.24
		Ca	39.73 ±	3.26
		Na	13.63 ±	2.45
834	Barite	Ba	73.04 ±	8.42
		S	20.94 ±	6.10
835	Anhydrite	Ca	51.15 ±	0.41
		S	46.10 ±	1.16
836	Gypsum	Ca	54.97 ±	2.42
		S	42.90 ±	4.50
837	Cryolite	Na	61.57 ±	1.10
		Al	34.60 ±	1.01
		Mg	2.91 ±	0.29
838	Cyanite	Al	55.16 ±	1.07
		Si	42.59 ±	2.80
839	Spodumene	Si	72.23 ±	1.176
		Al	25.84 ±	0.44
840	Amblygonite	P	60.03 ±	1.39
		Al	37.86 ±	0.62
841	Epidote	Si	52.10 ±	2.54
		Mg	37.85 ±	3.04
		Fe	8.15 ±	1.82
842	Rhodochrosite	Mn	87.36 ±	8.57
		Mg	5.82 ±	1.61

Table-1(cont.)

Number	Mineral	Element	%composition		Stand.dev
843	Asbestos- (Chyrosotile)	Si	52.51	±	0.97
		Mg	40.32	±	1.73
		Fe	2.65	±	0.39
		Na	1.49	±	0.31
844	Kaolin	Si	58.03	±	0.94
		Al	38.90	±	0.44
845	Talc	Si	66.59	±	2.01
		Mg	30.91	±	1.32
846	Bauxite	Al	73.83	±	3.93
		Fe	15.00	±	7.66
		Si	12.65	±	7.24
847	Azurite	Fe	42.11	±	3.23
		Si	25.60	±	3.24
		Al	22.31	±	3.93
848	Malachite	Cu	97.21	±	2.42
849	Sphalerite	Na	39.76	±	0.31
		Zn	37.21	±	0.44
		S	21.92	±	0.21
850	Scheelite	Ca	91.95	±	3.16
		Si	2.50	±	1.53
		Mn	1.59	±	0.56
		Fe	1.09	±	0.05
851	Cassiterite	Sn	94.21	±	3.20
		Si	1.99	±	0.56
852	Wollasionite	Ca	60.15	±	5.10
		Si	38.54	±	4.41
853	Zincite	Zn	54.51	±	5.10
		Na	41.94	±	5.06
		Mn	1.87	±	0.19
854	Serpentine	Si	44.73	±	1.77
		Mg	43.19	±	0.66
		Fe	7.08	±	1.08
		Al	3.80	±	1.30
855	Steatite	Ca	77.97	±	4.11
		Si	12.64	±	3.85
		Mg	8.94	±	1.38
856	Celestite				
	(Data Set I)	S	44.67	±	2.41
		Si	41.84	±	2.26
		Mg	5.25	±	0.39
		Al	4.96	±	0.36
		Na	2.18	±	0.24
	(Data Set II)	Sr	72.73	±	4.10
		S	25.74	±	3.18

Table-1(cont.)

Number	Mineral	Element	%composition	Stand.dev
857	Glauconite	Fe	42.35 ±	5.40
		Si	31.58 ±	17.32
		Ca	23.67 ±	16.31
		Al	10.09 ±	5.16
		K	3.90 ±	2.82
		Mg	2.04 ±	0.56
858	Opal	Si	96.61 ±	1.19
859	Obsidian	Si	71.46 ±	1.49
		Al	12.49 ±	0.81
		K	8.85 ±	0.46
		Na	4.01 ±	0.37
		Fe	1.61 ±	0.18
		Si	73.42 ±	2.23
860	Pumice	Al	12.81 ±	0.50
		K	6.53 ±	1.51
		Na	4.21 ±	0.37

* Determined at AL/OEA

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Appendix I

Q: QUANTIFY

REHNITE STUD# 832-E
Standardless Analysis
20.0 kV 38.5 Degrees

Refit _MGK' _MGK" _MNK' _MNK" _S K' _S K" _P K' _P K" _CLK' _CLK" _K K' _K K"
Refit _MGK _CAK" _NAK" _S K _P K _CLK _K K
Chi-sqd = 2.29

Element	Rel. K-ratio	Net Counts
Si-K	0.36230 +/- 0.00511	23825 +/- 336
Mg-K	0.00000 +/- 0.00000	0 +/- 0
Fe-K	0.03343 +/- 0.00412	778 +/- 96
Ca-K	0.38643 +/- 0.00354	17121 +/- 157
Mn-K	0.00184 +/- 0.00159	47 +/- 41
Na-K	0.00130 +/- 0.00125	34 +/- 33
Al-K	0.21470 +/- 0.00489	12299 +/- 280
S -K	0.00000 +/- 0.00000	0 +/- 0
P -K	0.00000 +/- 0.00000	0 +/- 0
Cl-K	0.00000 +/- 0.00000	0 +/- 0
K -K	0.00000 +/- 0.00000	0 +/- 0

AF Correction 20.00 kV 38.50 deg
No. of Iterations = 1

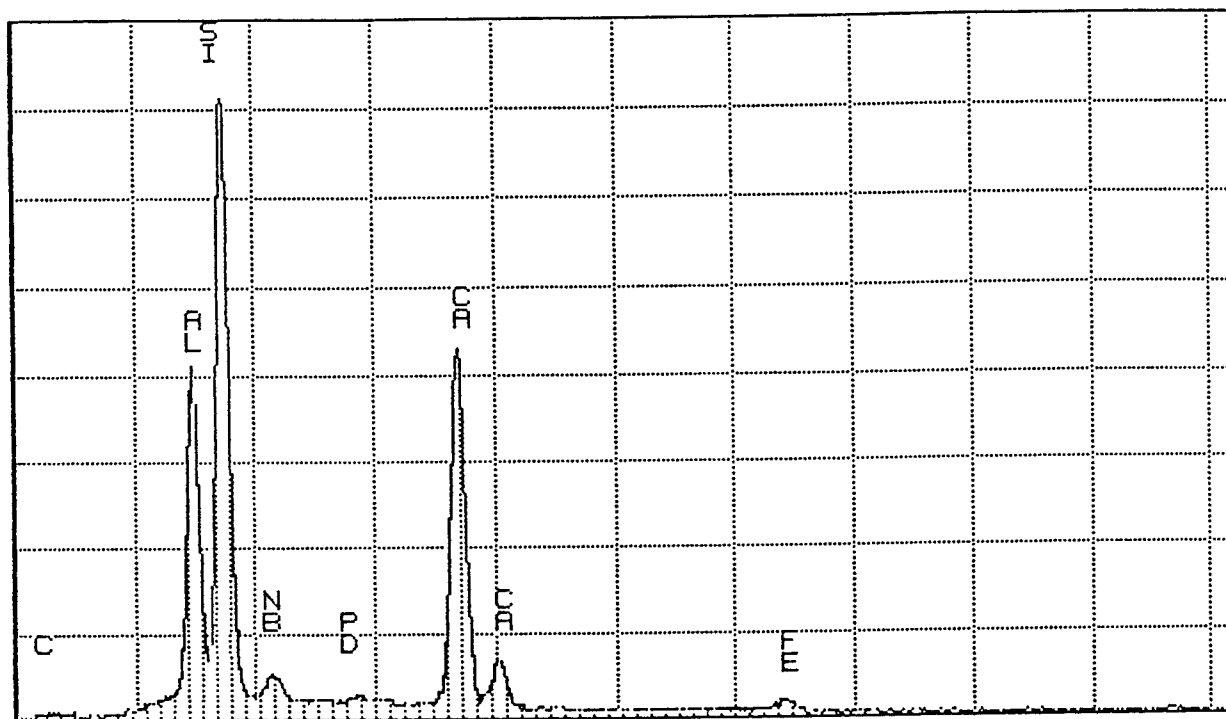
Element	K-ratio	Z	A	F	ZAF	Atom%	Wt%
Si-K	0.280	0.985	1.499	0.997	1.471	46.14	41.17
Mg-K	0.000	0.984	1.541	0.986	1.495	0.00	0.00
Fe-K	0.026	1.103	1.052	1.000	1.160	1.69	2.99
Ca-K	0.298	1.006	1.128	0.999	1.134	26.59	33.86
Mn-K	0.001	1.123	1.072	1.000	1.205	0.10	0.17
Na-K	0.001	1.009	1.981	0.993	1.986	0.27	0.20
Al-K	0.166	1.014	1.306	0.985	1.303	25.21	21.61
S -K	0.000	0.992	1.597	0.992	1.571	0.00	0.00
P -K	0.000	1.018	1.899	0.996	1.924	0.00	0.00
Cl-K	0.000	1.036	1.410	0.986	1.441	0.00	0.00
K -K	0.000	1.030	1.191	0.963	1.181	0.00	0.00
Total=							100.00%

Appendix I (cont.)

BROOKS AIRFORCE BASE

THU 24-JUN-93 14:18

Cursor: 0.000keV = 0



0.000

VFS = 2048 10.240

200

PREHNITE 832-E

A STUDY OF THE ROLE OF NITRIC OXIDE IN MILLIMETER
WAVE INDUCED CIRCULATORY SHOCK

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Final Report for:
Graduate Student Research Program
AL/OER at Brooks Air Force Base, TX

Sponsored by:
Air Force Office of Scientific Research
Bolling Air Force Base, Washington D.C.

August 1993

A STUDY OF THE ROLE OF NITRIC OXIDE IN MILLIMETER
WAVE INDUCED CIRCULATORY SHOCK

Maureen P. Soday
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Abstract

The purpose of this study was to investigate the possible involvement of nitric oxide (NO) in Millimeter Wave induced shock. Because most animal studies of shock use the anesthetic pentobarbital, it was first necessary to determine if the ketamine model used in this study was valid. To do this, dose-response curves of mean arterial pressure (MAP) and heart rate (HR) were plotted. It was shown that there was no significant difference between the two anesthetics. Furthermore, L-NAME showed a dose dependent increase in the both groups of rats. With this area secure, we used our ketamine model to study the effects of the NO inhibitor L-NAME in both irradiated (shocked) and non-irradiated (normotensive) rats. It was shown that L-NAME caused an increase in mean arterial pressure (MAP) in both groups, however, the effect was smaller in the shocked rats.

A STUDY OF THE ROLE OF NITRIC OXIDE IN MILLIMETER WAVE INDUCED CIRCULATORY SHOCK

Maureen P. Sodoy

An increase in the use of radiofrequency radiation emitters raises concern about the possible bioeffects and health hazards caused by these waves. A previous study of the bioeffects of microwave exposure conducted in this laboratory demonstrated that sustained irradiation of anesthetized rats with a frequency of 35 GHz (millimeter wavelength) induces a fatal state of circulatory shock (Physiologist 34: 246, 1991). This millimeter wave model may be useful for studying the physiological mechanisms involved in circulatory shock. Nitric Oxide (NO) was examined for its possible role in shock, specifically its involvement in visceral vasodilation preceding hypoperfusion.

In mammals, environmental heating causes vasodilation in peripheral blood vessels to release excess heat from the body into the environment. In response to this rerouting of blood to the skin, cardiac output increases and visceral vessels constrict (Rowell, 1986). This visceral vasoconstriction is mediated by α -adrenergic mechanisms (Proppe, 1980; Kregel and Gisolfi, 1989; Gisolfi et al., 1991) and under mild to moderate heat stress helps maintain arterial blood pressure. Severe hyperthermia, however, may lead to the medical emergency referred to as heat stroke which is marked by a precipitous fall in mean arterial blood pressure.

Kregel et al. (1988) observed that a selective loss of compensatory splanchnic vasoconstriction appears to trigger the circulatory collapse associated with severe

hyperthermia. This sudden splanchnic vasodilation, combined with continuing peripheral vasodilation, produces hypotension by decreasing both total peripheral vascular resistance and venous return; the latter ultimately results in decreased cardiac output.

During millimeter wave heating, the blood pressure of the anesthetized animal gradually increases to a peak followed by a decline. The decline in arterial blood pressure is preceded by mesenteric vasodilation, a response like that seen with heat stroke produced by environmental heating. NO, a known vasodilator, has been implicated as a mediator in several forms of experimental shock. In this laboratory, several Nitric Oxide synthesis inhibitors are currently being studied. These drugs include N-monomethyl-L-arginine (L-NMMA), N-nitro-L-arginine-methyl ester (L-NAME), and 3-Amino-Tyrosine. My involvement was principally in the study of L-NAME, an analogue of the NO precursor L-arginine. L-NAME competitively inhibits NO production by the enzyme NO synthase (Moncada et al., 1991). The purpose of this experiment was to determine whether a bolus injection of L-NAME ameliorates the hypotension produced by sustained Millimeter Wave irradiation. However, it was first necessary to determine whether responses to NO inhibition were dependent on anesthetic choice. Previous shock studies that implicated NO as the causative agent used rats anesthetized with pentobarbital (Pb) while ketamine (K) is used in our model. To determine whether a difference in anesthetic choice exists, dose response curves of MAP and HR response to L-NAME were plotted and analyzed.

Methods

Male Sprague-Dawley rats (350-450g) were anesthetized with either ketamine (150 mg/kg; i.m) or sodium pentobarbital (50 mg/kg; i.p.). A catheter was surgically placed in the carotid artery to measure MAP and an i.v. line was inserted into the jugular vein for drug administration. EKG (lead II) and respiration rate were monitored. Peripheral temperature was measured using left and right skin probes while core temperature was taken via colonic and tympanic probes and maintained at 37 ± 0.5 ° C. After a control period of 30 minutes in the normotensive rats, a bolus injection of L-NAME was given in one of several doses: 0 (0.9% NaCl vehicle), 0.1, 1.0, 2.5, 5.0, and 10.0 mg/kg . Measurement of MAP and HR were continued for 30 minutes. A bolus dose of L-arginine was then given in a concentration 20 times the dose of L-NAME to determine if the response to L-NAME could be reversed.

In rats made hypotensive by MMW exposure (35 GHz; SAR= 12.5 W/kg), irradiation was discontinued when MAP decreased to 75 mm Hg. Then a bolus injection of L-NAME was given at the same dose levels as the normotensive animals. Survival times following shock induction were noted.

Results

Dose response curves show no statistical difference ($p > 0.05$) between the two anesthetics. The pressor response to L-NAME increased in a dose dependent fashion for both K- and Pb-anesthetized rats (Figure 1). Furthermore, there was no decrease ($p > 0.05$) in HR in either groups of rats (Figure 2).

It was also shown that L-arginine reversed the pressor response caused by L-NAME in both K and Pb rats (Figure 3). L-NAME pressor responses in K-anesthetized rats were

smaller ($p < 0.05$) at any given dose in rats made hypotensive by MMW radiation than in the non-irradiated control rats (figure 4). L-NAME treatment did not increase survival time (figure 5).

Conclusion

Since the present data shows no difference in the pressor responses to L-NAME between normotensive K and Pb anesthetized rats, we feel that our use of K as an anesthetic agent in this model is valid. Furthermore, the ability of L-arginine to reverse the effects L-NAME shows that pressor responses in both groups of rats are the direct result of NO synthesis inhibition by L-NAME.

Upon exposure to MMW radiation there were no beneficial effects when L-NAME was administered in bolus to K-anesthetized rats at the point of shock induction. However, this does not necessarily rule out involvement of NO. To further this investigation we are studying different drug delivery methods as well as other NO inhibitors.

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Figure Legends

Figure 1. Dose-response curves (MAP) for L-NAME in K- and Pb-anesthetized rats. n=10 per dose for the ketamine group; n=6 per dose for the pentobarbital group.

Figure 2. Dose-response curves (HR) for L-NAME in K- and Pb-anesthetized rats. n=10 per dose for the ketamine group; n=6 per dose for the pentobarbital group.

Figure 3. Reversal of the pressor response to L-NAME by L-arginine. Control, MAP before L-NAME; L-NAME, value 30 min after L-NAME administration; L-arginine, maximum Δ MAP after L-arginine administration. *p<0.05 compared to control value; †p <0.05 compared to L-NAME value. n values in parentheses under doses.

Figure 4. Dose-response curves for L-NAME in K-anesthetized rats in the control (normotensive) state or following MMW- irradiation. n= 10 per dose in each group. *p<0.05.

Figure 5. Survival times of K- anesthetized, MMW irradiated rats following bolus injection of vehicle or L-NAME. n=10 per dose.

Figure 1

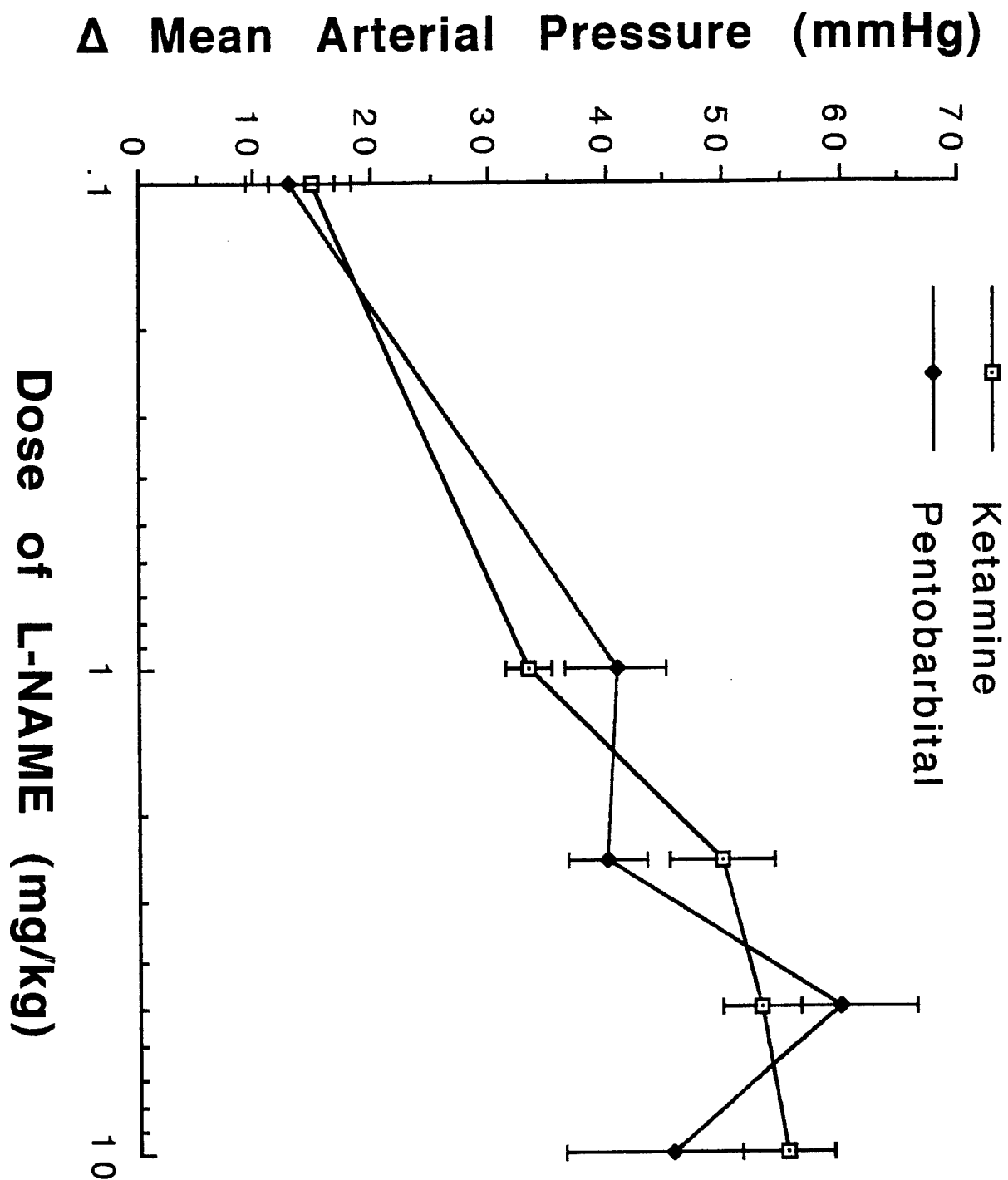


Figure 2

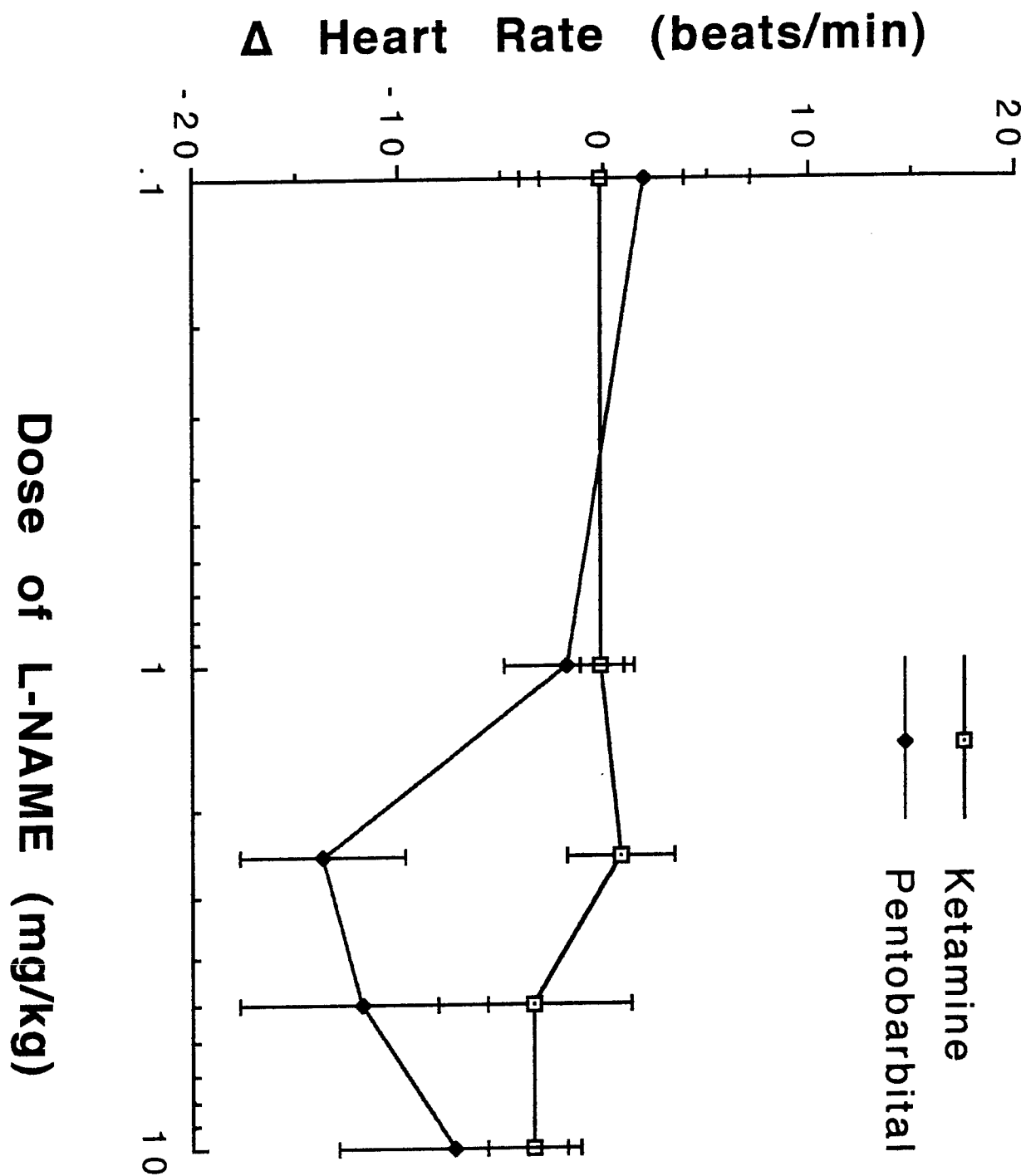


Figure 3

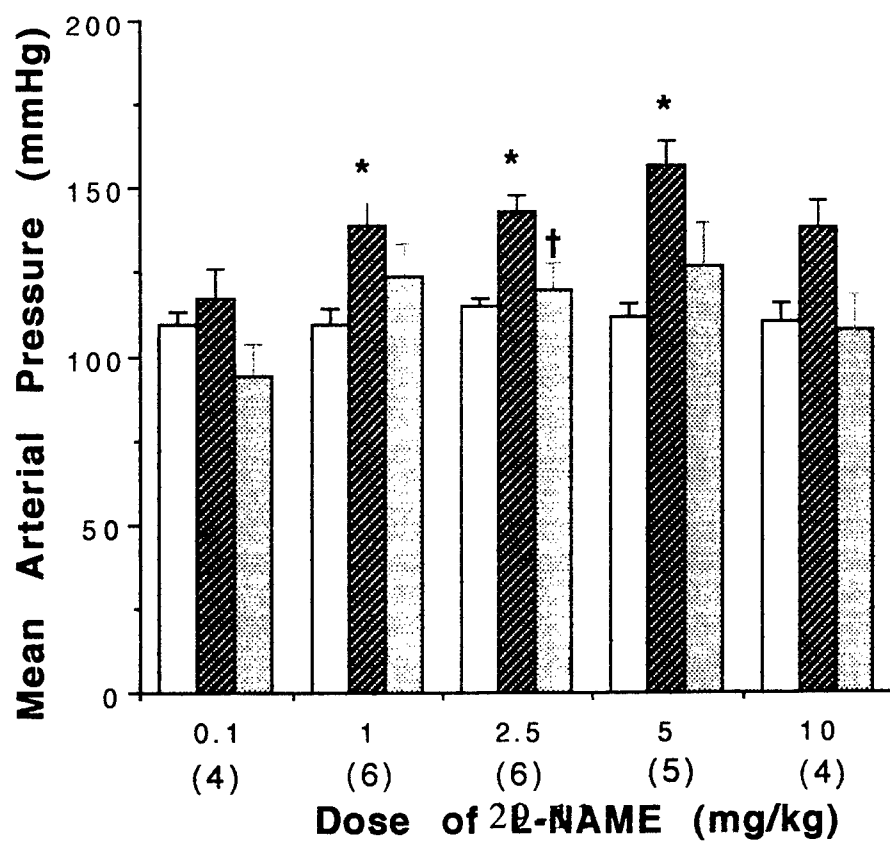
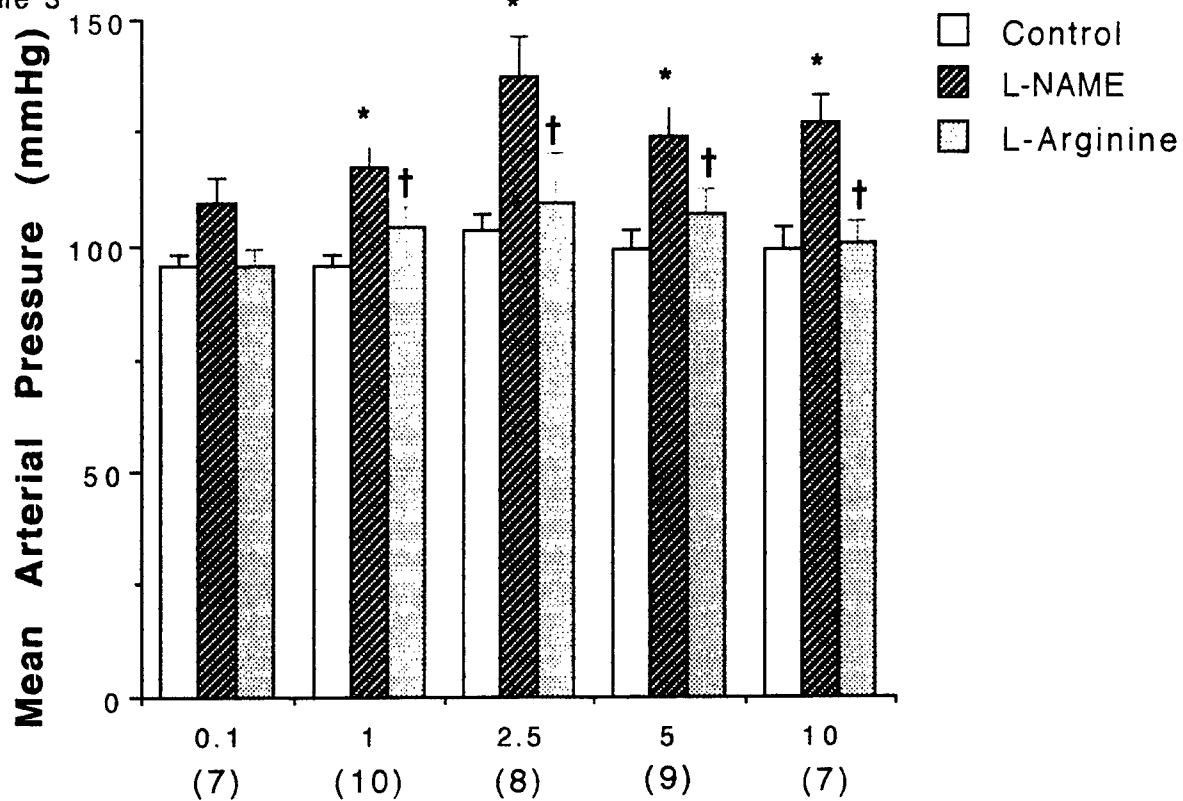


Fig. 4
Figure 4

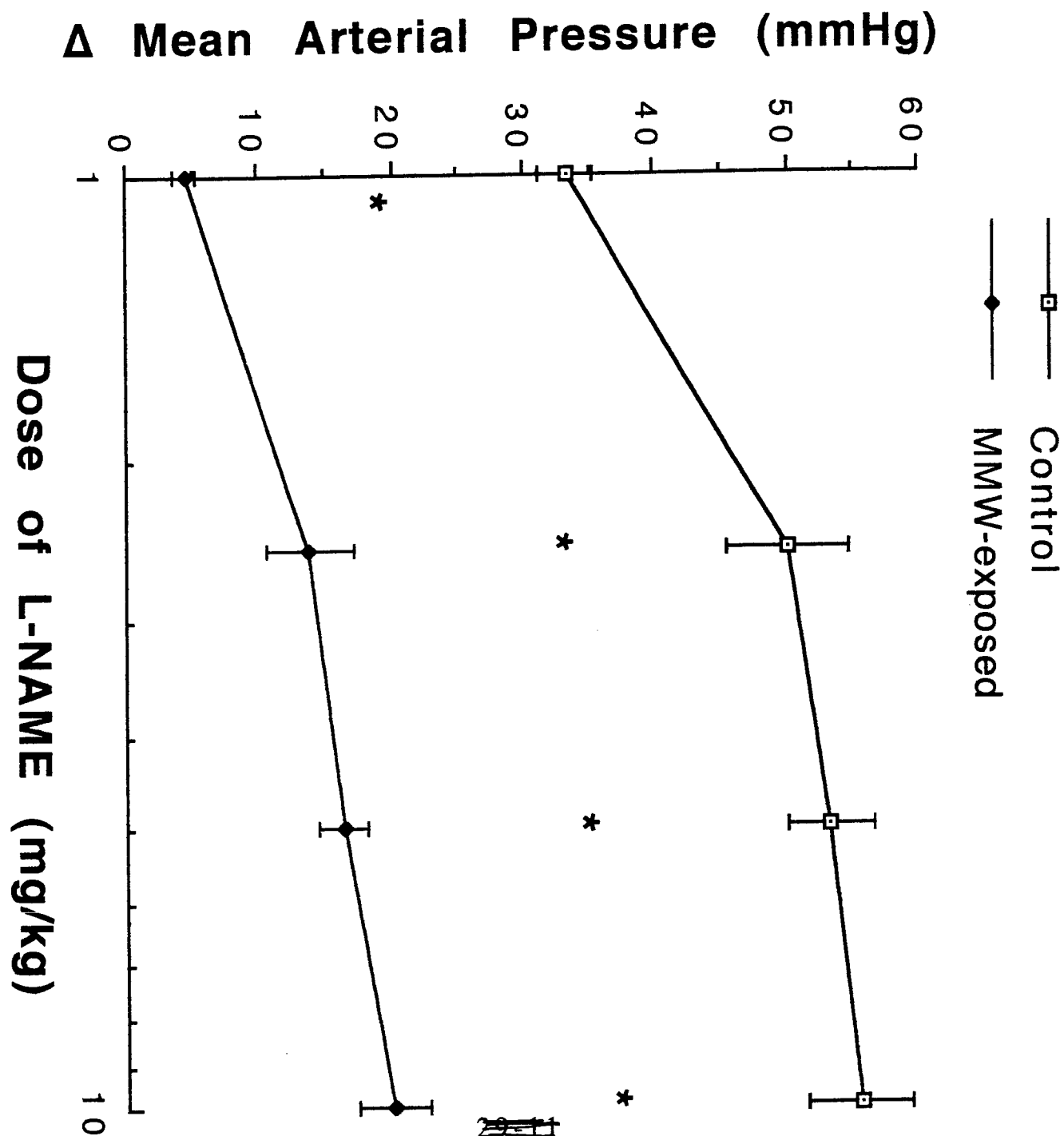
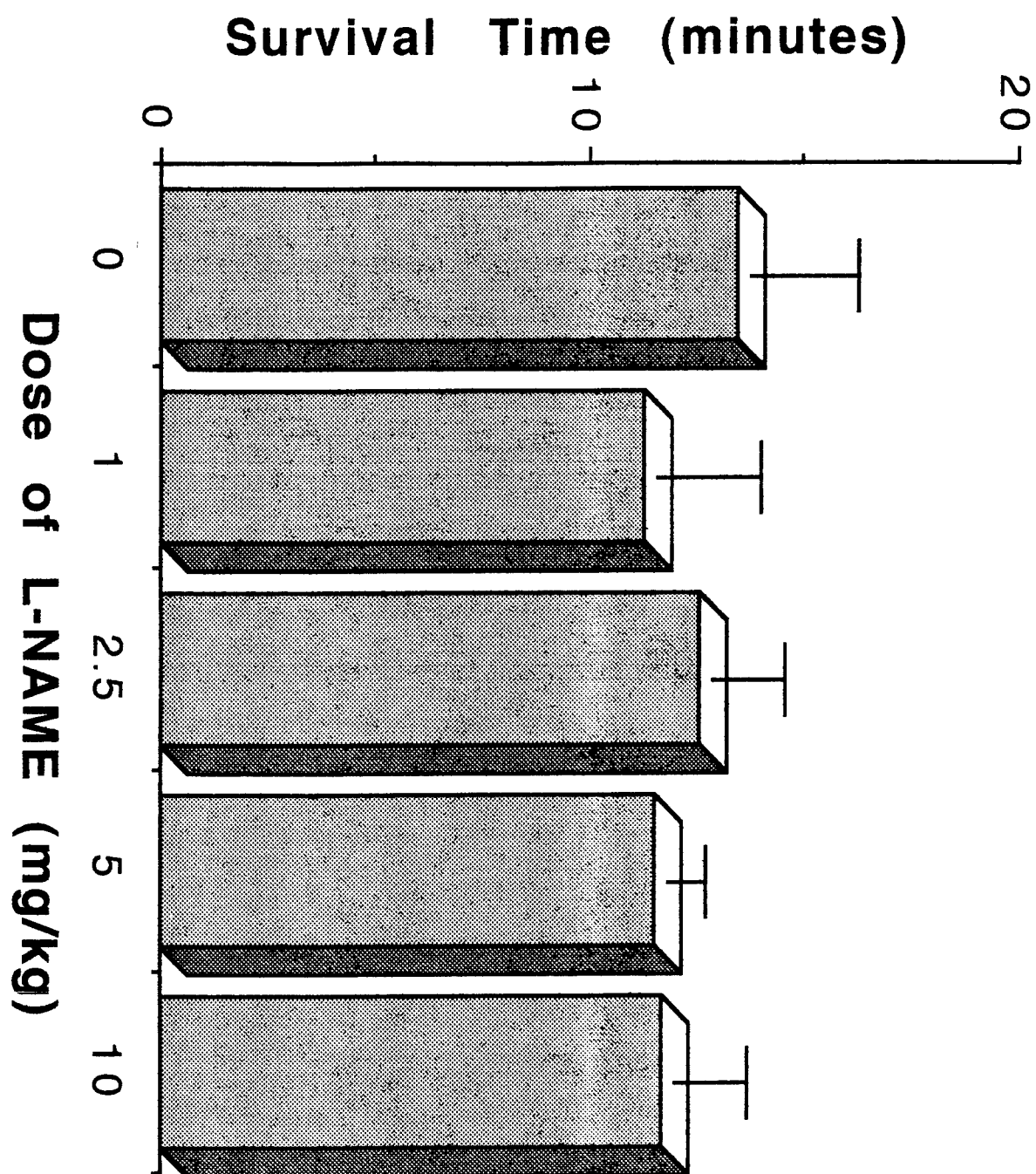


Figure 5



TRYPTAMINE AS A DERIVATISING AGENT FOR THE ANALYSIS OF
ISOCYANATES IN SPRAY PAINTING OPERATIONS

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ISOCYANATES IN SPRAY PAINTING OPERATIONS

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Abstract

Tryptamine was used as a derivatising reagent for the analysis of airborne hexamethylene diisocyanate (HDI) in monomeric and polymeric forms, using high performance liquid chromatography with fluorescence detection. Also, the use of solid sorbent tubes in actual field operations was compared directly to the results obtained from impingers.

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Ronald D. Sutcliffe

Introduction

Polyurethane coatings containing polyisocyanate activators have several outstanding features such as durability, color stability, chemical and abrasive resistance, and resistance to temperature extremes. All of these characteristics make these coatings very popular for exterior paints and their use will continue to grow.

The activators exist as polyisocyanate oligomers, either singly or in combination, of one of three diisocyanate monomers:

hexamethylene diisocyanate (HDI), toluene diisocyanate (TDI, which is a mixture of the 2,4 and 2,6 isomers), and 4,4'-diphenylmethane diisocyanate (MDI). In spray painting operations, the aliphatic-based (HDI) polyisocyanates are used in preference to aromatic-based polyisocyanates due to their greater resistance to discoloration by sunlight.¹ Due to its volatility and the reactivity of its isocyanate groups, the HDI monomer is a known occupational hazard and the American Council of Governmental Industrial Hygienists (ACGIH) has established a ceiling standard of 0.034 ppm and a time-weighted (8-hr) average (TWA) of 0.005 ppm.² A threshold limit value (TLV) has not yet been established for the oligomers of HDI due to its higher molecular weight and lower volatility.

However when in aerosol form, as during spray-painting operations, polyisocyanates may be inhaled. Recent reports suggest that polyisocyanates can cause occupational asthma during spray-painting operations.^{3,4} This has led some countries and the state of Oregon to set permissible exposure limits (PEL's) for all isocyanates, regardless of chemical form.^{5,6}

Of the methods for isocyanate analysis currently in use, only the Mobay Method 1.4.3 and the NIOSH Method 5521 appear to be suitable for analysis of polyisocyanates because they employ an impinger for sample collection.^{7,8} Though solid phase sorbents are preferred by field collectors, impingers are better than solid phase collection apparatus due to the superiority of liquid phase derivatization of aerosols.⁹ One problem with using either of these methods for the determination of polyisocyanates is that the conformation of the isocyanates must be known as the peak retention time alone does not necessarily confirm the existence of an isocyanate.¹⁰

Wu *et al.* have suggested using tryptamine (3-(2-aminoethyl)indole) as a derivatising agent to overcome this problem.¹⁰⁻¹⁴ The π -system of the indoyl moiety, which is responsible for both the florescent and amperometric activity, is isolated from the derivatising site of the amino group (figure 1). Therefore the response of the detector (fluorometric or amperometric) is only proportional to the amount of isocyanate being derivatised, and is not affected by the environment of the -NCO group. So the amount

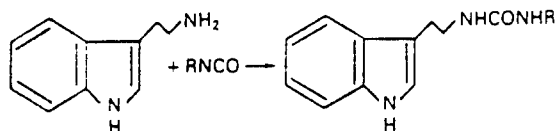


Figure 1: Derivatisation of isocyanate with tryptamine.

of total isocyanates in a samples can be determined using florescence or amperometric detection against a single standard for comparison. For this reason, the National Institute of Occupational Safety and Health (NIOSH) has developed a method for total isocyanate analysis based using tryptamine as a derivatising reagent.¹⁵

Recently, a study has been undertaken using tryptamine on solid sorbents for the determination of airborne isocyanates.¹⁴ Solid sorbents are preferred over impingers in the field due to their ease of use and reduced probability of spillage. Amberlite XAD-2 resin was determined to give results that compared well with those obtained using impingers. However, these results were obtained using an isocyanate (phenylisocyanate (PDI)) with a reactivity higher than that of isocyanates normally found in field samples. Moreover, no actual field tests were run; the tests were carried out using only a commercial test atmosphere generation system (TAGS).

In the following report, the use of tryptamine as a derivatising agent for HDI was investigated using both florescence and

amperometric detection. The efficiency of tryptamine coated XAD-2 resin in sampler tubes as a derivatising system was studied.

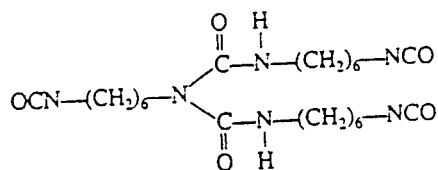
Experimental

Chemicals and Solid Sorbent

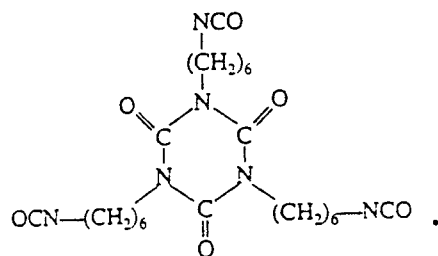
The 98% pure HDI monomer was purchased from Aldrich Chemical Co. (Milwaukee, WI.) and the 3-(2-aminoethyl)indole was purchased from Sigma Chemical Co (St. Louis, MO). The hexamethylene diisocyanate was purchased from the Eastman Kodak Co. (Rochester, NY). Tryptamine-derivatised HDI used as a calibration standard was synthesized at Southwest Texas State University, according to NIOSH protocols.¹⁵ Amberlite XAD-2 was purchased from Supelco Inc. (Bellefonte, PA). All other chemicals were reagent grade or better.

The sodium acetate buffer was prepared by dissolving 20.4 g sodium acetate in 2.0 L HPLC grade water, then adding 1.5 mL glacial acetic acid.

The bulk prepolymer standards were prepared from Deft 1 Coat, Component B; purchased from Deft Inc. (Irvine, CA.); which was the catalyst component of the paint used in the field samples. The catalyst consisted of 60% aliphatic polyisocyanate (a mixture of biuret trimer and isocyanurate of HDI, see figure 2) and 40% organic solvents (MSDS data sheet).



Biuret trimer



Isocyanurate

Figure 2: Prepolymers of HDI

Instrumentation

The high performance liquid chromatography (HPLC) system consisted of a Hewlett-Packard 1090 Series II chromatograph with autosampler and diode array uv-vis detector. A Hewlett-Packard 1049A electrochemical detector with glassy carbon working and Ag/AgCl reference electrodes operating at +0.8 V was used for amperometric detection. An ABI Analytical Spectroflow 90 fluorescence detector operating with an excitation wavelength of 275 nm was used for fluorescence detection. The column used was a LiChrospher 100 RP-18 5 μ m, 250 x 4 mm.

The mobile phase was a 50:50 mix of acetonitrile:sodium acetate buffer with a flow rate of 1 μ L/min.

The fluorescence detector (FD) settings were as follows: the photomultiplier tube was at 0.1 μA with a range of 1.0 $\mu\text{A/s}$. The response time was set at 1.0 s. The FD was not equipped with an emission monochromator and no filters were used, therefore the

emissions detected were for the entire range of the instrument, 190-700 nm, with a bandwidth of 5 nm.

Sampling

The impinger solution was prepared by diluting 0.225 g tryptamine to 500 mL with dimethyl sulfoxide (DMSO). Each impinger was filled with 20 mL of this solution before use and the volume measured after each run.

The solid sorbent tubes (5 mm interior diameter) were filled with treated XAD-2 resin to a height of 3 cm. The XAD-2 resin was mixed tryptamine dissolved in acetonitrile, then the solvent was evaporated in a rotary evaporator. The final coating was 1 mg tryptamine to 1.0 gram of resin.

Spray Painting Operations

The spray painting operations occurred from 1500 to 2300 hrs on 01 August, 1993 at Tinker Air Force Base, OK; involving the painting of a B-52 aircraft. Two hours were spent on cleaning and prepping and the remaining six hours spent painting the aircraft. The painters used Kraco Pro AA 4000 electrostatic paint guns for all operations. The paint was a Deft self-priming topcoat (Deft Inc. 8010-01-344-3222), which is a two component mixture. Component A is a 1-coat polyurethane coating and component B is a 1-coat aliphatic isocyanate (see above). The components are mixed 3 volumes of A to 1 volume of B.

All sampling was performed using side-by-side solid sorbent tubes and impingers. Initially six samplers were set up, four personal samplers and two area samplers. Three of the four personnel sprayed, the other operated the man lift, and the area samplers were set up on the east and west sides of the hanger dock approximately 15 feet from the wing tips of the aircraft. The ventilation system was placed in the exhaust mode for the first four hours of the operation, then changed to recirculation mode for the remaining four hours.

One and one-half hours into the operation, it was noticed that the impinger solution migrated into the sampling pumps of two of the painters; invalidating two impinger samples and one solid sorbent sample.

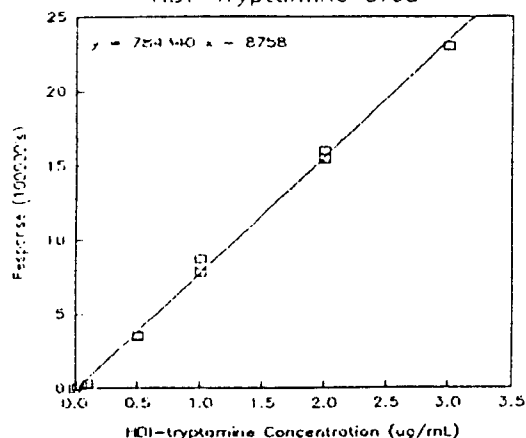
The remaining personal samples consisted of the following: one sample of four hours painting, one sample of two and one-half hours painting, one sample of four hours of manlift operation, and one sample of two and one-half hours of manlift operation. The two area samples ran without problems.

Results and Discussion

Isocyanate Standards

A plot of the fluorescence response versus HDI-tryptamine urea standards prepared according to the draft of the forthcoming NIOSH method (see reference 15) is shown in figure 3. The coefficient

Figure 3: Fluorescence Response for
HDI-Tryptamine Urea



of correlation is greater than 99% for the working curve with a linear response over the entire range studied (0.01-3.0 µg/mL) when 25 µL of sample is injected.

The analytical sensitivity of the method, measured by the slope associated with the FD response, is 7.84×10^5 counts/(µg/mL). The relative standard deviations for 14 working standards at 1.0 µg/mL was 0.840%.

A typical chromatogram for an HDI-tryptamine urea standard is shown in figure 4. The peak at 3.10 min is caused by the FD response to the unused tryptamine, while the peak at 5.40 min corresponds to the tryptamine derivatised HDI urea.

Polyisocyanate Standards

Standard solutions were prepared from the Deft hardener and the chromatogram obtained has three characteristic peaks. The largest peak, at 8.78 min, represents 95% of the total area (excluding reagent blank peaks and the peak caused by the HDI monomer) and is caused by the biuret trimer of HDI. Two smaller peaks, at 14.57 min and 16.74 min, are also observed and attributed to the

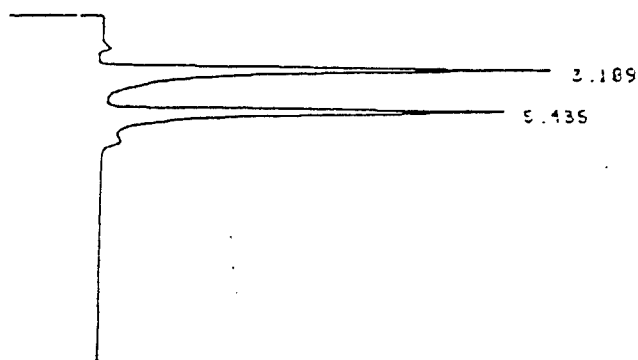


Figure 4: Chromatogram of the separation of 1.0 $\mu\text{g/mL}$ HDI-urea standard. Column, LiChrospher 100 RP-18 5 μm , 250 mm x 5 mm; mobile phase, acetonitrile: acetate buffer, pH 6 (50:50); flow rate at 1.0 mL/min; fluorescence detection, 275 nm (ex).

isocyanurate and higher molecular weight oligomers of HDI respectively, contribute 3.7% and 1.4% respectively.

Attempts at the determination of percent recovery of the impingers by direct spiking were unsuccessful. The probable cause of the inability to obtain results was that both the HDI monomer and the Deft hardener were dissolved in DMSO before being added to the derivatising reagent solution. It was later learned that DMSO dissolves a significant amount of water from the air and the water reacts with the isocyanate groups before the tryptamine has a chance to form the urea.¹⁶

Field Data

Figure 5 is a typical chromatogram of a sample obtained using an impinger and figure 6 is that of a sample obtained using a solid sorbent tube. Both clearly show the peaks attributed to tryptamine, HDI-tryptamine urea, and the biuret trimer of HDI.

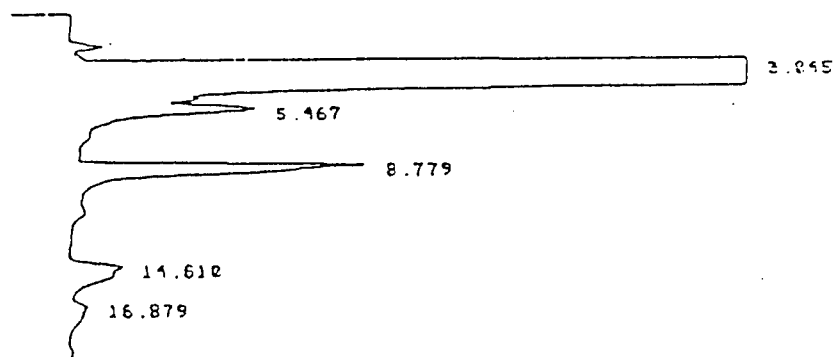


Figure 5: Chromatogram of the separation of sample obtained from impinger. Column, LiChrospher 100 RP-18 5 μ m, 250 mm x 5 mm; mobile phase, acetonitrile: acetate buffer, pH 6 (50:50); flow rate at 1.0 mL/min; fluorescence detection, 275 nm (ex).

However, the peaks due to the isocyanurate of HDI and those of the higher molecular weight oligomers are obscured in the chromatogram from the solid sorbent tube by the large peak at 16.02 min. Also present in the solid sorbent chromatograms are two large peaks at 27.4 min and 28.8 min which are also present in the blank.

The results of the analysis for HDI are shown in table 1. These results clearly indicate that while a tryptamine coated XAD-2 resin sorbent tube gives results comparable to that of an impinger for PDI, for a substance of lower reactivity the results are far from equitable. This is further enhanced by the results for the analysis for the biuret trimer of HDI (table 2). Here the results are given in "HDI equivalents", which is the equivalent amount of HDI (in μ g) having the same number of isocyanate groups available for derivatisation. This was done for comparison purposes as there are no specific limits for isocyanate oligomers or polymers.

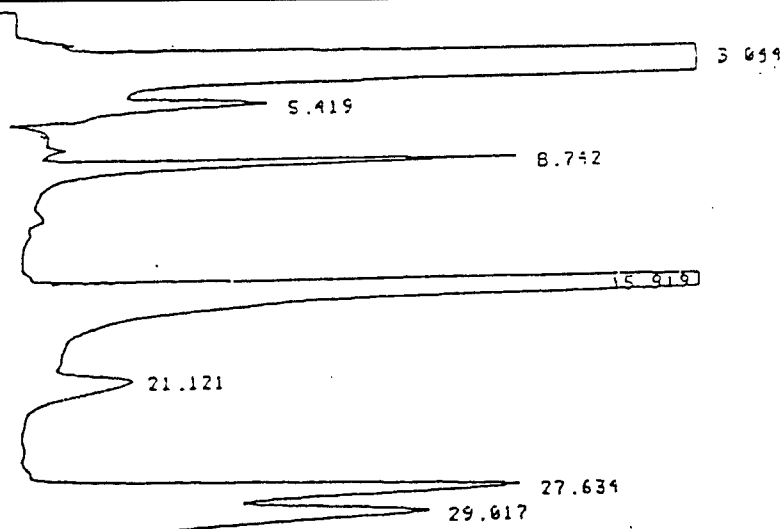


Figure 6: Chromatogram of the separation of sample obtained from solid sorbent tube. Column, LiChrospher 100 RP-18 5 μ m, 250 mm x 5 mm; mobile phase, acetonitrile: acetate buffer, pH 6 (50:50); flow rate at 1.0 mL/min; fluorescence detection, 275 nm (ex).

Eventually, if there are to be exposure limits for total isocyanates (as opposed to limits for specific isocyanate containing compounds), those limits would ideally be in amount of -NCO (in μ g or μ moles).

The data from both tables show that for compounds of moderate reactivity, such as those actually found in the field, tryptamine coated XAD-2 resin in solid sorbent tubes does not give results comparable to those of impingers. In all but four of the samples, the tubes gave significantly lower results than the impingers. For the HDI monomer, the percent recovery for the solid sorbent tubes, relative to the impingers, ranged from 126.6% to 6.3% with an average recovery of 52.2%. The average percent recovery for the biuret trimer was somewhat higher at 66.6% ranging from 31.8% to 119.1%; the recovery for A2-2 at 561.7% was disregarded. The

Table 1: Sampling Efficiency of Tryptamine in a Solid Sorbent Tube versus an Impinger for HDI

Sample	HDI (mg/m ³)		Recovery
	(solid)	(impinger)	
E1-1	0.0142	0.0256	55.4%
E1-2	0.0094	0.0243	38.7%
E2-1	0.0052	0.0831	6.3%
E2-2	0.0138	0.0393	35.1%
A1-1	0.0214	0.0169	126.6%
A1-2	0.0139	0.0283	49.1%
A2-1	0.0140	0.0142	98.6%
A2-2	0.0257	0.3308	7.8%

average percent recoveries were within the range expected and though low, they could be raised by changes in the sampling technique. For example, shorter sampling times or larger sorbent tubes could be used. Also a possible contributing factor to the low recoveries could be the fact that the air flow through the tubes was five times less than that of the impingers (0.2 L/min compared to 1.0 L/min). The area sample A2 gave exceedingly varied result from both impingers and tubes. This could be the result of the change in the ventilation system from exhaust mode to recirculation mode.

Though the solid sorbent tubes are inferior to liquid impingers for the derivatisation of isocyanates found in the field, the use of tryptamine in conjunction with fluorescence detection has shown to be an excellent technique for the analysis of isocyanates. All chromatograms were remarkably noise free, even down to very low concentrations. Using fluorescence makes it possible to lower the detection limit ten fold than that possible with uv detection. Also the tryptamine/fluorescence technique has shown to be an

Table 2: Sampling Efficiency of Tryptamine in a Solid Sorbent Tube versus an Impinger for Biruet Trimer of HDI

Sample	HDI equivalents* (mg/m ³)		Recovery
	(solid)	(impinger)	
E1-1	0.0143	0.0450	31.8%
E1-2	0.0227	0.0391	58.1%
E2-1	0.0738	0.1600	46.1%
E2-2	0.1250	0.1051	119.1%
A1-1	0.0702	0.0853	82.3%
A1-2	0.0857	0.1203	71.4%
A2-1	0.0940	0.1642	57.3%
A2-2	0.2202	0.0392	561.7%

* See text.

excellent method for the detection of polyisocyanates. Here, while only three of the HDI samples were over the NIOSH ceiling of 0.035 mg/m³, all but two of the polyisocyanate samples were above the NIOSH ceiling when measured in units of HDI equivalents. Airborne polyisocyanates were present in concentrations as high as 0.1250 mg/m³, 3.6 times higher than the exposure limit suggested by NIOSH. These aerosol polyisocyanates retain their reactivity and should therefore be considered as possible health hazards.

Conclusion

Based upon the evaluation of the data from the field samples and the laboratory standards, the following was observed:

1. The use of tryptamine in conjunction with fluorescence detector is an excellent technique for the detection of airborne isocyanates, whether in monomeric or polymeric form. The limit of detection is significantly lowered compared to other currently available methods.
2. Though the solid sorbent tubes gave poor results when compared to the impingers, these results could be

improved by several techniques, such as shorter sample times, larger sorbent tubes, or increased concentration of the derivatising reagent coating.

3. It is necessary to set exposure limits for polymeric isocyanates. Though of lower volatility than isocyanate monomers, during spray painting operations polyisocyanates are present in aerosol form in significant concentration to introduce a possible health hazard.

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CAPTURE AND ANALYSIS OF RAT'S 22-KHZ VOCALIZATIONS

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Abstract

Stressful stimuli often elicit 22 kHz ultrasonic vocalizations from rats. Ultrasonic vocalizations emitted by rats in startle chambers were digitally recorded in order to determine if startle-induced stress is manifest in the physical characteristics of these calls. Acoustical analysis of properties of rat ultrasonic calls was begun during the summer at the Armstrong Laboratory (OEDR) and is continuing in the bioacoustics laboratory at the University of Georgia.

Additionally, rhesus macaques (Macaca mulatta) were video taped and their vocalizations were recorded in various housing arrangements. The purpose was to broaden the data base of an on-going study comparing the expression of emotionality and stress in captive versus free-ranging groups of primates. This study is directed toward establishing a method for distinguishing between normal and abnormal levels of emotionality in laboratory

CAPTURE AND ANALYSIS OF RATS' 22-KHZ VOCALIZATIONS

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Introduction

Air Force and other armed forces personnel may be exposed to various physiological and psychological stressors (e.g., toxic chemicals, radiation). The Performance Extrapolation Branch, Radiofrequency Radiation Division, of the Armstrong Laboratory in San Antonio, Texas develops and validates animal models for predicting the effects of potential stressors on Air Force personnel. Development of methods to detect and assess levels of induced stress and emotional behavior have been the object of several programs of ongoing research in the Performance Extrapolation Branch. Rodents have been used successfully in previous models (Murphy et al, 1993). I, as a Summer Research Fellow, collaborated with Dr. S. C. Baker (AFSOR Summer Research Program Faculty Associate, James Madison University), Dr. B. E. Mulligan (Visiting Research Fellow, University of Georgia), and Dr. M. R. Murphy (AL/OEDR) by assisting in the recording and analysis of behavior of two animal species, rats and rhesus macaques.

Rats emit 22 kHz ultrasonic vocalizations (UV) in response to many different stimuli. Stressful situations have been found

to evoke UV from rats. Kaltwasser (1990, 1991) and Murphy et al (1993) demonstrated that rats will emit a 22 kHz UV when exposed to an acoustic startle.

Baker (pp 36-4, 1993) indicates that there are four advantages in using vocalizations as indicators of the internal state of the rat. (1) Vocalizations are responses to naturally occurring stressors. (2) Learning required for the rat to behaviorally respond to stimuli. (3) Vocalization measurement can be performed without physical contact with the animal and can be repeated without adverse effects. (4) In most experimental situations vocalization measurements can be accomplished without affecting the study protocol.

Method

Subjects

24 adult male Sprague-Dawley rats were used as subjects. These rats were from Charles River and were approximately 20 months old at the time of testing. The subject rats had been handled and tested in various projects. One project reportedly involved food deprivation though, the rats had been on ad lib feed for several months prior to this test. They had also been participants in a prior study on startle induced vocalization. The rats were maintained on a 12 hour light dark schedule and were tested in the light cycle during this procedure.

Equipment

The equipment for testing was located in a sound attenuated chamber. The acoustic startle was generated by a San Diego Instruments Model SIC startle apparatus. Rats were placed in clear plastic tubes which were mounted on the startle platform enclosed in the startle apparatus. The startles were 108 dB broadband signals of 40 msec duration.

A Bruel & Kjaer (B & K) Type 4135 microphone with attached cathode follower (B & K Type 2633) and a B & K Type 2610 amplifier were used to capture and amplify the signal. Frequency response of the B & K system extended to 200 kHz approximately flat, ± 1 dB to 100 kHz. The B & K system was calibrated relative to 20 microPa sound pressure using B & K Type 4228 pistonphone. A 250 Hz signal at 123.98 dB above reference sound pressure was used to calibrate the system. Correction factors due to ambient air pressure were obtained by using a B & K UZ0004 barometer.

Signals from the system was digitalized and stored using Global Lab data acquisition software (version 2.2) and a Data Translation A/D board (#2831-G).

Procedure

The B & K microphone with cathode follower was placed at the end of the plastic tube adjacent to the rat's head and affixed approximately 3.2 cm above air holes located at the end of the

tube. Prior to testing the subjects a 5 second calibration was performed using the pistonphone calibrator. Calibration was performed after every four rats.

For each rat there was a 5 minute habituation period once they had been placed in the startle chamber. After the habituation period a 20 second sample was taken within the first 7 minutes of the startle session. A second 20 second sample was taken between the elapsed time of 13 minutes and 20 minutes. The time differential between samples was to examine potential changes in vocalization rates over time. Once 2 samples had been obtained the rats were removed from the tube. Any matter in the tube was then removed prior to testing the next subject.

Results and Discussion

Vocalizations were obtained from twenty of the twenty four rats (83%). Even though four of the subjects apparently did not vocalize, the percentage that did was higher than reported by Kaltwasser (1990,1991). As previously noted, though, these rats had been previously tested in the startle apparatus. They had also been previously selected from a larger group based on vocalizations made when in the startle paradigm.

While final analysis of the results of these vocalizations are incomplete at this time some preliminary results seem to indicate that Kaltwasser (1990, 1991) obtained calls which were

acoustically similar to those obtained in this study. Further analysis of these vocalizations is continuing.

Other Research Activities

Video and auditory recordings were made of rhesus macaque (Macaca mulatta) monkeys in various housing conditions. The purpose was to expand the auditory data base of rhesus and to begin to develop a data base of facial expression, body posture, and behavior exhibited when vocalizing. The hypothesis is that vocalizations can be correlated to the "emotional" state(s) of the vocalizing animal. Additionally, we postulate that there is a correlation between vocalization and behavior and or body posture of the vocalizer.

Recordings were made in rooms in which the animals were singly housed. The rhesus in one room were subjects in ongoing research projects and attendants were transporting subjects into and out of the room during the recording session. A second room housed rhesus which had been subjects in research that was over and were being held pending a new assignment. A third recording was made of a breeding pair of rhesus. Results of these recordings have not been analyzed at this time.

I assisted Dr. S. C. Baker in the B & K Microphone/Bat Detector recordings. For further information on this see Baker (pp 36-14, 1993).

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