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CONTENTS

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PREFACE

The Rhodes College Science Journal is a student-edited, annual publication which recognizes the scientific achievements of Rhodes students. Founded twelve years ago as a scholarly forum for student research and scientific ideas, the journal aims to maintain and stimulate the tradition of independent study. We hope that in reading the journal other students will be encouraged to pursue scientific investigations and research.

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Dr. David Kesler
Beta Beta Beta Biological Honor Society
All professors supervising student research

Front cover photograph: Courtesy of Dr. D.H. Kesler and Reneé Pardieck

A COMPARISON OF INSECTICIDE SUSCEPTIBILITY AND ESTERASE ACTIVITY USING A RESISTANT STRAIN OF THE GERMAN COCKROACH, *BLATTELLA GERMANICA*, (DICTYOPTERA:BLATTELLIDAE)

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ABSTRACT A resistant strain of the German cockroach, A&M (Texas A&M), was evaluated to determine the relative susceptibility to Dursban, a chemically unstable organophosphate insecticide. A refined method was employed to test the susceptibility of the A&M German cockroach to a controlled dosage of Dursban. The survivors of this A&M population (renamed S&W) were housed and allowed to reproduce. The S&W population was then tested for susceptibility to Dursban. A comparison of the LD_{50} values of the A&M and S&W populations reveals an increase in insecticide resistance in the S&W survivors. An analysis of esterase activity on animals resistant to organophosphate insecticides was completed on the S&W population selecting male nymphs for the experiment. These data were compared to the esterase activity of the original A&M population (McCanless and Domon, 1989). The enzyme assay revealed that the esterase activity of the S&W population was greater than the esterase activity of the original population.

KEYWORDS *Blatella Germanica, resistance, esterase*

INTRODUCTION

The German cockroach, *Blattella Germanica*, is a common pest to many parts of the world. Because these cockroaches, like many insects, are known to carry dangerous pathogens, it has been necessary to employ the use of insecticides in order to control their growth. However, this has become increasingly difficult due to the resistance the cockroach populations have acquired to many of the common insecticides (Koehler and Patterson, 1986). This all too often leads to results in increasing application and the use of multiple pesticides, leading to economic and environmental problems. In 1956, a common method, the jar test, was developed in detecting insecticide resistance in the German cockroach (Keller et al., 1956). This method was adopted by the U.S. Armed Forces Pest Control Board (1959) and the World Health Organization (1970). In 1983, a survey of German cockroaches from various apartment complexes in Gainesville, FL revealed no chlorpyrifos (Dursban) resistance based on the jar test (Milio et al., 1987). However, further testing suggested that the jar test can produce inaccurate results when assessing chlorpyrifos susceptibility in field populations of German cockroaches (Milio et al., 1987). Using an unpublished method proposed by Bancroft and Jones, we produced a strain of German cockroaches resistant to chlorpyrifos. This resistance revealed a decrease in mortality in comparison with the original population. Previous analysis of esterases indicated a higher enzyme activity in resistant strains when compared with the susceptible strains (Prabhakaran, 1993). Thus, to achieve a quantitative assessment of this resistance, enzyme assays were employed to compare the levels of esterase activity in our resistant and susceptible populations.

METHODS

animal care

A population of Texas A&M German cockroaches was kept in a 32 gallon container in an incubator at a constant temperature of 27° C and constant relative humidity of 52% with a continuous supply of commercial dogfood and water. The containers were cleaned on a regular basis. A thin film of 1:1 mineral oil and petroleum jelly around the top of the containers prevented the roaches from escaping.

LD_{50} procedures

A 19.6 x 19.6 cm^2 area was marked off on a glass plate. The desired dosage of a 1% Dursban solution was applied to the plate and covered with acetate strips. 40 animals were anesthetized with CO_2 and placed on the plate. These animals were covered in groups of 10 with petri dishes coated on the inside with 1:1 mineral oil and petroleum jelly. When the animals resume consciousness, the acetate strips were removed exposing them to the pesticide for one hour. After one hour, the animals were placed in recovery chambers and observed for a 48 hour period. The survivors were placed in there respectable housing units.

selection for resistance

LD_{50} procedures were carried out on male nymphs of the Texas A&M population. Mortality rates at a dosage of 15.6 mg/cm^2 produced survivors that were allowed to regenerate for several months. This strain was renamed S&W. LD_{50} procedures were carried out on male nymphs of the S&W population. LD_{50} values and mortality rates were collected for both populations.

esterase determination

Male animals similar in weight were homogenized in .04 M sodium phosphate buffer (1 ml of buffer per 10 mg of roach). Each sample was added to a reaction mixture containing 5 ml of ß-napthyl acetate, acetone, and .04 M sodium phosphate buffer. The concentration of ß-napthyl acetate was determined for A_{560} described by Jones, 1994. A_{560} was monitored and esterase activity was analyzed as described by Prabhakaran, 1993.

RESULTS

The original A&M population was selected for insecticide resistance and plotted as a function of probit mortality versus log dosage (Table 1, Fig. 1). The LD_{50} value (%50 mortality) was determined to be 7.32 mg/cm^2. The S&W population was selected for insecticide resistance and plotted as a function of probit mortality versus log dosage (Table 2, Fig. 2). The LD_{50} value was determined to be 12.3 mg/cm^2.

An analysis of esterase activity was completed in the S&W population. This data was compared to the assessment of esterase activity of the original A&M population done by McCanless and Domon, 1989 (Table 3). An increase in esterase activity was observed in the S&W population.

DISCUSSION

Organophosphates (OP) are cholinesterase (ChE) inhibitors which exert their toxic action on the nervous system of vertebrates as well as insects. This action is the result of the inhibition of ChE involved in the hydrolysis of acetylcholine (ACh) (Bevan and Thompson, 1983). Dursban® (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), or chlorpyrifos, is an organophosphate commonly used as an insecticide.

An analysis of our results reveals remarkable differences between the % mortalities, LD_{50}s, and esterase activities of the A&M and S&W populations respectively. There is an obvious decrease in the mortality rate between the A&M and S&W population at the dosage of 15.6 mg/cm^2, %66.4 and %40.4 resp. The LD_{50} value likewise increases in the S&W population (12. mg/cm2) in comparison to the A&M population (7.32 mg/cm^2). The LD_{50} values and mortality rates between the susceptible and resistant populations indicate an induction of resistance within the progeny (Table1, Table 2). A corresponding increase in the level of esterase activity

was evident in the S&W population when compared to the A&M (Prabhakaran, 1993) (Table 3). Although it has not been clearly established, these results suggest that there is either a modification in the efficiency of the enzyme(ChE) or increased production of the enzyme. Nonetheless, it has been shown that building a population of German cockroaches resistant to chlorpyrifos is possible through the proliferation of animals surviving a moderate exposure to Dursban (15.6 mg/cm2). Furthermore, we found a relevant correlation between insecticide resistance and increased esterase activity in the resistant (S&W) population. The capacity of a cockroach to develop a noticeable immunity to a common insecticide (such as Dursban) illustrates a serious problem in controlling these pests. Our findings thus provide information for further research on the involvement of esterases in the resistance mechanism of German cockroaches.

SELECTION FOR RESISTANCE IN THE ORIGINAL A&M POPULATION

concentration (mg/cm^2)	log dosage	%mortality	probit mortality
0.00	---------	2.5	3.04
15.6	1.19	**66.4**	5.432
31.2	1.49	92.5	6.4395
62.4	1.80	99.5	7.5758

Table 1. These are the data obtained and derived from the A&M population's exposure to Dursban at various levels of insectide concentration.

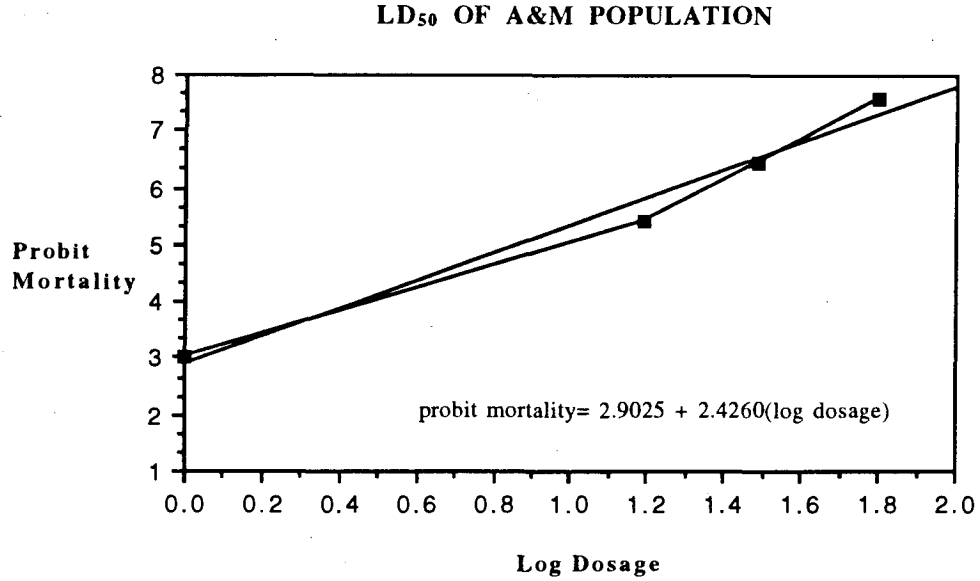

Figure 1. This figure can be used to determine the LD_{50} for the original Texas A&M population.

SELECTION FOR RESISTANCE IN THE S&W POPULATION

concentration (mg/cm^2)	log dosage	%mortality	probit mortality
0.00	---------	1.6	2.8556
15.6	1.19	40.4	4.7570
31.2	1.49	89.7	6.2646
62.4	1.80	91.7	6.3852

Table 2. These are the data obtained and derived from the S&W population's exposure to Dursban at various levels of insectide concentration.

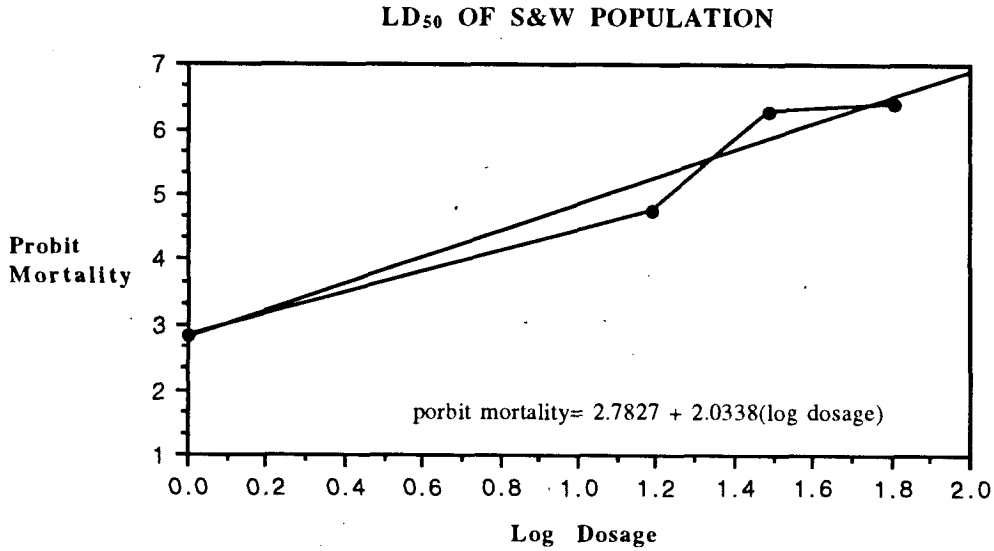

Figure 2. This figure can be used to determine the LD_{50} for the resistant S&W population.

ANALYSIS OF VARIANCE IN THE A&M AND S&W POPULATIONS

strain	N	mean
A&M	32	3.3648
S&W	25	7.5692

Table 3. The means for both populations were derived from the absorbance values (560 nm) collected during the esterase assay. This data was analyzed to determine the relationship between the mean and esterase activity according to Prabhakaran, 1993.

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REFERENCES

Bevan, J.A. & J.H. Thompson. 1983. Essentials of Pharmacology. Pennsylvania, Harper and Row, Publishers, Inc.

Jones, Bobby. 1989. (Unpublished data). Memphis, TN., Department of Biology, Rhodes College.

Keller, J.C., P.H. Clark & C.F. Lofgren. 1956. Results of USDA sponsored research in cockroach control. Pest Control 24: 12; 14; 17-20.

Koehler, P.G. & R. S Patterson. 1986. A comparison of insecticide susceptibility in seven nonresistant strains of the German cockroach. J. Med. Entomol. Vol. 23, no 3.: 298-299.

McCanless & Domom. 1989. (Unpublished data). Memphis, TN., Department of Biology, Rhodes College.

Milio, J.F., P.G. Koehler & R.S. Patterson. 1987. Evaluation of three methods for detecting chlorpyrifos resistance in German cockroach populations. J. Econ. Entomol. 80: 44-46.

Prabhakaran, S.K. 1993. Activity and electrophoretic characterization in insecticide-resistant and susceptible strains of German cockroach. J. Econ. Entomol. 86: 1009-1013.

CHARACTERIZATION OF CLATHERIN FROM BOVINE BRAIN

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Abstract

The purpose of the research was to attempt to detect the presence of clathrin in *Achlya ambisexualis*; however, due to problems with the samples of bovine brain coated vesicles obtained from prior studies, the analysis of the clathrin in these coated vesicles was attempted. Throughout the research several protein samples were used in numerous electrophoresis studies to determine the molecular weight. Also, western blots were performed in order to determine whether clathrin was actually present in the bovine brain samples. The molecular weight was calculated to be 162 kDa, approximately 18 kDa less than the accepted molecular weight of clathrin. It was also determined from the western blots that clathrin was present in the samples because of the positive antibody reactions observed because of the presence of the epitope of the clathrin. Also the isolation and storage protocols were reviewed to determine any flaws which could result in inaccurate experimentation and results.

Introduction

Clathrin coated vesicles of eukaryotic cells serve important functions within the cell, including various types of intracellular transport processes. These vesicles were first isolated by Barbara Pearse in 1975 from the brains of pigs. They have been found to form by the invagination of the clathrin-coated membrane segments of the membrane in the Golgi apparatus and lysosomes (Morris 1988). Since this time, many different functions of clathrin have been identified, and a few are listed as follows: receptor-mediated endocytosis, membrane retrieval after exocytosis, transfer of lysosomal enzymes from the trans-Golgi to lysosomal compartments, and the development of exocytotic storage vesicles (Linder 1992). Clathrin is also involved in the trafficking and targeting of many proteins (Xie 1988).

Clathrin has been found to consist of three heavy chains, along with three light chains; the structure of clathrin has been found to be a triskelion (Morris 1989). The heavy chains of clathrin vary in weight, depending on the source of the clathrin, but the weight of the protein ranges from about 170 kDa in brown algae (Hang 1987) to about 190 kDa in some species of tobacco (Mersey 1986). The clathrin normally found in the bovine brain has a molecular weight of 180 kDa. This 180 kDa component of clathrin has as its sole responsibility to promote clathrin assembly (Lindner 1992).

The coat of the coated vessicle is composed primarily of clathrin, along with several other proteins, such as HA-I and HA-II adaptor complexes (Morris 1989). When clathrin is combined with these adaptors, a very organized structure on the surface of the membrane is formed (Zhou 1992).

Clathrin and the vesicles that this protein covers are important to all organisms because of the many functions that are listed above. By analyzing these vesicles, a better understanding of the intracellular active transport system can be gained, and more conclusions as to the complete role of clathrin within the individual may be drawn.

Materials and Methods

Samples:

Samples of bovine brain clathrin-coated vesicles were recovered from previous experiments that were performed on the same subject. The samples had been stored at -70 degrees Celsius; however, these samples had undergone two thaws due to electrical failures at the storage site. Samples that were harvested from several different dates were used for this experiment:

Sample 1: August 1, 1990
Sample 2: February 8, 1991
Sample 3: April 16, 1992

These samples were chosen because they had been processed fairly recently and had been harvested according to a proven scientific method. They were all originally taken from bovine brain that was collected from a local abattoir and were processed immediately, according to the procedure described by Nandi, et al. (1982).

Electrophoresis:

Gel electrophoresis was performed on the samples in order to verify the molecular weight of clathrin and to check the physical quality of the chosen samples.

Protocol A: Two polyacrylamide mini-gels (3.25"x2.5"x1mm) were prepared according to the method described by Laemmli (1970). The denaturing gel electrophoresis was performed using a 7.5% sodium dodecyl sulfate polyacrylamide gel, or SDS-PAGE. New molecular weight standards were prepare. The standards were made in a 3:1 ratio of 4X SDS 2ME buffer to protein, which were boiled and aliquoted in 20µl portions. The aliquots were frozen at -20 degrees Celsius and thawed just before use.

The gels were loaded with 10 µl of sample which had been denatured in boiling water for three minutes before loading. The proteins were separated with 150 volts for between 45 minutes to one hour. The gels were stained in Coomassie Brilliant Blue staining solution and the excess stain was removed with a 30% methanol, 10% acetic acid destaining solution.

The molecular weights of the proteins were determined by comparing the distances migrated by the proteins and the molecular weight standards. The logarithms of the molecular weights were determined and these values were plotted against the distances travelled by the proteins. The slope of the line on the graph was represented by a third order polynomial which was used to calculate the actual molecular weights. This graph can be seen on the following page in **Figure 1**. The electrophoresis procedure was repeated several times in order to clarify the results obtained from the first attempt.

Protocol B: Two 7.5%, large-scale SDS-PAGE gels (8"x6"x1.5mm), as described by Laemmli (1970), were also performed using the bovine brain samples; this was done, hoping that the larger gel size would allow the proteins to migrate a greater distance than in the mini-gels, thereby yielding more accurate results. The procedure from Protocol A was followed regarding gel preparation and sample volume, and the gels were run at the University of Tennessee, Memphis under the guidance of Dr. Syamal K. Bhattacharya and Dr. Hua Ju Li.

Protocol C: 4% to 20% SDS-PAGE large-scale gradient gels were also run on the samples, using the procedure according to Hames (1981) and Walker (1984). These gels were also run at UT Memphis with the hope of clarifying the results of the original mini-gels and secondary large-scale gels.

Western Blotting:

Western blotting procedures were run according to Towbin, et al. (1979). The Western Blots were performed to determine the presence of clathrin in the bovine brain samples.

Protocol: Two SDS-PAGE mini-gels were prepared and electrophoresis were performed so that the proteins could be transferred to a nitrocellulose membrane. The proteins were transferred for 3.5 hours with a 200 mAmp current. Before the immunoblot procedure the membrane was stained in 0.1% Ponceau S protein stain and the molecular weight standards and one lane of the sample were saved for possible molecular weight determinations of any positive immunoblotted proteins. The primary antibody used was Goat Anti-Clathrin, (Sigma# C-8034); the secondary antibody used was Peroxidase Conjugated Anti-Goat IgG, (Sigma #A-5420). The Western Blot procedure was repeated on numerous occasions in order to obtain the best data. In each of the Western Blots one lane was always treated in the absence of primary antibody.

The protocol was modified somewhat and the one followed was provided by Dr. Terry Hill. The blocking solutions used consisted of 4% Carnation milk powder or 0.25% Knox gelatin diluted in Phosphate buffered saline (PBS), pH 7.4; while the dilution of antibodies was carried out in different "buddy protein" solutions consisting of 0.5% milk powder and 0.25% gelatin. The first two rinses of the membrane were done in solutions of PBS with Tween-20 (PBST), while the final rinse before the addition of the substrate was carried out in PBS because Tween has been known to interfere with the reaction between the peroxidase and its substrate, 4-chloro-1-napthol, (Sigma # C-6788).

In the first Western Blots the primary antibody was diluted 1:200 and 1:500, while the secondary antibody was diluted 1:1000. This Western Blot was to determine the best concentration of primary antibody. The blocking of the nitrocellulose membrane and the dilutions of the antibodies were performed with the previously stated milk powder solutions.

The following Western Blots utilized the best dilution of primary antibody, 1:500, and a secondary antibody dilution of 1:1000. In these Western Blots the blocking and antibody diluting agents were varied between milk powder and gelatin for comparison.

Results

The gel electrophoresis all showed the same basic results, none of which were consistent with the known value for the molecular weight of clathrin. The resulting values for the molecular weight can be found in Table 1; the known weight is 180 kDa.

Table 1

Experimental Molecular Weights	Average Molecular Weight
165.5 kDa	161.9 kDa
162	
162	**Standard Deviation**
162	+/- 1.3 kDa
158	
162	

New protein samples and molecular weight standards were prepared because we thought that a mistake might possibly have been made while preparing the materials for the electrophoresis. The samples were prepared in a 3:1 ratio of protein to sample buffer. The procedure was run again, yielding almost the same results; upon completion of two more runs, the average molecular weight was found to be 162 kDa, similar to the previous results, but not close to the accepted value of 180 kDa.

We decided to perform a large scale electrophoresis, in hopes that it might clarify the results that we had already obtained. However, the
larger gels did not prove to be any more effective in yielding accurate results. The procedures were run as described by Laemmli (1970). It wasvery similar to the mini-gel technique. This type of electrophoresis was performed two times, with both gels showing that the molecular weight of clathrin was 162 kDa.

The gradient gels also did not better adjust our findings. The gradient gels were set up according to Hames (1981) and Walker (1984). The first run of the gradient gel showed that the molecular weight was 162 kDa, just as the mini-gels did. Another run was performed; it was set up exactly as the first one had been. This time the molecular weight was found to be 158 kDa. The results of the gradient gels can be found in **Figure 2.**

The Western Blotting results were positive for clathrin; these results can be found in **Figure 3**. It was determined that the primary antibody concentration which produced the best results was 1:500. The concentration of 1:200 was too concentrated and even with three washes of the membrane the background was not clear. The level of secondary antibody was 1:1000, which produced adequate recognition of the primary antibody. The blockers used showed that the milk powder was too strong, and that the gelatin was not specific enough. When the milk powder was used, a faint band was seen, while the gelatin showed stronger bands, many of which were not specific.

The primary antibody was recognizing the epitope on clathrin which we had earlier determined to weigh 162 kDa. All of the lanes which had
lacked primary antibody stained negative; therefore, it is determined that the problem is not with the secondary antibody or the substrate.

Discussion

The problem that was encountered in this study of clathrin coated vesicles was not in determining the presence of clathrin in *Achlya* , but rather in proving that the accepted molecular weight of clathrin is indeed 180 kDa. All of our testing has shown it to be about 162 kDa. The reasons for this discrepancy between the two values are not known, but there are several possible explanations. Since all of the protein samples used were at least two years old, it is possible that degradation has occurred and that all of the 180 kDa mass is no longer present. If this is the case, it brings up another question, however. Namely, if degradation has occurred, why would the samples all have degraded to the same degree, (162 kDa), but in differing amounts of time? How could the sample from August of 1990 have degraded the exact same amount as the sample from April 1992? The only way that this would be an acceptable solution would be if there is an 18 kDa component of clathrin that is especially susceptible to degradation. There are no observable explanations as to why this particular part of the molecule would be degraded when no other parts of it are, other than the conformation of the protein or its amino acid sequence; since there has been nothing proven in respect to this finding, we are not accepting this theory as solution to the problem. The presence of proteolytic enzymes appears to be the best explanation for the degradation of clathrin. There is probably a certain enzyme present in the bovine brain that is responsible for clathrin breakdown.

The protease inhibitors probably were not stable due to the length of time since the isolation of the samples. Beynon and Bond (1989) reported the life of the inhibitors, when unfrozen, to be several hours for leupeptin, at least a day for pepstatin, and one hour at pH 7.5 for PMSF. As a result of repeated periods of freezing and thawing and two known ultra-low freezer

failures; it is hypothesized that the inhibitors were not functioning according to the specifications of the distributors. The failure of the protease inhibitors was not a result of the prior research of Anne Finney because the concentrations of inhibitors added in her protocol were in accordance with the manufacturer's specifications. Finney did not experimentally show that the molecular weight of clathrin is 180 kDa; she determined it to be 178 kDa. Based on Finney's laboratory notebook, she followed the protocol correctly, allowing her to gain more accurate results. It is hypothesized then that the degradation of the proteins occurred after Finney made her measurements. Gina Rhodes also has experimented with coated vesicles, and her research showed the molecular weight of clathrin to be 196 kDa. It is uncertain why her calculations were higher than the known molecular weight, 180 kDa, for clathrin. Her research notebook shows that she possibly did not freeze the samples immediately after the isolation and purification procedures. Perhaps with the samples not being frozen immediately, the inhibitors became unstable and decayed beyond their effective protective half-life.

Dr. Syamal K. Bhattacharya and Dr. Hua Ju Li neither could explain the findings of this experiment. Both of these research scientists at the UT Health Sciences Center were confounded when we presented this problem to them. Dr. Li thought we might have been looking at a clathrin-like molecule. This can not be true though because the samples that we used were the exact samples used by other people in previous research. Dr. Bhattacharya also thought that we might not be looking at clathrin. When we informed him that other experimenters had found clathrin in these same samples, he responded that the problem must have come out of the techniques that were employed by us throughout our research with the protein samples or that the clathrin we were working with had been degraded.

We hypothesize that clathrin had been degraded to 162 kDa due to proteolysis either as a result of the conformation of the protein or the amino acid sequence. Our results are variant from the accepted weight of clathrin because of several possible reasons. These include using a sample that was not originally frozen immediately after isolation and purification steps, using samples that had undergone periods of thawing due to electrical failure, or by not replenishing the protease inhibitors throughout the purification steps in the protocol. We feel that new samples need to be isolated and the electrophoresis and Western Blotting procedures repeated to determine if clathrin, which corresponds to the 180 kDa weight, can be obtained. Also, the stability and half-life of the protease inhibitors needs to be investigated further in order to find the best possible way to use these protease inhibitors in future isolations of the clathrin samples.

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The authors would like to thank Dr. Terry Hill for the opportunity to conduct research in his laboratory at Rhodes College. Much valuable knowledge was gained by the investigators, and for that we are grateful. We would also like to thank Dr. Syamal K. Bhattacharya and Dr. Hua Ju Li for allowing us the use of their laboratory facilities at the University of Tennessee, Memphis. We would also like to thank Dr. Hill for his assistance and criticism of this manuscript.

Literature Cited

Beynon, R.J. and J.S. Bond, eds. Proteolytic Enzymes: A Practical Approach. Oxford: IRL Press, 1989.

Finney, A. (1991). "Isolation of Clathrin-Coated Vesicles from Achlya ambisexualis." Rhodes College, Biology 452: pp. 1-18.

Finney, A. (1992). "Isolation and Characterization of Clathrin From *Achlya ambisexualis*." Rhodes College, Biology 452: pp. 1-11.

Hames, B.D. and D. Rickwood, eds. Gel Electrophoresis of Proteins: A Practical Approach. Oxford: IRL Press, 1981.

Hang H.X., M. Bomsel, C. dePaillerets, H. Weintraub, and A. Alfsen. (1987). "Cellular biochemistry (immunology)--homology between antigenic determinants of bovine clathrin and brown algae *Laminaria digitata* clathrin." *C.R. Acad. Sci. Paris.* 304: 465-68.

Laemmli, U.K. (1970). "Cleavage of structural proteins during the assembly of the head bacteriophage T4." *Nature* 227: 680-85.

Lindner, R. and E. Ungewickel. (1992). "Clathrin-associated Proteins of Bovine Brain Coated Vesicles." *J. Biol. Chem.* 267: 16567-73.

Morris, S., Hannig, K., and E. Ungewickell. (1988). "Rapid Purification of Clathrin-coated Vesicles by Free-Flow Electrophoresis." *Euro. J. Cell Biol.* 47:251-58.

Morris, S., Mann, A., and E. Ungewickell. (1989). "Analysis of 100-180- kDa Phosphoproteins in Clathrin-coated Vesicles from Bovine Brain." *J. Biol. Chem.* 265: 3354-57.

Nandi P.K., Irace, G., VanJaarsveld, P.P., Lippoldt, R.E., and H. Edelhoch. (1982). "Instability of coated vesicles in concentrated sucrose solutions." *Proc. Natl. Acad. Sci. U.S.* 79: 5881-85.

Pearse, B.M.F. (1975). "Coated vesicle from pig brain: purification and biochemical characterization." *J. Molec. Biol.* 97: 193-98.

Rhodes, G. (1991). "Western Blot Detection of Clathrin From Achlya ambisexualis Coated Vesicle Isolates." Rhodes College, Biology 452: pp. 1-12.

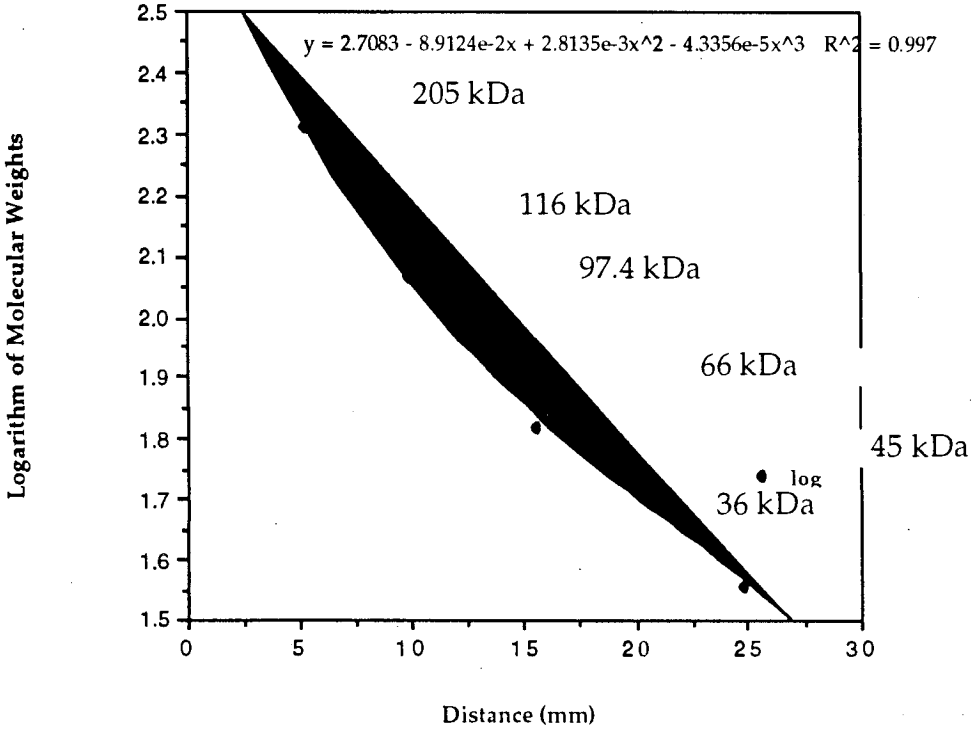
Towbin H., Staehelin, T., and J. Gordon. (1979). "Electrophoretic Transfer of Proteins From Polyacrylamide Gels to Nitrocellulose Sheets: Procedure and Some Applications." *Proc. Natl. Acad. Sci. U.S.* 76: 4350.

Walker, J.M. (1984). "Proteins." *Meth. in Molec. Biol.* 1: 57-62.

Xie, X., Stone, D., and Racker, E. (1988). "Purification of a Vanadate-sensitive ATPase from Clathrin-coated Vesicles of Bovine Brain." *J. Biol. Chem.* 264: 1710-14.

Zhou, S., Tannery, H., Yang, J., Puszin, S., and Lafer, E. (1993). "The Synapse-specific Phosphoprotein F1-20 Is Identical to the Clathrin Assembly Protein AP-3." *J. Biol. Chem.* 268: 12655-12662.

Figure 1: Molecular Weight Determination



THE COMPOSITION OF THE RAT INCISOR AS AFFECTED BY GNAWING ACTIVITY

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INTRODUCTION

The dental make-up of the rat includes two types of teeth, incisors and molars. Each set consisting of one incisor and three molars, a rat has four sets of teeth. This means that rats are monophyodont in dentition (Massler & Schour, 1949). Two sets lie next to and are mirror images of the other in both the upper and lower jaws. Incisors, in both upper and lower jaws, are formed anterior to the molars. The relationship between the upper jaw to the lower jaw is analogous to that of the human overbite, where the lower incisors are just posterior to the upper incisors (Figure 1).

Much like the development of a human tooth, the rat molar grows for a finite period and stops. In rats, this period is approximately the first 125 days of life (Massler & Schour, 1949). After this time, rat molars function, essentially, as permanent teeth. A record of the effects of various stimuli on their growth would be limited to the time during which growth occurs.

The incisors, however, offer more flexibility of observation. The rat incisor is unique in that it grows, calcifies, and erupts continuously throughout the life of the rat (Massler & Schour, 1949). Eruption occurs from the point of contact with the jaw, at the base of the incisor, into the oral cavity. The consequence of this is that the tips of the incisor must be broken off or worn away so that the rat's mouth does not become overwhelmed by the incisor's length; causing death from starvation (Schour, 1938, cited in Massler & Schour, 1949) (Figure 2). Incisor tips are worn accidentally or through attrition, the functional wearing away from gnawing at objects such as food. The unique characteristic of continuous growth causes the incisor to be of particular interest to this experiment where the composition of the incisor was observed according to the effects of altering dietary stimuli.

The rat incisor is primarily composed of dentin, enamel, a pulp cavity, and cementum (Figure 3). Enamel is derived from the ectoderm germ layer, while dentin, pulp, and cementum are derived from the mesoderm (Burkitt, et al., 1993). Incisor formation begins in utero but does not erupt into the oral cavity until 8 days of age (Addison & Appleton, 1915). During formation of the incisor, epithelial cells differentiate into tall, columnar cells called ameloblasts which are responsible for the production of enamel (Burkitt, et al., 1993). This stimulates the odontoblasts, also columnar cells, to differentiate. Dentin is made from odontoblasts (Burkitt, et al., 1993). Ameloblasts and odontoblasts cease producing enamel and dentin once the incisor has erupted (Herzberg & Schour, 1941).

The incisor is longitudinally divided into two sides, labial and lingual. The labial side is the anterior-most side of the incisor that is convex and lies proximal to the rat's "lips." The lingual side is the posterior-most side of the incisor that is concave and lies proximal to the rat's tongue.

Enamel is found only on the labial portion of the incisor (Massler & Schour, 1949). Enamel is the hardest substance a body can make, containing less that 1% organic material (Burkitt, et al., 1993). Each active ameloblast produces a column of organic enamel matrix. This matrix is mineralized into a hard translucent substance composed of highly calcified prisms (Burkitt, et al., 1993). Formation and mineralization of enamel is not a uniform process (Burkitt, et al., 1993). Erupted enamel is pigmented.

The composition of the dentin matrix is much like that of bone and is produced by

odontoblasts (Burkitt, et al., 1993). Calcification of dentin and enamel is rarely homogenous (Massler & Schour, 1949). This is significant because they are formed independent of one another since they are from two different germ layers.

A cross-section of a rat incisor is composed mainly of dentin with an enamel cap at the labial edge. Cross-sectional size is determined by an epithelial ring that outlines the future tooth and increases proportionally with the growth of the rat for at least the first 150 days of life (Herzberg & Schour, 1941). Cementum is found at the lingual, mesial, and distal edges (Schour & Steadman, 1935). Perhaps the cementum functions as added support to the exposed regions of dentin that are not covered by enamel. Since neither dentin nor cementum are as strong as enamel, a longitudinal slice of the incisor shows that its tip is bevelled. The bevelling results as incisal stress wears away at the weaker area of the incisor. Thus, the lingual portion is worn away leaving the incisor tip to come to a point at its labial edge. From a longitudinal view, the length spanning the first indication of lingual 'wearing away' to the tip is referred to as the bevel.

Keeping the incisor's morphology in mind, we can reflect on the life cycle of the incisor and begin to understand the importance of continuity of growth to this experiment. There are four stages in the incisor's life cycle: growth, calcification of deposited matrix, eruption of the formed and calcified tooth into the oral cavity, and functional attrition (Massler & Schour, 1949). It has been observed that an equilibrium of the stages is maintained by occlusal stress, a force that is provided by contact between upper and lower incisors (Butcher & Taylor, 1951). Schour (1934) and Hoffman (1939) recorded that "when a tooth is fractured so as to remove the incisal stresses which normally oppose the eruptive forces, its eruption is increased up to two and three times the normal rate and the tooth elongates" (cited in Massler & Schour, 1949). Thus, there appears to be some signal to stimulate the rate of eruption when the upper and lower incisors are not in contact, a survival mechanism since the rat will starve if it cannot gnaw at its food. The normal adult incisor length remains relatively constant, as attrition rate is compensated by an approximately equal eruption rate (Massler & Schour, 1949).

Altering the diet of a rat will vary its rate of incisor eruption (Downs, 1931, cited in Massler & Schour, 1949). A relatively hard diet will increase the attrition rate of incisor tips, so occlusal contact is lost, which will, in turn, stimulate the rate of eruption to restore the desired level of occlusal stress. A relatively soft diet will not be as demanding on the incisors, the tips won't be as inclined to break, the rate of attrition is decreased relative to the hard-diet rat, and the rate of eruption is retarded (Butcher & Taylor, 1951). It is this last information that we are interested in. Our experiment questions if incisor contact is rarely maintained under the influence of an extremely hard diet, does the metabolism of the incisor compromise itself by depositing less dentin and enamel in order to allow a sustained, extraordinarily fast rate of eruption to achieve contact?

Raising two groups of rats on diets of different consistencies allows for a comparison to be made of the deposition of dentin and enamel. Ideally, the anatomical maintenance is governed by the theory of "form fits function" to minimize energy expenditure and maximize the conservation of an organism's limited resources. Thus, it would seem that a more rigorously utilized incisor would produce more dentin and enamel to strengthen the tooth to withstand abnormally high demands. On the other hand, does such a demand cause the premature eruption of an incisor that has not had time to produce as much dentin and enamel? Our experiment will examine how a rat accounts for the principle of form fitting function according to differences of gnawing activity, assuming that a more mechanically demanding diet will impose an increase in the rates of attrition and eruption.

METHODS

Thirty-three rats were sacrificed at three or five months and categorized as control or experimental. The controls were fed rat chow and water ad lib. The experimentals were fed the same diet plus raw hide chew bones, assuming a 'harder' diet. Right upper and lower incisors were extracted, embedded in resin, and made into slides to be analyzed under a light microscope in conjunction with the Macintosh NIH image program. Measurements were taken of dentin and enamel areas as well as of four loci segments outlining a cross-hair pattern on the right mesial (I), labial (II), left mesial (III), and left mesial (IV) sides of the dentin and one focus segment bisecting the enamel cap (V).

Comparisons between controls and experimentals were made for the upper and lower incisors of rats in the following groups: rats of the same age at three and five months (C3*E3 and C5*E5), controls of different ages (C3*C5), and experimentals of different ages (E3*E5). Seven variables were examined: dentin area, enamel area, loci I-IV which outlined a cross-hair grid in the dentin, and locus V which bisected the enamel cap. Loci I-IV were numbered clockwise with locus I bisecting the left mesial side, locus II bisecting the labial side, locus III bisecting the right mesial side, and locus IV bisecting the lingual side. For each comparison, the F test was used to determine if variables were equal between groups. Only one data point, between C3*C5 at locus V for the lower incisors, failed to meet this criterion and was examined using a separate test for unequal variances (Table I).

Next, two group, unpaired, two-tailed T-tests were performed. Instead of a 0.05 probability level, a more stringent probability of 0.007 (0.05/# of comparisons) was used to determine statistically significant differences. This more conservative probability level accounts for the repeated comparison within groups (Table II).

RESULTS

The most apparent observation we can conclude from the comparison is that of an age effect. Measurements of the seven variables were consistently significantly different with the most demanding criterion, at the 0.007 level, among comparisons of E3*E5 and C3*C5 for both the upper and lower incisors. This only confirmed the postulation by Herzberg and Schour (1941) that the cross-sectional size of the tooth continues to increase for approximately 150 days (about 5 months) after birth.

No other such consistently different patterns were detected. However, sporadic significantly different variables provided motive to speculate on possible trends indicated by the T-tests. Across the board, there was virtually no difference among measurements of the III and IV loci, the region that would have been chiseled away to form the bevel (Figure 9). In general, the upper incisors showed the least difference for all seven variables, with the exception of the E3*E5 upper incisor comparison.

Being prompted by the fear that small sample size had masked statistical significance of the comparisons and seeking to examine these trends from all angles influenced a final comparison of the data by reporting the mean value of each variable for both the upper and lower incisors (Table III). These mean values could be evaluated on a larger versus smaller basis for corresponding variables on control and experimental rats of the same ages, control rats of different ages, and experimental rats of different ages, as before. All but 2 of 28 five month-old rat mean values were found to be greater that their three month-old counterparts. Also, 22 of 28 experimental rat mean values were found to be larger than their control counterparts. This trend does support our hypothesis, though it does not carry the weight of statistical significance.

DISCUSSION

The results and trends derived from our data are best summed up by an affirmation that older rats have larger incisors that younger rats. However, this conclusion was intuitive from the start, from the 1940's even. Our results do not specifically support the hypothesis that a harder diet, imposing increased mechanical demands on the ever-growing incisors, will provoke the formation of a more sturdy tooth with greater dentin and enamel deposition. Nor do our results support the antithesis of the hypothesis that a harder diet will provoke the formation of a weaker tooth, assuming an increase in the rate of attrition that would increase the rate of eruption and prevent the deposition of sufficient amounts of dentin and enamel. Examination of the mean values of the seven compared variables does show the trend of a larger experimental value than that of the corresponding control, which warrants further examination of larger samples of rats. Thus, the value of this experiment lies within the speculations and predictions that we can cast on what the data means, suggesting directions for future research.

Requiring the utmost attention are the assumptions built into this experiment that the raw hide chew bones truly provided a harder diet and that the rate of attrition, thus of eruption, was affected. Future experimenters should weigh the bones before, during, and after the experiment to see how often and to what extent the rats gnawed the chew bones, insuring that the bones did influence the hardness of the diet. The rates of eruption may be measured by marking the teeth at the base and measuring their growth daily (Butcher & Taylor, 1951). The rate of eruption should also be measured for controls to discover if merely gnashing or grinding at their own teeth might serve to establish some base rate of eruption. The rate of attrition may be determined by measuring from the same mark to the tip of the incisor (Butcher & Taylor, 1951). It is conceivable that the experimental's incisors in this study underwent no more stress than the control's incisors that might have been self-inflicted with stress.

Another question might be asked of what IS a normal rat diet? Does it consist of banana peels and mushy slop or of tough, sun-dried meat and things that must be gnawed through to get to? Surely data will be inconsistent depending on how experimenters define variables as opposed to how those variables exist in nature.

Small sample sizes may have also had a profound effect on our data. Sixty-six teeth is a large sample until it is divided in half for the upper and lower incisors before being further divided into four separate comparison groups by age and experimental or control.

The trends proposed by examination of the mean values are quite encouraging that something more is happening that our data is capable of manipulating. Hopefully, this experiment reveals a future direction toward uncovering how form fits function, the economy of nature.

WORKS CITED

Addison, W.H.F. and J.L. Appleton. "The structure and growth of the incisor of the albino rat." Journal of Morphology. 26: 43-96. 1915.

Burkitt, H.G., B. Young, and J.W. Heath. Wheater's Functional Histology--A text and color atlas. 3rd ed. Churchill Livingstone: New York. 1993.

Butcher, E.O., and A.C. Taylor. "The regulation of eruption rate in the incisor teeth of the white rat." Journal of Experimental Zoology. 177: 165-188. 1951.

Hammett, F.S. and E.S. Justice. "The geometrical symmetry of growth of the upper incisors of the albino rat." The Anatomical Record. 26: 141-144. 1923.

Herzberg, F. and I. Schour. "The pattern of appositional growth in the incisor of the rat." The Anatomical Record. 80: 497-506. 1941.

Massler, M. and I. Schour. "The teeth." The Rat in Laboratory Investigation. E.J. Farris and J.Q. Griffith, eds. Hafner Publishing Company: New York. 1949.

Schour, I. "The effect of tooth injury on other teeth. I. The effect of a fracture confined to one or two incisors and their investigating tissues upon the other incisors in the rat." Journal of Physiological Zoology. 7: 304-329. 1934.

Schour, I. and S.R. Steadman. "The growth pattern and daily rhythm of the incisor of the rat." The Anatomical Record. 63: 325-333. 1935.

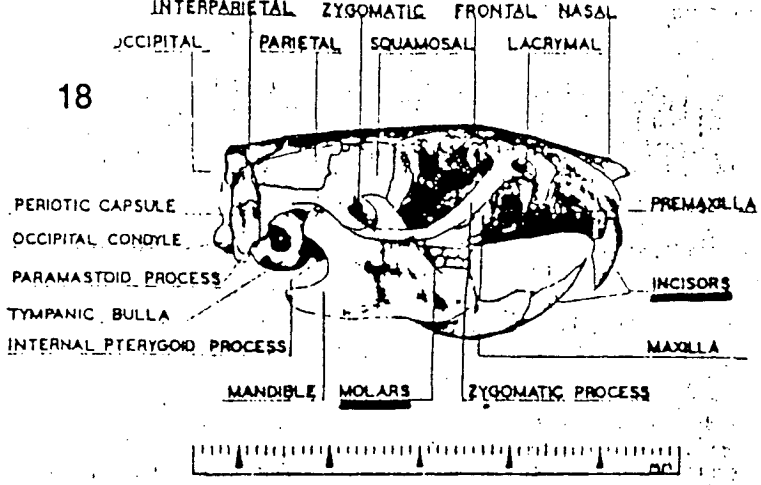

Figure 1: Right lateral view of a rat skull

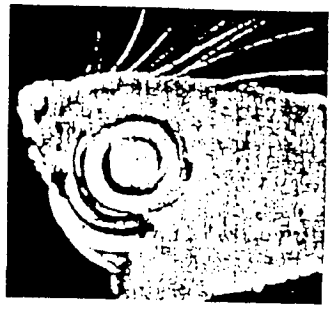

Figure 2: Spinal elongation of rat incisors due to failure of attrition

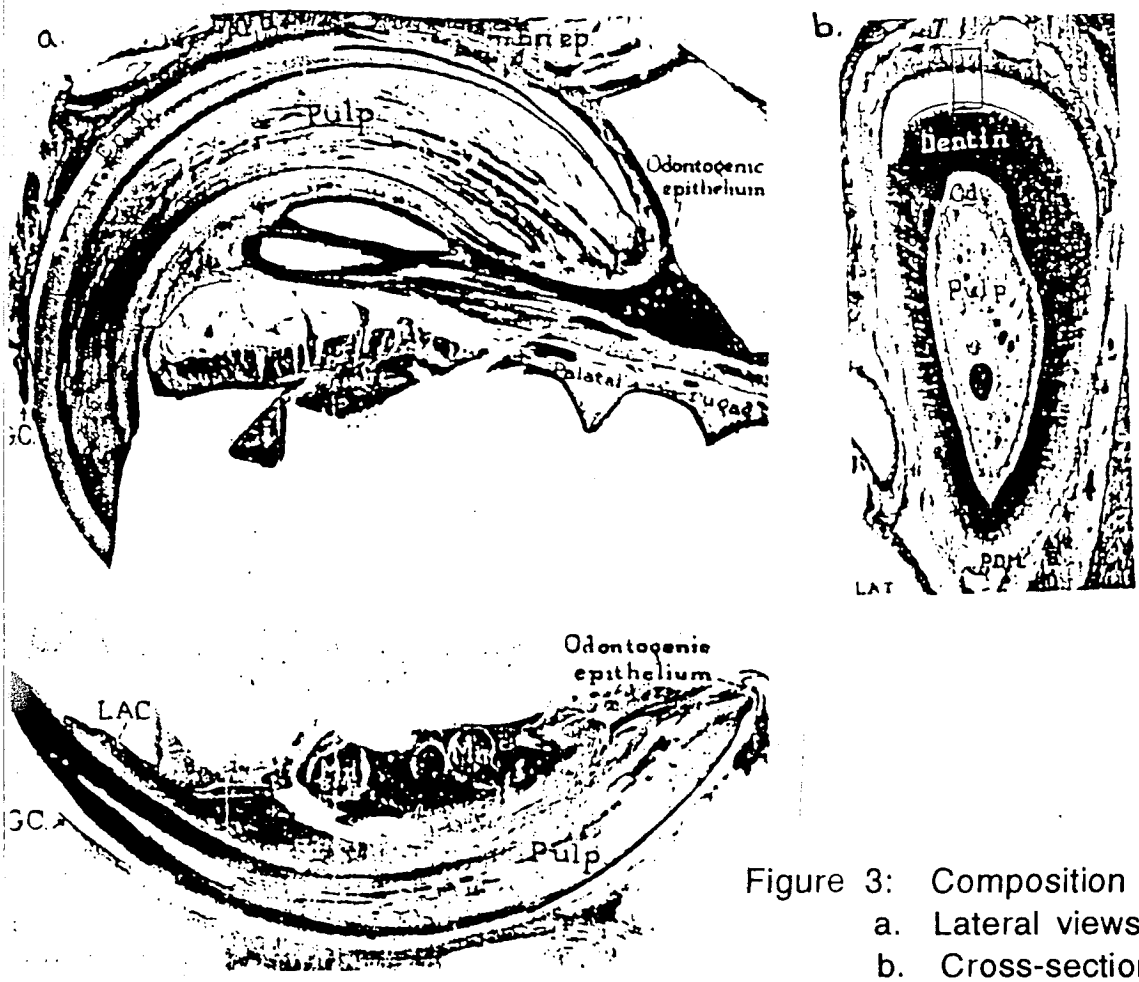

Figure 3: Composition of rat incisors
a. Lateral views
b. Cross-sectional view (enamel occupies the clear space above dentin

TABLE I: F_{max} tests

UPPER	E3*C3	E5*C5	E3*E5	C3*C5
	(4.03)	(5.82)	(4.43)	(5.82)
Dentin	2.37	1.06	1.62	3.61
Enamel	1.07	2.14	3.86	1.93
I	1.27	1.30	0.945	1.75
II	1.51	1.26	2.23	1.17
III	1.53	1.27	1.62	1.20
IV	1.50	1.75	1.05	1.12
V	1.25	3.20	1.00	4.00

LOWER	E3*C3	E5*C5	E3*E5	C3*C5
	(4.03)	(5.82)	(4.43)	(5.82)
Dentin	1.08	1.31	2.84	3.43
Enamel	2.00	2.50	1.33	1.67
I	2.09	1.92	3.47	1.15
II	1.24	1.52	1.44	2.71
III	1.22	2.06	1.64	1.52
IV	2.21	1.90	1.22	3.44
V	2.50	3.67	1.38	6.67

TABLE II: T-tests
*0.007 probability level

	E3*C3 *upper*	E3*C3 lower	E5*C5 *upper*	E5*C5 lower	E3*E5 *upper*	E3*E5 lower	C3*C5 *upper*	C3*C5 lower
Dentin	1.87 0.0781	2.44 0.0275	-0.53 0.6040	1.29 0.2200	-2.52 0.0237	*-6.03 0.0001	*-5.29 0.0001	*-5.30 0.0001
Enamel	0.82 0.4240	-0.32 0.7530	-0.99 0.3400	2.57 0.0245	-2.75 0.0147	*-9.02 0.0001	-3.72 0.0023	-3.15 0.0072
I	1.18 0.2560	0.72 0.4840	0.17 0.8680	3.70 0.0030	0.26 0.8020	*-4.25 0.0007	-0.55 0.5910	-1.92 0.0755
II	0.45 0.6580	2.05 0.0560	1.08 0.3030	1.80 0.0963	-3.84 0.0016	-3.34 0.0045	-2.67 0.0184	-2.35 0.0340
III	0.26 0.8020	0.24 0.8145	0.98 0.3490	0.57 0.5810	-2.47 0.0261	-2.08 0.0554	-1.38 0.1890	-1.43 0.1750
IV	1.44 0.1680	1.35 0.1945	1.92 0.0793	1.43 0.1790	-1.20 0.2493	-0.52 0.6140	-0.25 0.8060	0.12 0.9090
V	-1.70 0.1070	-0.12 0.9050	-0.17 0.8697	2.08 0.0595	-2.93 0.0102	-2.49 0.0252	-1.19 0.2530	-0.16 0.8730

TABLE III: Mean measurement values

UPPER	E3	E5	C3	C5
Dentin	2.77	2.98	2.66	3.04
Enamel	0.193	0.211	0.187	0.217
I	0.626	0.619	0.598	0.613
II	0.732	0.845	0.721	0.805
III	0.633	0.709	0.626	0.673
IV	0.717	0.751	0.672	0.682
V	0.084	0.099	0.092	0.100

LOWER	E3	E5	C3	C5
Dentin	1.73	1.99	1.66	1.91
Enamel	0.200	0.253	0.202	0.230
I	0.529	0.613	0.515	0.550
II	0.613	0.674	0.583	0.630
III	0.520	0.564	0.515	0.550
IV	0.611	0.621	0.589	0.587
V	0.123	0.135	0.124	0.125

Garner Lake Study: March 12- April 12 An Examination of Oxygen, Phosphorus, Chlorophyll Concentrations, Temperature, Light Intensity, and E. Coli

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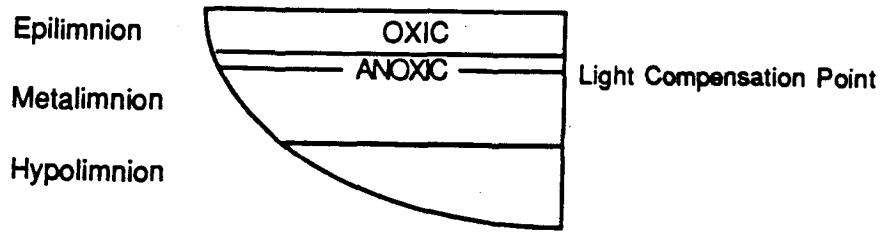
ABSTRACT Twenty-five years have passed since the development of Garner Lake began. In this time, the ecological community and especially the aquatic community have been impacted. Due to the negligence o f the developers, more than 40 lakeside acres were clear-cut and left vacant and unretained. Four sewage pump stations have been erected around the lake's perimeter, of which at least one is known to leak into the lake. Concerned residents of Garner Lake have attempted to resolve these problems, but have met with little success. Over 14 tons of phosphate fertilizer were dumped into the lake in the hope of re-establishing the clarity of the water, but no significant improvement has been noted over the last year.

The purpose of this study is to provide base-line information on Garner Lake for future reference. By observing the physical and chemical factors involved in lake systems, an understanding of the condition of lake and the external factors shaping its changing behavior can be achieved. In this study, temperature, oxygen, phosphorus, light, and chlorophyll concentrations were determined and bacterial testing was conducted. Through the interaction of these factors, insight into the characterization of Garner Lake and the various properties and behavior of the lake system can be gained.

INTRODUCTION Every lake environment demonstrates a typical pattern of behavior which can be attributed to the properties of a water molecule. Due to the structure of a water molecule, water density increases between the freezing point and four degrees Celsius. This allows for vertical mixing in the spring and fall as the temperature changes. Beginning in the early spring, the lake's surface is gradually warmed, causing the warmed surface water sinks to cooler depths as the temperature exceeds four degrees Celsius. A similar reverse process occurs in the fall as the water on the bottom of the lake approaches four degrees Celsius and then rises to the surface creating an inverted temperature profile (Ricklefs 1993). This vertical mixing allows for heat to be redistributed evenly throughout the water column. Spring and fall overturns, typically caused by winds, result in deep vertical mixing, bringing nutrients from the bottom sediments and allowing oxygen to penetrate lower depths. The end result of both processes is the summer and winter stratification, respectively.

The stratification of the lake allows it to be defined in three different regions- the epilimnion, metalimnion, and hypolimnion. In the epilimnion, the shallow surface waters, photosynthesis and gas exchange between the air and water occur, producing high oxygen concentrations. This region of a lake is set apart from the deeper regions of the lake because of its high productivity and diversity. The metalimnion receives less light than the epilimnion yet oxygen is still produced above the light compensation point. Below this level, light is no longer able to penetrate through the water. The hypolimnion is a deep, cold region formed where currents are at a minimum and oxygen has been depleted (Cole 1979). Here, carbon dioxide concentrations are high due to detritus-oxidizing bacteria depleting oxygen through respiration. Climatic changes cause these regions to be alternately mixed and stratified yearly.



Although all lakes demonstrate this general pattern of behavior, each individual lake can classified according to certain physical and chemical factors. Among the more important factors are temperature, light intensity, oxygen, phosphorus, and chlorophyll concentrations.

Gases are typically associated with the atmosphere. However, they also play an important role in aquatic systems where they exist in a dissolved state for the purpose of facilitating biological and physiological reactions (Cole 1979). One of the most important gases in an aquatic lake system is oxygen. Oxygen is a prerequisite for most forms of life and serves as an indicator for the sustainability and abundance of a habitat. Within a lake, oxygen is produced as a by-product of photosynthesis. In most lakes, phytoplankton and algae contribute to the bulk of the oxygen supply due to the tremendous amounts of chlorophyll present within these populations (Cole 1979). Since chlorophyll is essential for photosynthesis and thus the production of oxygen, a measurement of chlorophyll concentrations indicates a lake's productivity potential.

Lake production can also be measured by determining phosphorus concentrations. Phosphorus is a major cellular component of organisms, vital to the operation of energy transport systems and the production of nucleic acids, but it is often present only in small quantities in biological ecosystems. Since it is usually less available than the biological demand, it is commonly viewed as a limiting factor in the productivity of freshwater organisms. Phosphorus occurs in several forms: soluble phosphate, soluble organic, and particular organic (Reid 1961).

Natural lakes display a wide range of productivity because they rely not only on the recycling of nutrients form within the lake, but also on obtaining nutrients from outside the lake (i.e. rainfall, sewage, runoff, etc.). In nature, plants obtain phosphorus directly from the soil or water and transform it into phosphate esters. Animals excrete excess phosphorus from their systems in the form of urine, and phosphatizing bacteria complete the cycle by changing the phosphorus contained in dead organic matter to phosphate ions absorbed by plants. Phosphorus can be released from sediments, macrophytes, and algae (Wetzel 1990). This cycle occurs continuously within the lake and is referred to as internal loading.

External loading takes place when phosphorus is added via precipitation, sewage leakage, phosphate fertilizers, and agricultural runoff. Lawns immediately adjacent to the lake (within 300 ft.) are also a possible source of nutrient runoff. Fertilizer applied in the winter is not readily absorbed, and many nutrients are lost in runoff. Phosphate fertilizer applied to lakes is quickly absorbed (Henry 1992). The disappearance of fertilizer is often associated with an increase in plant production because more phosphorus is available to meet biological demand.

One particular component of external loading that concerns the human population of Garner Lake is sewage leakage and bacterial contamination. Water contaminated by human feces can have a variety of pathogens present (Hill 1993). Fecal contamination is most commonly tested by the presence of *Escherichia Coli*. *Escherichia Coli* is only found in abundance in the intestinal track of warm-blooded animal; therefore it is assumed that water without *E. Coli* does not have fecal contamination and no fecal pathogens are present (Gainly and Lord 1952).

MATERIALS AND METHODS

Field Techniques:

Standard techniques were used to insure accuracy and precision. Before each trip, 45 300-ml. BOD bottles were rinsed with 1 ml. of concentrated HCl. BOD bottles were then labeled and organized according to depth and how the samples were to be tested. A 10-m rope was attached to the Van-Dorn water sampler and labeled in one meter increments. A 1-m rope was attached to the secchi disk and labeled in one centimeter increments. Likewise, the LI-COR 185B light probe was attached to a cord labeled in ten centimeter increments.

The samples were obtained form approximately 40 ft. east of the spillway at 2-m intervals from the surface to a bottom depth of 10 meters. Three BOD bottles were filled at each depth for chlorophyll, two for phosphorus, and two for oxygen. The oxygen samples were fixed with 1 ml. of $MnSO_4$ and 1 ml. of NaOH + KI and mixed thoroughly. Then 1 ml of concentrated H_2SO_4 was added to the bottles. Water temperature was taken at 2-m intervals. Light intensity was measured with the light probe, and read at every ten centimeters. Turbidity was measured with the secchi

disk. E. Coli was tested at five sites near known pump stations (see map of Garner Lake). Samples were collected in sterile bottles. All samples were placed in an ice chest for transport back to lab, and the bottle numbers and depths were logged in a journal.

LAB TECHNIQUES:

Oxygen

100 ml. of each of the fixed water samples were transferred with volumetric pipette to a 250-ml Erlenmeyer flask. The sample was then titrated with 0.005M standardized sodium thiosulfate solution until a pale "straw" color was observed. Stabilized starch solution was added until the solution turned a uniform blue color. The sample was then titrated rapidly to a colorless end point with the 0.005M sodium thiosulfate solution. The oxygen concentration was calculated as follows:

$$mg02/l = \frac{(\text{ml titrant})(\text{molarity of thiosulfate})(8000)}{(\text{ml sample titrated})\ (\text{ml of bottle} - 2/\text{ml of bottle})}$$

Chlorophyll

900 ml. of water from each depth was filter using a vacuum pump and a glass fiber filter. The filter paper was then ground into a fine paste with acetone and a tissue grinder. The mixture was then centrifuged for approximately fifteen minutes. Each centrifuge tube was leveled off to 5 ml. with additional acetone.

One clean cuvette was filled with acetone and placed in the Gilford 2600 spectrophotometer to serve as a blank. Acetone from the centrifuge tube was decanted in to a second cuvette and placed in the spectrophotometer. The absorbance of the chlorophyll solution was read at the following wavelengths: 772, 665, 663, 645, and 630nm. The spectrophotometer was zeroed on the blank for each wavelength. After the absorbencies were taken, one drop of 4N HCl was added to the cuvette. The solution was thoroughly mixed and read for absorbance at 750, 665, and 663nm. The cuvettes and tissue grinder were rinsed with acetone between samples.

Soluble Phosphorus

The samples were kept cool until the analysis was conducted always within one hour of sample collection.

Materials:

Six solution were necessary for the testing of soluble and total phosphorus. They were as follows:

1) Ammonium molybdate solution--15 g of ammonium paramolybdate were dissolved in 500 ml. of distilled water. Stored in an amber polyethylene bottle.

2) Concentrated sulfuric acid--140 ml. of acid were added to 900 ml. of distilled water. Stored in a glass-stoppered bottle.

3) Ascorbic acid-- 27 g of L-ascorbic acid were dissolved in 500 ml. of distilled water. Made fresh daily. Unstable compound.

4) Potassium antimonyl-tartrate-- 0.34 g of potassium antimonyl-tartrate were dissolved in 250 ml. of distilled water. Heat needed. Stored in glass bottle.

5) Composite reagent-- 100 ml. ammonium molybdate, 250 ml. sulfuric acid, 100 ml. ascorbic acid, and 50 ml. potassium antimonyl-tartrate solution. Made daily.

6) Phosphate standard solution-- 0.2197 g of oven-dried potassium dihydrogen phosphate was dissolved in distilled water. Diluted to one liter. Stored in dark bottle with 1 ml. of chloroform. Diluted to make appropriate standards using the equality of 1ml = 50 ug of phosphate ions.

Methods:

Two 100 ml. samples from each depth were transferred to Erlenmeyer flasks and heated to

between 15 and 30 degrees Celsius. Then 10 ml. of composite reagent were added to the solution while stirring. Then samples sat for a minimum of ten minutes and a maximum of two hours while the reaction took place. Then the absorbencies were read taking care to zero the spectrophotometer as before only this time using water as the blank. All measurements were read at 885 nm. Then a sample of the lake water was measured without the composite reagent to determine the turbidity of the water. The measured amount of the samples minus the difference of the two cuvettes and the turbidity factor represented the soluble phosphorus concentrations.

Total Phosphorus
Materials:
1-7) same as for soluble
8) Potassium persulfate solution-- 5% w/v potassium persulfate dissolved in distilled water. Made daily.

Methods:
First the 100 ml. samples (taken from the same bottles as the soluble samples were placed in 250-ml Erlenmeyer flasks with 16 ml. of the 5% potassium persulfate solution, and then they were autoclaved for 30 minutes at 121 lbs of pressure. After cooling, the volume of each sample was adjusted to 120 ml. using a 150 ml. graduated cylinder. The liberated phosphate ions were then analyzed using the methods for soluble phosphorus.

E. Coli
Materials and Methods:
Using a sterile 1-ml pipette, 0.1 ml. of lake water was added to three single strength lactose broths (SSLB) and 1 ml. to three other SSLB --fifteen tubes. Using a sterile 10-ml pipette, 10 ml. of a lake water sample were added to three double strength lactose broths (DSLB) --fifteen tubes. A fresh sterile pipette was used to transport each from sample from the different water sites. The broths were then incubated at 37 degrees Celsius for 24-48 hours.

Broths that produced at least 10% gas in the Durham tube (type positive) at 24 hrs, were then streaked onto a plate of EMB agar and incubated at 37 degrees Celsius for 24 hrs--12 EMB plates. After 48 hrs, the number of broths that were type positive was determined and recorded. From these data the most probable number (MPN) of coliforms was determined. When the EMB plates matured they were inspected for colonies having a dark center and especially those with a greenish metallic sheen. From one such colony, both a SSLB and a tryptic soy agar (TSA) slant were inoculated--15 SSLB and 12 TSA. This was performed for each EMB from each of the five sights. After 24 hrs, the SSLB broths were again observed for gas production. If no gas was produced, the testing ended. If it was, the appropriate slant was used to inoculate the SIM agar deep and the Simmon's Citrate slant--12 of each. After 48 hours, a thin layer of Kovac's reagent was added to the SIM agar samples and observed for a color change of the reagent. If it turned red, then the culture tested positive for indole. Likewise, the Simmon's slants were observed for a change from green to blue which meant the culture was positive. Those that tested positive for the indole and negative for the Simmon's slants were assumed to be *E. Coli.* However, the samples were still run on a Gram negative stain to confirm the results.

RESULTS AND DISCUSSION
Oxygen and Temperature
Garner lake demonstrated the typical behavior of temperature and oxygen. Early measurements of water temperature were almost vertically homogeneous. Since the results were obtained after the winter thaw, the shift away from the inverted temperature profile was expected (Figure 3). By March 19 (Day 7), a premature stratification of temperature was seen. This was disrupted by a strong cold front that arrived after March 26 (Day 14). This front brought cold temperatures and high winds that mixed colder surface water deeper. This mixing allowed oxygen to penetrate to lower depths (Figure 4).

Chlorophyll, Light, and Secchi Disk

"Estimating the concentration of chlorophyll-a remains the most common method for assessing the phytoplankton biomass..."(Wetzel In Axler and Owen 1994). Figure 7 shows the extinction coefficient on each of the six days that samples were taken. The extinction coefficient shows the depletion of light as it passes through the water to lower depths. Extremely productive, turbid waters usually have a low extinction absorption (0-4), indicating that the longer wavelengths required by chlorophyll-a for photosynthesis are not transmitted very far in depth (Cole 1979). Figure 7 shows the highest absorbance coefficient on 3/15. This can be viewed in conjunction with the high chlorophyll-a concentrations on the same day (Figure 6). In a study done on Reelfoot Lake in western Tennessee, a similar trend was observed. Chlorophyll-a concentrations of 36 ug/L (Garner 15-30 ug/L) were recorded and an overabundance of micro-organisms was found. These micro-organisms severely reduce the water quality and the amount of light that can reach certain depths (McCullough et. al. 1985).

Garner Lake also demonstrated secchi measurements (0.48m) comparable to those taken at Reelfoot Lake (0.3m). According to the USEPA methods of trophic-state classification, Garner Lake can be classified as eutrophic (Lind 1993). Eutrophic lakes are very fertile usually due to the external loading and recycling of nutrients from sediments (Kortman and Henry 1990). At Garner Lake, high external loading may be a result of the erosion of the vacant clear-cut plot as well as other areas without seawalls.

Phosphorus

The phosphorus measurements further verified the eutrophic lake classification. Soluble phosphorus ranged from 200-250 ug/L and the total phosphorus was much higher with a maximum of 2000 ug/L. According to Lind et al. (1993), eutrophic lakes have a total phosphorus concentrations above 20 ug/L. The addition of 8 tons of phosphate fertilizer a few years ago no doubt has increased internal phosphorus cycling.

The ratio of particulate phosphorus to soluble phosphorus was much higher on April 12 (Day 32). Oxygen concentrations were also high, and ferric ions should have been present to combine with soluble phosphorus (Reid 1961). Soluble phosphorus decreased over the short time in which the lake was tested and the temperature began to increase. Consumption of phosphorus by phytoplankton, and sedimentation of inorganic complexes probably resulted in this trend (Wetzel 1975).

When averaged over all dates, particulate phosphorus was the highest between four and six meters below the surface (Figure 2). There was no temperature stratification or discontinuity at four or six meter to explain these data. Unlike particulate phosphorus, we saw no pattern of soluble phosphorus with depth.

E. Coli and Coliform Testing

The presumptive portion of the coliform test determines gas production from lactose. The formation of gas within 24-48 hrs is sufficient evidence of the presence of coliforms. The three lactose tubes, received a different volume of water to be tested. This was done in order to be able to gauge the degree of contamination in the lake water. Results from the test tubes that tested positive and what volumes were used to inoculate each made it possible to infer approximately how many coliforms were present (Table 2).

Table 2. Most Probable Number of Colonies from Presumptive Coliform Test

	Number of Tubes Positive				95% Confidence Limits	
SITE	10ml	1.0ml	0.1ml	MPN/100ml	lower	upper
1	3	3	0	240	36	1300
2	3	3	3	>1100	150	4800
3	3	3	0	240	36	1300
4	3	3	1	460	71	2400
5	3	3	1	460	71	2400

According to the Joint Committee of the Conference of State Sanitary Engineers and the Engineering Section of the American Health Association, natural surface waters should be within the "minimum limits of coliform indices of not more than 240 to 500 per 100 ml. "(Abel Wolman 1969). Compared to these limits, all sites samples April 12 at Garner Lake exceeded the recommended minimum, especially site 2. A coliform test was also performed on April 21 by the Environmental Testing and Consulting Inc. ETC tested samples from the same sites by filtering water samples through a membrane. The membrane was then grown in a medium and inspected for green colonies. Results from this test were much lower, yet neither data should be disregarded (Table 3). The life span of coliforms is approximately a week; therefore variances could be due to the amount of coliform input from fecal contamination.

Table 3. Total Coliform Count

Site	Coliform/100m
1	4
2	11
3	10
4	8
5	5

The confirmative portion of the test employed several specialized selective and differential media to verify the presence of E. Coli in the water. Sites one and two were confirmed to be fecally contaminated by the final results of this test (Table 3).

Table 3. Results of IMViC Tests for *E. coli* Confirmation

Test:	I	M	V	C
E. coli	+	+	-	-
Site: 1	+	+	-	-
2	+	+	-	-
3	-	-	+	+
4*				
5	-	+	-	+

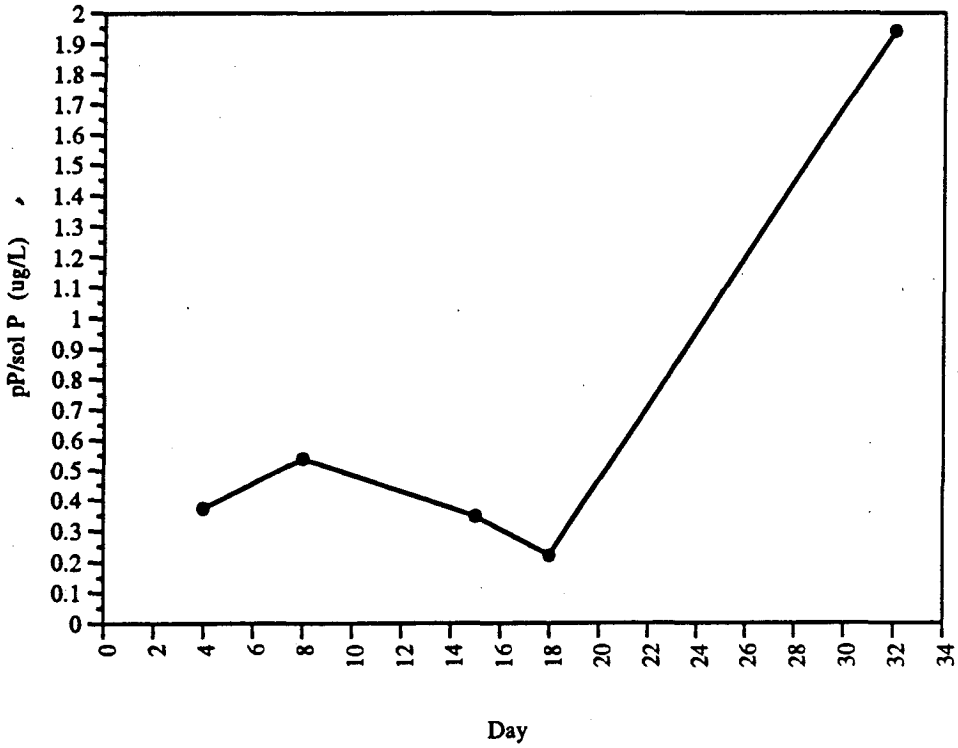
* sample failed earlier in presumptive testing

The results of coliform and E. Coli testing showed that Garner Lake was fecally contaminated on April 5, 1994 when samples were taken. Only one confirmed siting of sewage leakage has occurred around site 2 on a previous date. This raises questions of why and how often these two sites are being contaminated.

CONCLUDING STATEMENTS

Since the development of Garner Lake, many changes have occurred within the lake system. The purpose of this study was to try to understand these changes by observing the lake from beneath the surface. Various physical and chemical factors interact in the typical cycling of a lake, yet outside disturbances can disrupt the community. The changes that have occurred at Garner Lake cannot be eliminated overnight, yet through monitoring and other measures, further disruptions to the lake system can be prevented. The Garner community can use low phosphorus fertilizers on areas within 300 ft of the lake and try not to over-fertilize other areas to prevent an excess of nutrient runoff into the lake. The enforcement of seawalls and stricter enforcement of sewage contamination regulations will inhibit further pollution and possible biohazard contamination. Through continuous monitoring the lake can be better understood, and a strategy to maintain the lake properly can be formulated.

Figure 1. Relationship of Particulate Phosphorus to Soluble Phosphorus



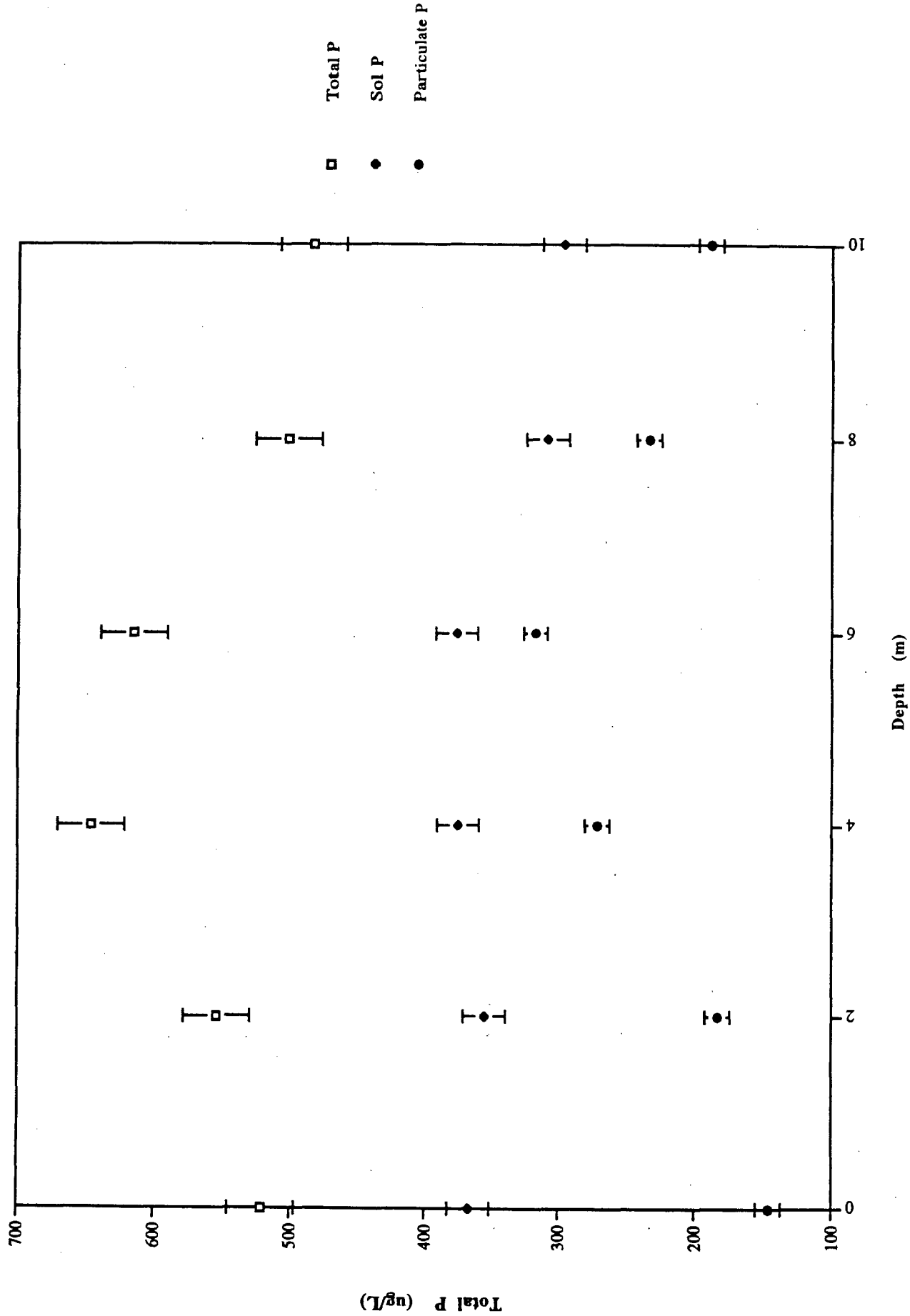


Figure 2. Phosphorus Average Per Depth

Figure 3. Temperature Profile of Garner Lake
March 12 - 26, 1994

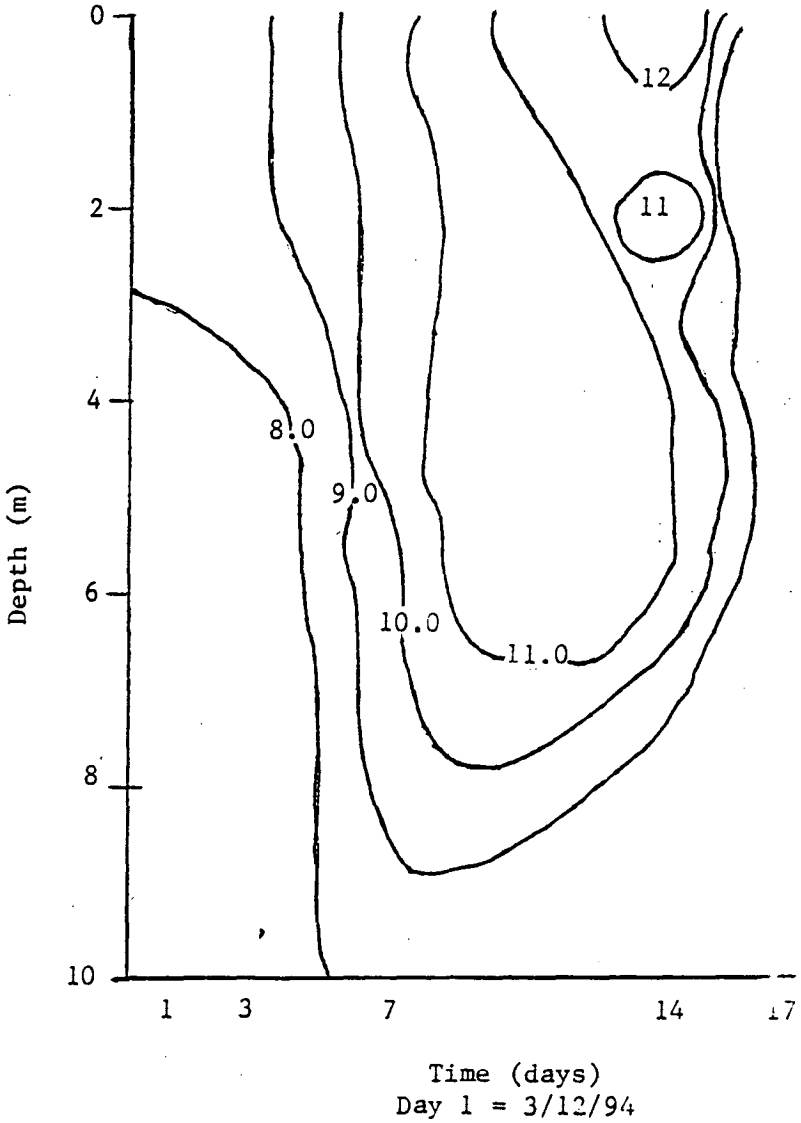


Figure 4. Oxygen Profile of Garner Lake
March 15 - April 12,1994

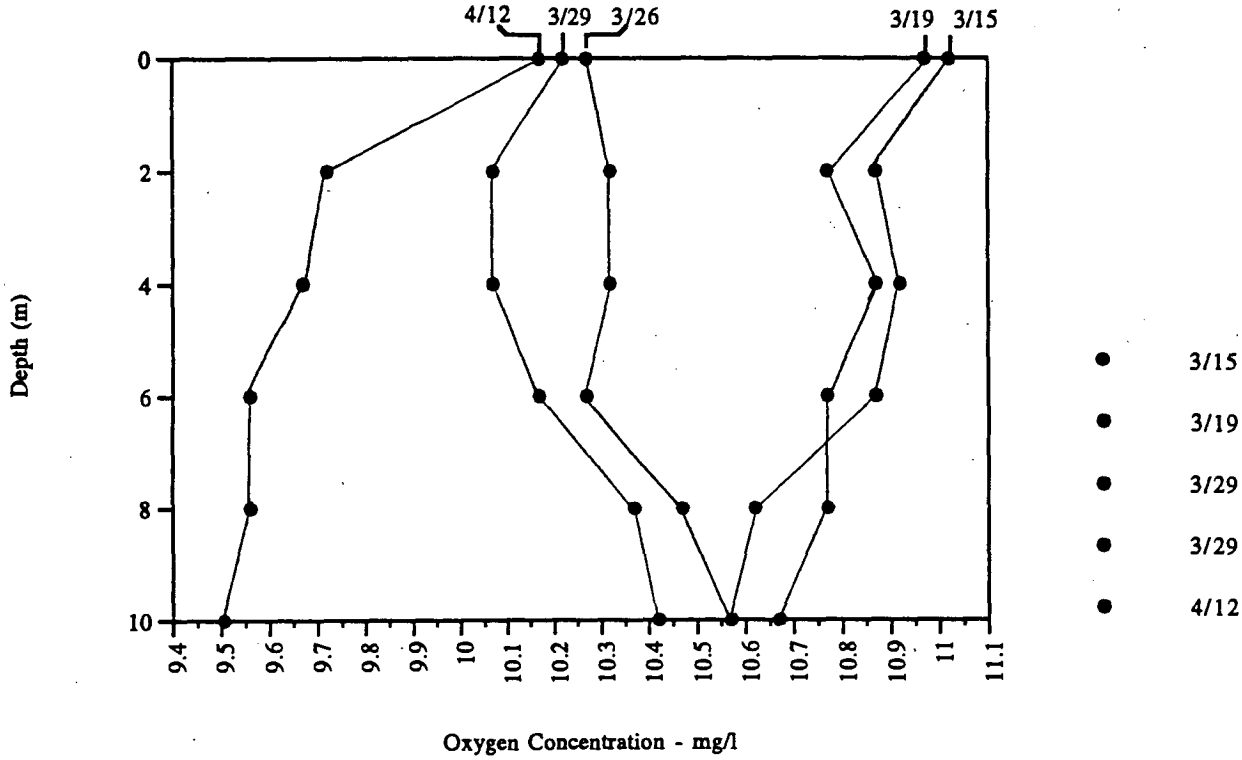
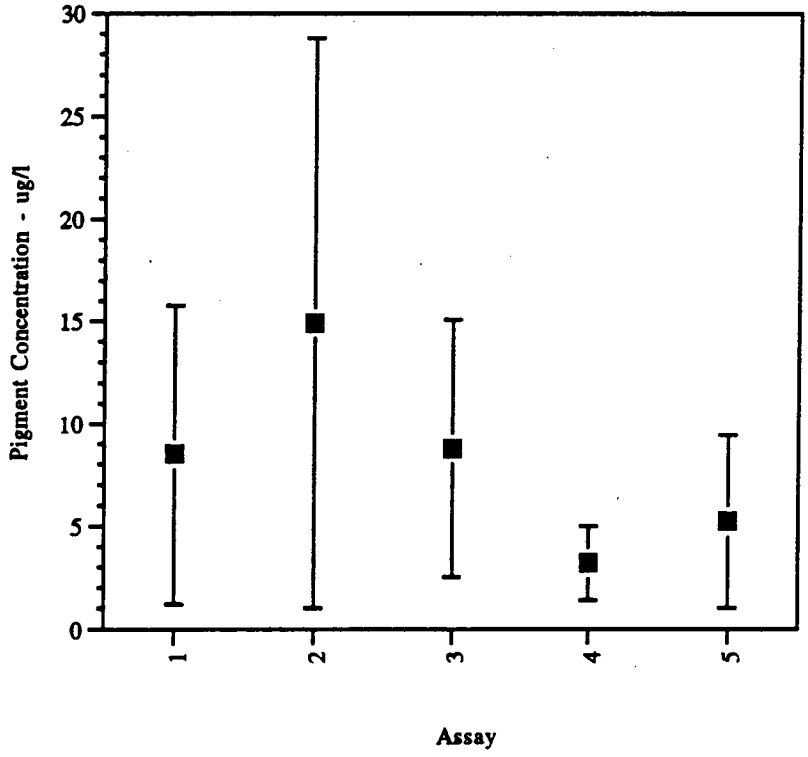
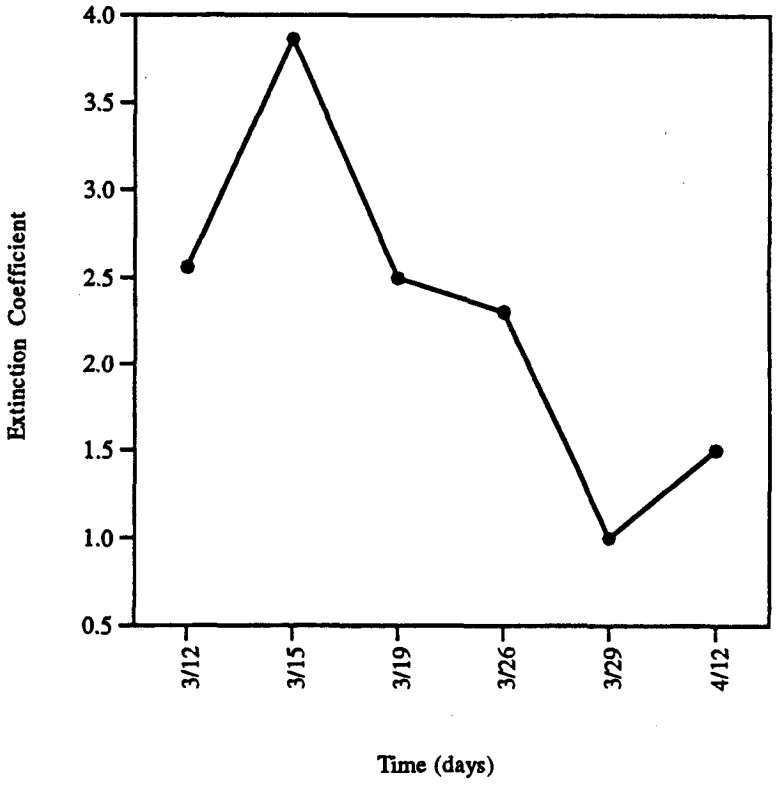


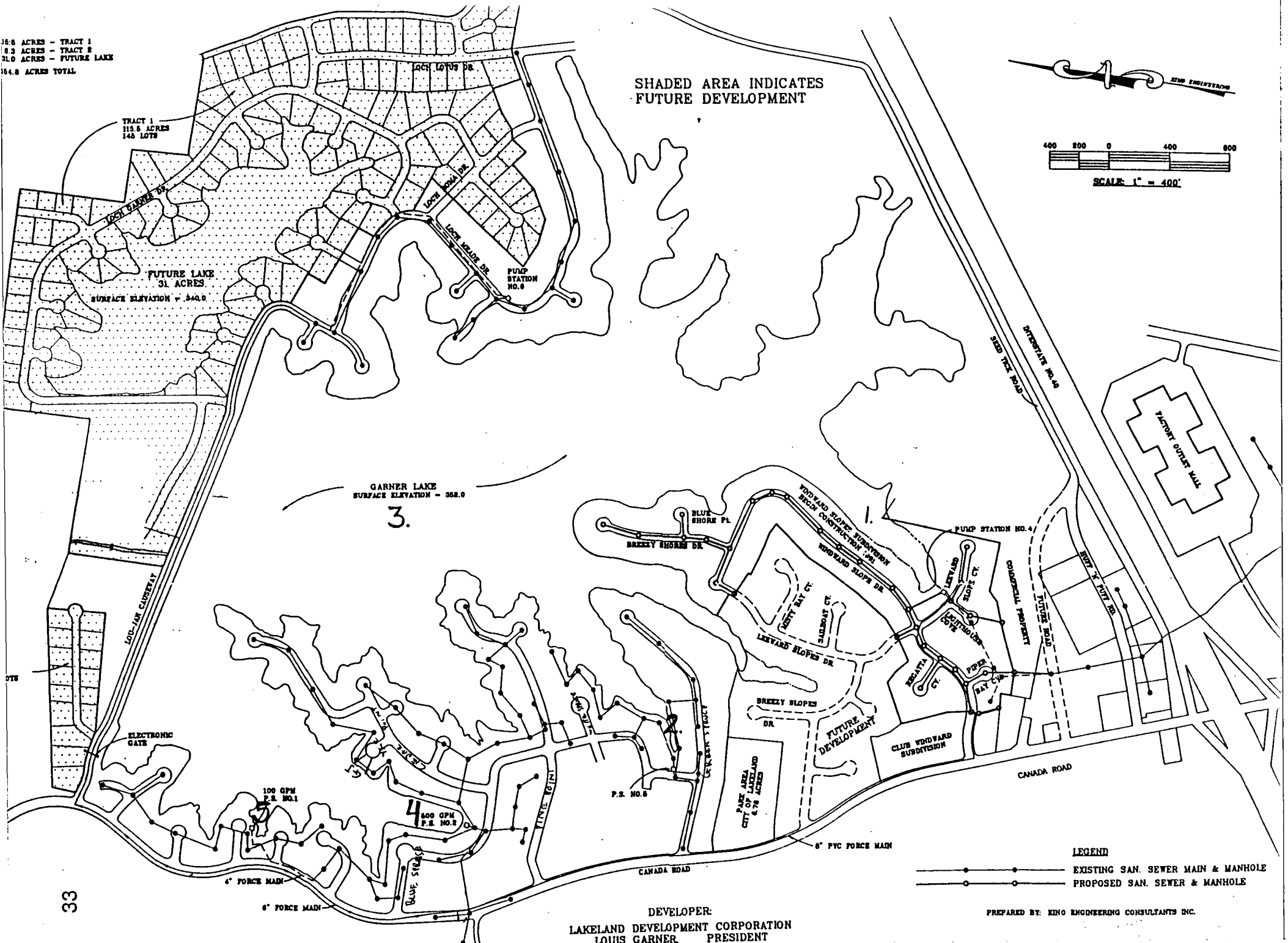
Figure 5. Chlorophyll a Concentration of Garner Lake



Assay 1 3/15/94
Assay 2 3/19/94
Assay 3 3/26/94
Assay 4 3/29/94
Assay 5 4/12/94

Figure 6. Extinction Coefficient




16.6 ACRES - TRACT 1
8.3 ACRES - TRACT 2
31.0 ACRES - FUTURE LAKE
164.8 ACRES TOTAL
SHADED AREA INDICATES
FUTURE DEVELOPMENT
KING ENGINEERING
400 200 0 400 800
SCALE: 1" = 400'
TRACT 1
115.6 ACRES
148 LOTS
LOCH LOTUS DR.
LOCH GARNER DR.
LOCH IONA DR.
LOCH ARKAIG DR.
PUMP STATION NO.6
FUTURE LAKE
31 ACRES
SURFACE ELEVATION = 340.0
GARNER LAKE
SURFACE ELEVATION = 358.0
3.
1.
2.
4
5
LOG-JAM CAUSEWAY
ELECTRONIC GATE
100 GPM P.S. NO.1
600 GPM P.S. NO.2
P.S. NO.6
BLUE SHORE PL.
BREEZY SHORES DR.
WINDWARD SLOPES SUBDIVISION
BEGIN CONSTRUCTION 1981
WINDWARD SLOPE DR.
LEEWARD SLOPES DR.
MISTY BAY CT.
SAILBOAT CT.
LEEWARD SLOPES CT.
LOCATING LOOP
PUMP STATION NO.4
COMMERCIAL PROPERTY
FUTURE ROAD
BUTT 'N' PUTT RD.
SEED TECK ROAD
INTERSTATE NO.49
FACTORY OUTLET MALL
REGATTA CT.
PUDER BAY CIR.
BREEZY SLOPES DR.
FUTURE DEVELOPMENT
CLUB WINDWARD SUBDIVISION
PARK AREA CITY OF LAKELAND 4.78 ACRES
CANADA ROAD
8" PVC FORCE MAIN
4" FORCE MAIN
6" FORCE MAIN
BLUE SHORE
PINE POINT
LEGEND
EXISTING SAN. SEWER MAIN & MANHOLE
PROPOSED SAN. SEWER & MANHOLE
PREPARED BY: KING ENGINEERING CONSULTANTS INC.
DEVELOPER:
LAKELAND DEVELOPMENT CORPORATION
LOUIS GARNER PRESIDENT
33

Bibliography

Axler, R. P. and C. J. Owen. 1994. Measuring Chlorophyll and Phaeophytin: Whom Should you believe? Lake and Reserv. Manage. 8(2): 143-151.

Cole, G. A. 1979 The Textbook of Limnology. 2nd ed. C. V. Mosby Co., St. Louis, 11, 231-240.

Gainey, P. L. and T. H. Lord. 1952. Microbiology of Water and Sewage. Prentice-Hall, Inc., New York, 183-201.

Hill, T. W. 1993. Microbe Public Health Handout.

Hutchinson, G. E. 1957. A Treatise on Limnology. John Wiley and Sons, Inc., New York, 735-40.

Kortman, R. W. and D. D. Henry. 1990. Mirrors of the Landscape. Conn. Inst. of Water Resources. 70-71.

Lind, O. T., T. T. Terrell, and B. L. Kimmel. 1993. Problems in Reservoir Trophic-state Classification and Implications for Reservoir Management. Comp. Reserv. Lim. and Water Quality Manage. 57-67.

McCullough, J. D., T. E. Venneman, A. A. Hartung, and D. M. Wedding. 1985. Occurrence of the Rotifer Trochosphaera solstitialis (Thorpe 1893) in Reelfoot Lake, Tennessee. Journal of the Tenn. Acad. of Sci.60(1):20-21.

Reid, G. K. 1961. Ecology of Inland Waters and Estuaries. Reinhold Publishing Corp., New York, 187-192.

Ricklefs, R. E. 1993. The Economy of Nature. 3rd ed. W. H. Freeman, New York, 70-73.

Ruttner, F. 1970. Fundamentals of Limnology. University of Toronto Press, Toronto, 89-91.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Company, Philadelphia, 743 pp.

Wolman, A. 1969. Water, Health and Society. Indiana University Press, Bloomington and London, 113-122.

AN INVESTIGATION OF THE STEADY-STATE LIMITING CURRENTS IN SOLUTIONS WITH AND WITHOUT SUPPORTING ELECTROLYTE USING MICROELECTRODES

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ABSTRACT

The ratio of limiting currents obtained from various concentrations of solutions of $Ru(bpy)_3(PF_6)_2$ and $Ru(phen)_3(PF_6)_2$ with and without electrolyte were compared to values predicted Cooper, Bond, and Oldham (*J. Electroanal. Chem.*, *331* (**1992**) 877-895). The results obtained support the predictions of Cooper, Bond, and Oldham and contradict the experimental results obtained by these authors.

INTRODUCTION

The use of cyclic voltammetry with microelectrodes, or electrodes with the smallest dimension less than or equal to about 25 micrometers, allows the probing of highly resistive, low electrolyte solutions. The use of a small radius electrode allows the IR drop, which distorts the signal at larger electrodes, to be sufficiently small so that electrochemistry in low electrolyte solutions can be performed.

In cyclic voltammetry, the potential adopted by the working electrode is varied at a constant rate to a certain potential (E) limit. Then, a reverse sweep returns the electrode at a constant rate to the original starting potential, constituting one cycle. The current (i) which flows is measured as a function of the applied potential.

The use of a microelectrode allows a special condition called a steady-state to arise. Under steady-state conditions, the relationship between E and i involves neither time nor frequency. A steady-state arises when the profile of redox-active species being presented to the electrode does not change over time. Such homogeneity can only arise when all dimensions of the electrode are very small (on the scale of microns). Consequently, microelectrodes are a very useful tool for a variety of applications of voltammetry.

The foundation for our research project was an article written by J.B. Cooper, A.M. Bond and K.B. Oldham (1). Cooper et.al. were interested in producing a model for predicting current values obtained in solutions of very low levels of electrolyte. In their article, they derive equation (1), which enables the calculation of a limiting current ratio (I_l/I_d). The following equation (1),

$$\frac{I_l}{I_d} = [Z+1]\left[1 + \frac{Zz}{Z-z}\ln\left[\frac{z[Z+1]}{Z[z+1]}\right]\right] \qquad (1)$$

allows the direct calculation of the current ratio (I_l/I_d), given only the charge of the reactant (Z) and the charge of the product (z). The migratory current term (I_l) arises in low or no electrolyte solutions, in which migration of the electroactive species toward, or away from, the electrode makes a significant contribution to the overall limiting current. The diffusive current term (I_d) is relevant in solutions containing a large concentration of electrolyte. When the ionic concentration is large, the fraction of the redox species migrating to the electrode surface is small, so transport occurs primarily by diffusion. In the experimental testing of this equation, however, Cooper et. al. tested the validity of this equation and found that several of the test compounds did not agree with the predicted values for the ratio.

The one electron oxidation of $Ru(bpy)_3(PF_6)_2$ was predicted to have a ratio of 0.88 by

equation (1). However, in the experimental trials, Cooper et.al. obtained a ratio of 0.65. However, the one electron oxidation of $Ru(phen)_3(PF_6)_2$ followed the predicted value of 0.88 in their experiments. The authors attributed the discrepancy with the $Ru(bpy)_3(PF_6)_2$ compound to an increased electric field surrounding the electrode surface, causing a greater migration away from the electrode. This greater electric field arose from the more positive potential required to oxidize the $Ru(bpy)_3(PF_6)_2$. This led to a greater double-layer effect in proportion to that of the depletion region, effectively decreasing the current in a more highly resistive, low electrolyte solution.

The electric double-layer refers to a region at or near the electrode surface where the layer of charge on the electrode surface is directly opposed and balanced by the oppositely charged layer of ionic species. The large potential drop across the double-layer leads to a migratory current away from the electrode surface. Usually, the current contribution of the double-layer is miniscule compared to that of the depletion region. The depletion region refers to the distance from the electrode surface into solution which the electrode can sample, given its steady-state condition. The length of the depletion region is given by ten times the radius of the electrode used; the length of the double-layer depends upon the redox species and the solvent used, and is somewhat less than 100Å under our experimental conditions.

It was predicted that the discrepancy in the actual I_l/I_d ratios for the 0.8 mM $Ru(bpy)_3(PF_6)_2$ compound and the 3.2 mM $Ru(phen)_3(PF_6)_2$ compounds was due not to a greater double-layer effect in the $Ru(bpy)_3(PF_6)_2$ compound, but to the different concentration of the redox species used in the experiments of Cooper et. al.

MATERIALS AND METHODS

Reagents

The original stock of $Ru(phen)_3(PF_6)_2$ was synthesized via a metathesis reaction from $Ru(phen)_3(Cl)_2$ (Aldrich) and NH_4PF_6 (Aldrich). The product was then recrystallized from hot ethanol in a low yield (<15%). The yield was improved to around 90% in a subsequent metathesis and recrystallization by dissolving the crude product in a minimum of acetone and adding diethyl ether dropwise until precipitation of the compound was complete. This recrystallization procedure was used to purify the crude $Ru(bpy)_3(PF_6)_2$ solution produced in a metathesis reaction of $Ru(bpy)_3(Cl)_2$ (Aldrich) and NH_4PF_6 (Aldrich, recrystallized). Acetonitrile (Burdick and Jackson Distilled in Glass) was dried over 4Å molecular sieves. Tetra-n-butylammonium hexafluorophosphate (TBAH, GF Smith, recrystallized three times) was used as supporting electrolyte. All other reagents used were of at least reagent grade quality.

Electrochemical Measurements

Platinum disk microelectrodes of various sized diameters (6, 10, and 25 microns) were used. The construction of these electrodes is detailed elsewhere (2). Measurements were made using conventional three-compartment cells separated by medium porosity sintered glass disks. Measurements were taken after placing the cell in a Faraday cage. The solutions were degassed by bubbling a steady stream of prepurified nitrogen through the solution for 20 minutes and keeping the solution under a blanket of nitrogen for the duration of the experiment. All potentials were measured versus a sodium saturated calomel electrode (SSCE) without regard to the liquid junction potential. Voltammetric measurements were taken on either a Princeton Applied Research 273 or a Bioanalytical Systems CV-27 with a PA-1 low current module. The recording devices were the PAR LY 1400 and the Soltec VP-6432S x-y recorder respectively. At least four replicate scans were taken for each solution.

RESULTS AND DISCUSSION

Figures 1-4 show representative cyclic voltammograms for both the $Ru(phen)_3(PF_6)_2$ and $Ru(bpy)_3(PF_6)_2$ compounds with and without supporting electrolyte.

Figure 1 shows a typical voltammogram of 0.78mM $Ru(phen)_3(PF_6)_2$ in acetonitrile (MeCN). From the five measured limiting currents (I_l) obtained for this compound, the mean I_l = 1.96±0.02nA. A typical voltammogram from a 0.81mM $Ru(phen)_3(PF_6)_2$ and 0.1001M TBAH in MeCN solution is pictured in Figure 2. Its mean limiting current was found to be 2.24±0.01nA. After normalization to concentration and propagation of error, the ratio of currents obtained from these two solutions is $0.89_9 \pm 0.02_0$, which agrees with the value of 0.88 obtained from equation 1.

Figure 3 represents a voltammogram of 0.77mM solution of $Ru(bpy)_3(PF_6)_2$ in MeCN. It was later determined that the offset present at 0.00V was due to an impurity of the above Ru compounds present in the frits of the electrochemical cells. A mean limiting current of 2.02±0.01nA was calculated from the five scans taken. A typical voltammogram of a 0.78mM $Ru(bpy)_3(PF_6)_2$ solution in 0.1019M TBAH in MeCN is given in Figure 4. The mean limiting current for the 0.78mM solution was 2.45±0.02nA. After normalization to concentration and propagation of error, the ratio of currents calculated for these two solutions was $0.83_5 \pm 0.01_5$. As shown in Table 1, this value agrees within uncertainty with the 0.88 value given by equation 1.

As stated earlier, Cooper et.al. explained the 0.65 ratio for the 0.8mM $Ru(bpy)_3(PF_6)_2$ by citing a greater double-layer effect in the $Ru(bpy)_3(PF_6)_2$ compound. We decided to explore the possibility of a double-layer effect by designing an experiment to test whether or not shrinking the depletion region in proportion to the double-layer would have an appreciable effect on the limiting current obtained. A smaller depletion region near the surface of the electrode would cause both a greater contribution of the double-layer to the overall limiting current, and a reduction in the overall limiting current obtained. This effect is most pronounced in solutions containing low levels of supporting electrolyte, causing an overall decrease in the I_l/I_d ratio. A convenient way to make the depletion region smaller is to shrink the radius of the microelectrode used.

To investigate the double-layer effect, 0.8mM solutions of $Ru(bpy)_3(PF_6)_2$ and $Ru(phen)_3(PF_6)_2$ were made with and without electrolyte, and voltammograms were taken for each solution with microelectrodes of diameters 6, 10, and 25 micrometers. When the resulting data were analyzed, the I_l/I_d ratio fluctuated about the values given in Table 1 within the given uncertainty, and were statistically the same value, 0.81±0.04 and 0.89±0.04, for $Ru(bpy)_3(PF_6)_2$ and $Ru(phen)_3(PF_6)_2$ respectively. Thus, a case cannot be made for an effect of the double-layer on the overall I_l/I_d ratio.

It was decided to pursue the possibility that a lower I_l/I_d ratio occurred at lower concentrations of redox species. Table 1 summarizes all data obtained for I_l/I_d ratios of the $Ru(phen)_3(PF_6)_2$ and $Ru(bpy)_3(PF_6)_2$ compounds. As shown in the table, no trend is seen, as all values for both compounds agree with the value of 0.88 predicted by equation 1. Thus, the initial attempts to replicate the Cooper et. al. experiment with 0.8mM $Ru(bpy)_3(PF_6)_2$ were unsuccessful. Moreover, the results obtained support equation (1). Investigation of concentrations above and below the 0.8mM $Ru(bpy)_3(PF_6)_2$ solution showed no statistical difference between any values. The hypothesis that the Cooper et. al. result of 0.65 was due to the concentration of the counter ion was not supported by the data in Table 1.

REFERENCES

1. J.B. Cooper, A.M. Bond, K.B. Oldham, *J. Electroanal. Chem.*, *331* (**1992**) 877-895.
2. R.T. Robertson, B.D. Pendley, *J. Electroanal. Chem.*, *374* (**1994**) 173-177.

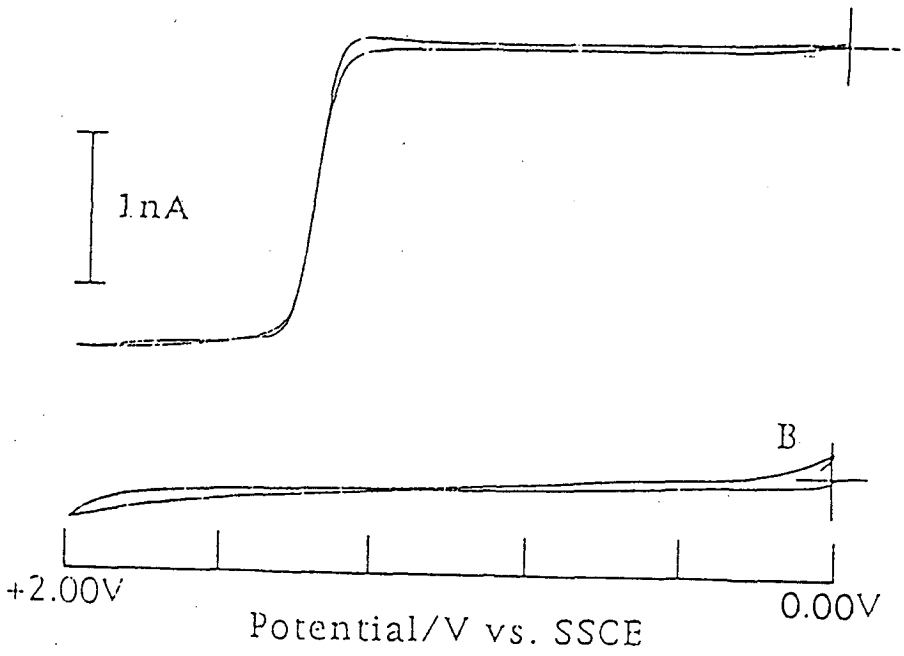

Figure 1: (A) Cyclic voltammogram using a 10mm diameter platinum microelectrode in a 0.78mM $Ru(phen)_3(PF_6)_2$ solution in MeCN. The scan rate was 25 mV/s. (B) Blank

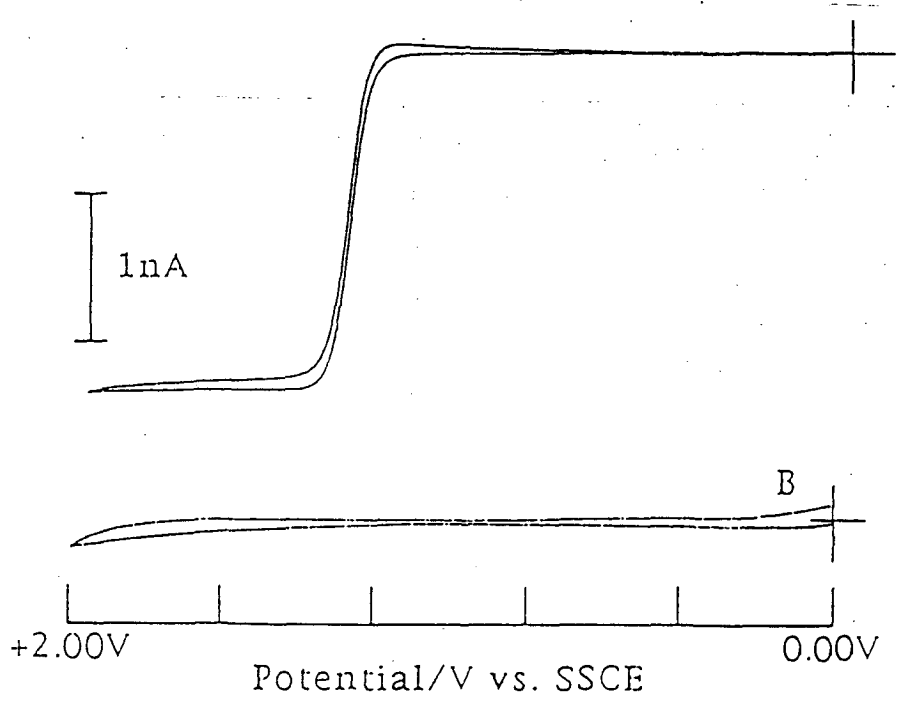

Figure 2: (A) Cyclic voltammogram using a 10mm diameter platinum microelectrode in a 0.81mM $Ru(phen)_3(PF_6)_2$ and 0.1001M TBAH solution in MeCN. The scan rate was 25mV/s. (B) Blank

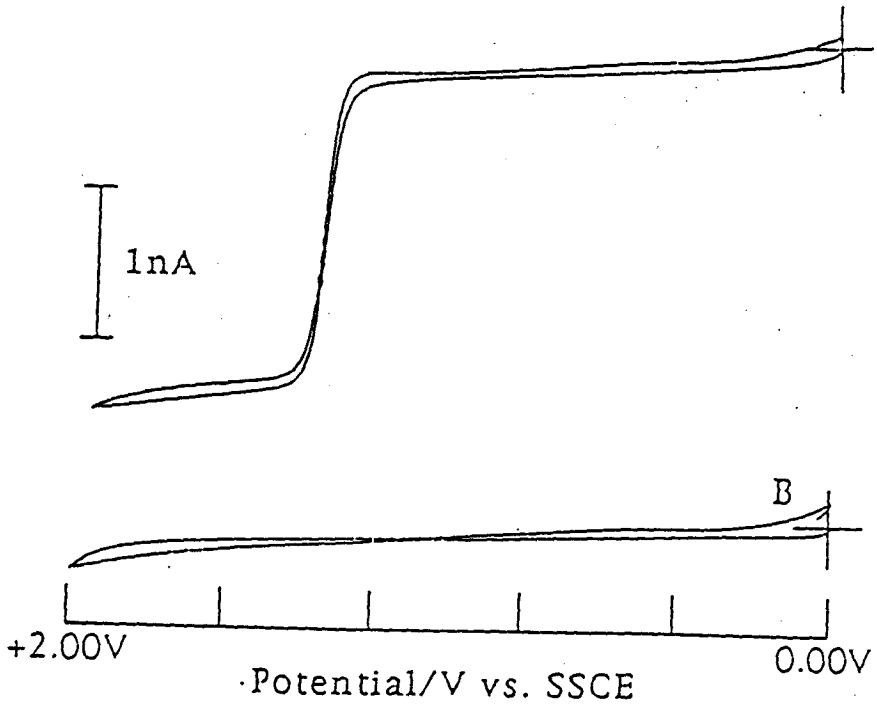

Figure 3: (A) Cyclic voltammogram using a 10mm diameter platinum microelectrode in a 0.77mM $Ru(bpy)_3(PF_6)_2$ solution in MeCN. The scan rate was 25mV/s. (B) Blank

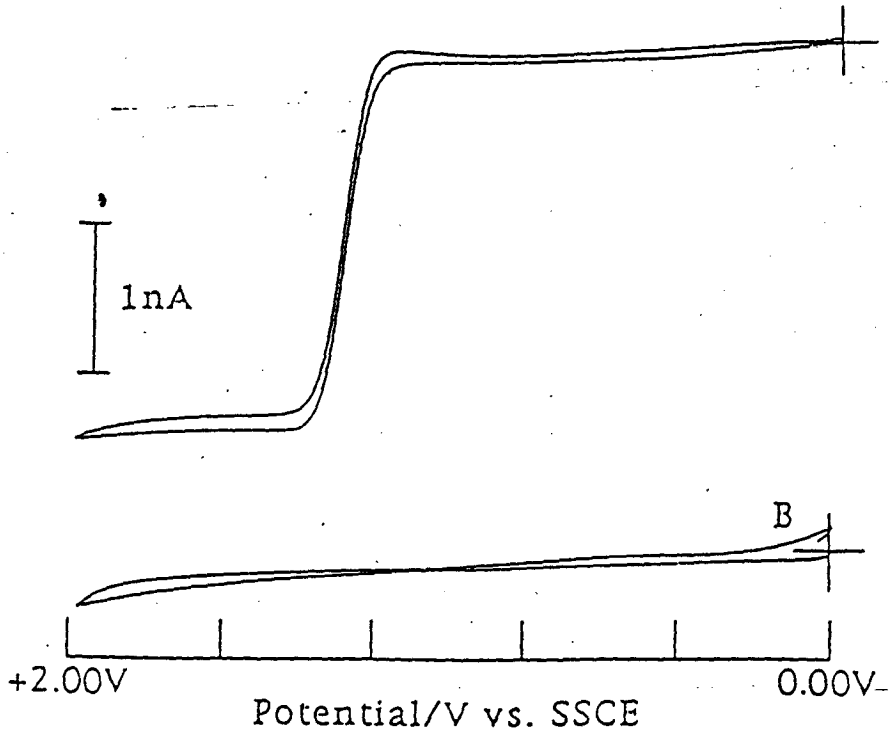

Figure 4: (A) Cyclic voltammogram using a 10mm diameter platinum microelectrode in a 0.78mM $Ru(bpy)_3(PF_6)_2$ and 0.1019M TBAH solution in MeCN. The scan rate was 25mV/s. (B) Blank

TABLE 1: I_l/I_d ratios

	I_l/I_d ratios	
Conc/mmol l^{-1}	$Ru(bpy)_3(PF_6)_2$[a]	$Ru(phen)_3(PF_6)_2$
0.2	0.82±0.07	
0.5	0.88±0.03	
0.8	0.80±0.05	0.91±0.04
1.2	0.90±0.01	
2.0	0.91±0.01	
3.2	0.90±0.02	0.88±0.01

[a]Values are reported as a mean ± one standard deviation.

Isolation and Quantitation of RNA from the filamentous fungus
Achlya ambisexualis
Mathew Kraus and Morris Rivera

Achlya ambisexualis is a filamentous, multinucleated, unicellular water mold. Its asexual life cycle begins with an encysted spore, which either germinates or alternates between motile zoospore and re-encysted forms before ultimately germinating by means of a germ tube (Thomas, 1989). The developing mycelium grows by longitudinal extension of its hyphal tips (Hill, 1979). There is potential for unlimited growth as long as appropriate nutrients and environmental conditions are present. Under scarce nutritional conditions it will use stored nutrients for spore formation, completing the cycle.

Another phenomenon associated with scarce nutritional conditions is that of lateral hyphal branching. This behavior has been observed during addition of either amino acid based nutrients after starvation or hormones from female mycelium (Mullins, 1973). Mullins noted that an increase in enzymatic cellulase levels accompanied branching. He suggests the increased cellulase levels are responsible for local weakening of hyphal walls, allowing turgor pressure to initiate primordial branch tips (Mullins, 1973). In order to study the true nature of cellulase's role in branching it will be necessary to isolate the gene(s) which produce it and regulate its expression.

Two ways to isolate these genes for manipulation are through the production of a cDNA library or a genomic library. Of these two techniques, it was decided that the cDNA library would be more beneficial because the cellulase encoding sequences represent a greater fraction of an mRNA population than of a population of genomic sequences (Lindquester, personal communication). This paper details experiments conducted in order to develop a procedure for the isolation of RNA that will provide enough RNA to support the production of a cDNA library.

MATERIALS and METHODS

Fungal strain

The strain of fungus used was *Achlya ambisexualis* Raper E87 male.

Sporulation and Inoculation

Sporulation was carried out under sterile conditions, in the following manner. Three colonies were transferred from 3-day-old peptone yeast extract glucose (PYG) medium (Manavathu, 1976) plates and placed in 100 ml of defined liquid medium (DLM) (Mullins, 1965). After 2 hours on a shaker at room temperature, the DLM medium was decanted and replaced with 100 ml of .05% calcium gluconate starvation medium and placed on a shaker for 14-17 hours.

Spore concentrations were counted on a hemacytometer after triturating to reduce zoospore motility. If higher concentrations were necessary to avoid excessive dilution of the inoculation medium, spores were pelleted by centrifugation and resuspended in smaller volumes of .05% calcium gluconate. Flasks of DLM growth medium were then inoculated with varying volumes of spore sample to achieve the desired concentrations of spores to be grown as described below.

Harvesting and Freezing

Mycelia were harvested by vacuum filtration with a Buechner funnel on Whatman's 5.5 cm, #1 filter paper. Harvested mycelia were then weighed and transferred into a plastic 50 ml centrifuge tube, capped, and immersed in liquid nitrogen. After they were frozen, the samples were stored at -80°C.

Assaying of Medium for Glucose and Cellulose

Glucose assays were performed by adding 1 ml of sample to 1 ml of dinitrosalicylic acid (DNSA) reagent. The mixture was boiled for five minutes and then it was cooled by adding 10ml of type I water. The solution was mixed together by inversion of the tube and then a reading was taken at 540nm. The standard used for this assay was 0.5g glucose per 100ml.

Cellulase activity was assayed viscometrically, using carboxymethyl cellulose as a substrate, at pH 6.6, 30° C.

RNA Extraction and Electrophoresis

RNA was extracted by modification of LeJohn's method (1984). Frozen mycelia were ground into a fine powder in liquid nitrogen with an RNase-free, pre-cooled mortar and pestle. The homogenized powder was resuspended in 4.5 ml/g culture weight suspension buffer (pH 7.5), and 0.9 ml/g culture weight 10% SDS and gently rocked. After two minutes an equal volume of phenol/chloroform/isoamyl in a 25:24:1 ratio was added to extract proteins and lipids. This solution was centrifuged at 4,000 g's for 2 minutes, and the lower, organic layer was discarded. Extraction and centrifugation with phenol/chloroform/isoamyl alcohol was repeated until no white debris was observed at the aqueous organic interface. RNA was precipitated in 1/10 volume sodium acetate and 2.5 volumes 100% ethanol. This solution was cooled at -80°C for ten minutes, and the RNA was pelleted by centrifugation at 6,000 g's for ten minutes. The supernatant was discarded and the pellet resuspended in water treated with diethylpyrocarbonate (DEPC).

An absorption reading of a 20 ul final sample : 1 ml water solution was taken at 260 nm. Concentration of RNA in the final sample was then calculated by the following equation, which relies on a standard of 40ug/ml of RNA per unit of absorption:

[RNA] = (absorption) x (40 ug/ul) x (test sample dilution factor)

The extent of protein contamination was calculated by taking another absorption reading at 280 nm. A ratio of 2/1 for absorption at 260 nm/absorption at 280 was considered purity.

RNA samples were run on a 1% agarose gel containing 2.2M formaldehyde at 5 volts per centimeter of gel for four hours. The nucleic acids were stained by soaking the gel in a solution containing 0.5ug/ml ethidium bromide.

RESULTS

Effects of Agitation During Growth

The purpose of the first experiment was to determine if there was a significant difference in hyphal mass and appearance in shaken versus unshaken cultures.

Mycelia were sporulated as previously described. Erlenmeyer flasks containing 20ml each DLM were inoculated with 200,000 spores, giving a final concentration of 10,000 spores per milliliter. All flasks were placed in a 25°C incubator/ shaker. Half of the flasks were shaken reciprocally at 100 rpms. The remaining flasks were not shaken. The mycelia were harvested and weighed every five hours for 30 hours. Hyphal morphology was also observed under phase contrast microscopy at every harvesting interval to follow such trends as hyphal length, vacuolation and branching.

There were little apparent difference in mycelial mass between the two series of flasks. The one salient difference noted was that unshaken mycelia grew in an evenly spread mate, while shaken mycelia grew in uneven clumps and globs. The unshaken method was thus adopted in order to maintain colonies of even, homogeneous growth.

Effects of Spore Concentration and Determination of the Rate of Growth and Consumption of Nutrients

The purpose of the second experiment was to determine if the rate of culture growth differs significantly as a function of the number of spores employed. The data from this exercise were also used to determine rate of growth and nutrient consumption.

Spores were obtained as previously described. Flasks containing 20ml of DLM were inoculated with 200,000 spores as the lower series, and 2,000,000 spores as the higher series. Both series were left unshaken in a 25°C incubator. Grown mycelia were harvested, weighed and frozen at 12 hour intervals over a period of 72 hours. Filtrates were frozen at -4°C and later assayed for glucose concentrations.

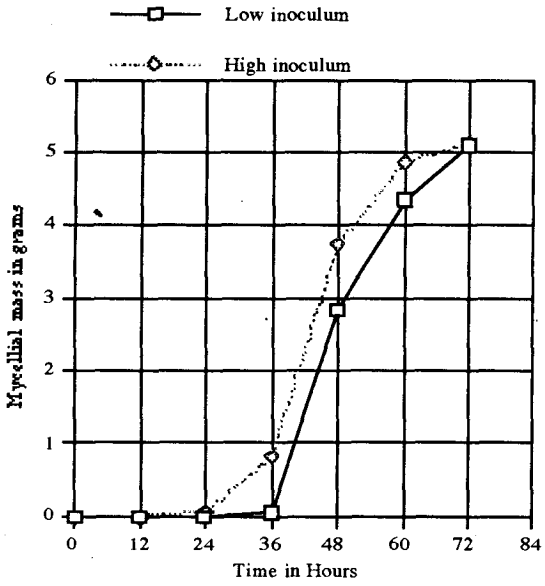

Figure 1: Comparative growth curves of high-inoculated verses low-inoculated cultures. The high inoculum elicited a more compact growth curve, beginning earlier (at 24 hours), also showing an earlier tapering at 60 hours.

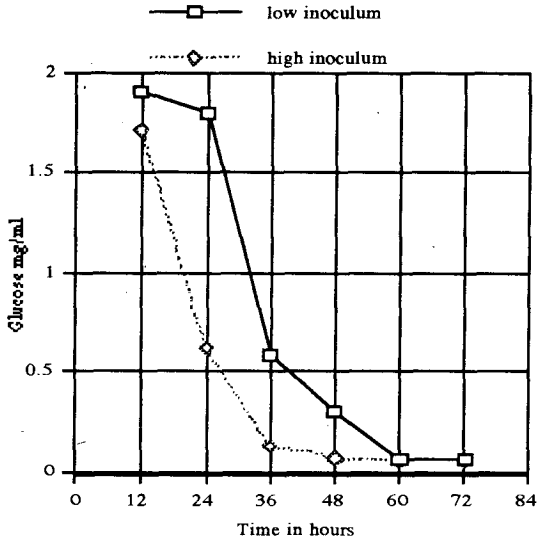

Figure 2: Glucose levels over time. The graph shows that as the mycelia were activily growing that they were consuming nutrients out of the medium. Nutrient depletion of the medium occurred at 60hrs. indicating readiness for induction of branching.

The data obtained showed no evidence that hyper-inoculated cultures were at a disadvantage to lower concentrated ones. On the contrary, the hyper-inoculated flasks showed an accelerated growth curve with earlier initiation of growth and a more compacted growth curve, tapering at about 60 hours (Figure 1). Likewise, glucose assays showed glucose depletion consistent with mycelial growth, with nutrients reaching asymptotic depletion at 60 hours for higher-concentrated cultures, and 48 hours for lower-concentrated cultures (Figure 2). Due to the fact that the hyper-inoculated flasks had a more compacted growth curve, a faster depletion of nutrients, and a greater number of hyphal tips, the higher-inoculation method was used in all subsequent experiments.

Effects of Induction on Branching

The purpose of the third experiment was to observe branching in induced verses non induced controls, with the rational that branching was directly proportional to cellulase

levels (Mullins 1973). Explant growths were used instead of suspended growths due to the greater ease of observing branching in individual hyphal tips.

Three colonies of mycelia were grown on a PYG plate for three days. Four 2-3mm by 2-3mm agar explants with mycelial growth were removed and evenly placed in a Petri dish containing 40ml of 0.2% peptone DLM. This was repeated for four dishes. The first two explants were allowed to grow for 72hrs. The mycelia in one Petri dish were washed with type I water and the medium replaced with 0.2% peptone as described above. The second Petri dish was left undisturbed. The second pair of Petri dishes were allowed to grow for 96 hours, unshaken, at 25°C and then the above procedure was repeated.

Through microscopic observation of hyphal tips an increase in hyphal branching was observed in the explants that underwent induction. In light of this evidence it was decided that 0.2% peptone was an adequate inducer of branching, and was employed as a means to boost cellulase mRNA levels in all following experiments.

Optimization of the Timing of Induction and Correlation of Branching and Cellulase Secretion

The purpose of the fourth experiment was to test Mullins' data that induced branching is accompanied by an increase in the level of secreted cellulase (1973), and to determine an optimal time for induction.

Petri dishes containing 20ml each of DLM were inoculated with 2,000,000 spores each and incubated at 25°C. After 36 hours branching was induced in two plates in the following manner. The medium of each was decanted, and the hyphae washed three times with type 1 water. Thirty milliliters of 0.2% peptone was added to each, and 1.5 ml from each plate was immediately removed and stored as a reference. After two hours, an additional 1.5ml was removed from each plate for assaying. The above procedure was repeated for the 48 hour and 60 hour growth plates.

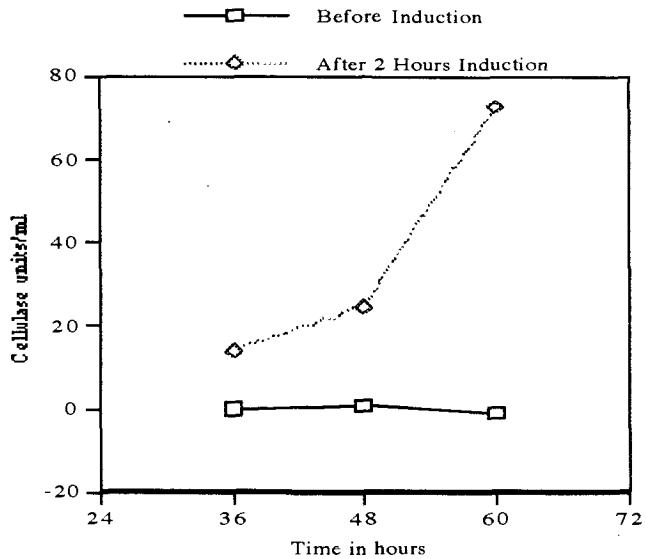

Figure 3: Cellulase levels in induction medium over time. The graph shows the highest levels of cellulase at 60hrs which corresponds with increased hyphal branching.

The results from the cellulase assays showed that older cultures secreted more cellulase than younger ones after induction (Figure 3). The 60 hour induced mycelium had the largest amount of secreted cellulase. It was reasoned that this was due to a combination of age and increase in mycelial mass.

DISCUSSION AND CONCLUSION

The following is the experimentally determined optimal procedure for RNA isolation and extraction from *Achlya ambisexualis*.

The sporulation was performed as previously stated and the plates were inoculated with 2,000,000 spores per each 20ml volume of DLM. The plates were incubated unshaken at 25°C. After 60 hours the DLM medium was aspirated away and the mycelia were washed three times with type I water, after which .2% peptone was added to induce branching and increase cellulase mRNA transcription. After 2 hours of induction the mycelia were harvested, weighed, and frozen. RNA was extracted and quantified as stated above, yielding a final concentration of 1.03 ug/ul of RNA. This lead to a ratio of 500 ug of RNA per one gram of wet mycelial weight. This is comparable to LeJohn's extraction of 530 ug of RNA per gram of wet weight from *Achlya klebsiana* under similar conditions (1984).

RNA was electrophoresed , with 20 ug of sample per well. That RNA was in fact extracted is evident in the characteristic two bands of ribosomal RNA (28s and 18s) on a backdrop smear of multiple sized mRNA (Figure 4). Furthermore, even distribution of the smear indicates that little RNA was degraded in the isolation and extraction procedures.

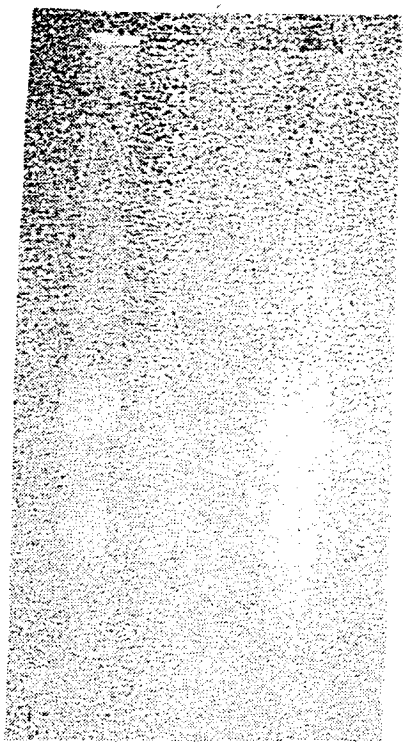

Figure 4: Electrophoresis gel of RNA isolated from *A. ambisexualis*. The two lanes shown contain duplicate samples.

The developed procedure appears to be an acceptable and suitable method for extraction of enough competent RNA to supply several working cDNA libraries of *Achlya ambisexualis*, without the need to grow mass quantities of cultures. Immediate follow-up experiments will investigate optimal conditions for purification of poly (A)+ mRNA from the total RNA .

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The authors would like to thank Dr. Terry Hill and Dr. Gary Lindquester for their guidance and technical expertise in this endeavor. We thank Dr. Lindquester for entrusting us with his lab and for guiding us in molecular techniques, without which this project would not have been possible . We would also like to thank Dr. Hill for performing the glucose and cellulase assays and for entrusting us with his beloved Achlya, without which this project would not have been conceived.

Literature Cited

Hill, T.W., J.T. Mullins. 1979. Association of latent cellulase activity with a membrane fraction from vegetative hyphae of *Achlya ambisexualis*. Mycology, 71: 1227-1239.

LeJohn, H.B. 1985. Degradation of RNA and Poly (A)+ RNA during isolation from *Achlya klebsiana* prevented by alkaline pH not Ribonuclease inhibitors. Experimental Mycology, 9: 64-69.

Manavathu, J.T., D.D. Thomas. 1976. Non-penetrating thiol reagent mimics cytochalasin in inhibition of secretory protein synthesis. Biochemical and Biophysical Research Communications, 71: 280-285.

Mullins, J.T., Alma Barksdale. 1965. Parasitism of the chytrid *Dictyomorpha dioica*. Mycologia, 67: 352-359.

Mullins, J.T. 1973. Lateral branch formation and cellulase production in the water molds. Mycologia, 65:1007-1014.

Thomas, D.D. 1989. A method for preparing *Achlya* cysts with high zoosporogenic potential. Mycological Research, 92: 233-235.

IMMUNOCYTOCHEMICAL VERIFICATION OF ANTI-DYSTROPHIN ANTIBODIES IN NORMAL AND MDX MICE

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a degenerative lethal X-linked disease involving the skeletal muscle system which is charac-terized by the absence of sarcolemmal dystrophin, a 400 kDa protein (1-3). While Becker muscular dystrophy (BMD), a milder form of the disease, is a result of an abnormal form of dystrophin (4). This abnormal form of the protein is thought to be a size-altered form of dystrophin (5). The dystrophin protein is composed of four regions homologous to other known cytoskeletal proteins (6-8). Dystrophin is anchored to trans-membrane dystrophin associated glycoproteins (DAG) by its carboxy terminus (9-10) on the sarcolemmal membrane (11).The interaction between dystrophin and DAG appears to play important physiological roles in the regulation of transmembrane slow-calcium channels (12-13). Dystrophin is effectively labeled using the three different Monoclonal Antibodies (mAb), DYS1, DYS2, and DYS3, whose epitopes correspond to the middle, rod region, the C-terminus, and N-terminus respectively.

According to, Novocastra Laboratories, DYS1 mAb is specific for human, mice, rat, rabbit, and dog skeletal, cardiac, and smooth muscle. DYS2 has been shown to react with skeletal, cardiac, and smooth muscle from normal mouse, rat, rabbit, and dog tissues. Novocastra stated that DYS2 was not specific for mdx mouse tissue. Novocastra reported that DYS3 is a human specific mAb and that no reactivity between other species muscle occurred.

The goal of this project was to test the validity of the supplier's reports regarding the specificity of the mAb and to qualitatively assess the effectiveness using immunohistochemical analysis of normal (C57/BL) and mdx mice. The mdx mouse was chosen because it lacks the dystrophin protein and provides an excellent negative control for sarcolemmal dystrophin.

MATERIALS AND METHODS

The immunocytochemical analysis of the skeletal muscle was accomplished by using normal (C57/BL) and mdx mice. Three anti-dystrophin mAbs were obtained from Novocastra Laboratories Ltd., and one polyclonal dystrophin Ab (pAb) raised by Dr. H.J. Li (LiAb). Fetal calf serum (FCS) was used as the blocking reagent. The secondary Ab was a goat anti-mouse-biotin Ab for the three mAbs obtained from Novocastra, while for LiAb, the secondary Ab was goat anti rabbit-biotin. The secondary Ab was cross-reacted with Streptavidin-Texas Red conjugate obtained from GIBCO BRL. The blocking agent, primary Ab, secondary Ab, and Streptavidin label were all diluted using TBS (20mM Tris-HCl, 150mM NaCl, at pH 7.5).

The quadriceps muscle was removed from the normal and mdx mice and frozen in liquid nitrogen pre-chilled 2-methylbutane. Cryostat tissue sections 8μm thick were collected and fixed onto slides for fluorescent staining. The tissue samples were fixed with absolute alcohol for 5 minutes. The slides were blocked with 10% FCS for 15 minutes. The blocking agent was then dumped and the primary anti-dystrophin antibodies were then incubated on the tissue sections for 1 hour at room temperature. The mAbs obtained from Novocastra were diluted 1:20 while LiAb was diluted 1:2000. The samples were washed thoroughly 5 times with TBST (TBS + 0.1% Tween-20). Both of the biotin conjugated secondary antibodies were diluted 1:200 and the goat anti mouse Ab was incubated on the tissue sections which were labeled with the Novocastra mAbs, and the goat anti-rabbit Ab was incubated onto the slides containing LiAb. The secondary Ab was

incubated for 45 minutes also at room temperature. The visualization step was accomplished using Streptavidin-Texas Red conjugate diluted 1:500. The fluorescent label was incubated at room temperature on the tissue sections for 45 minutes. The slides were washed 5 times with TBST and sealed with 50% glycerol and cover slips. The tissues were analyzed using an immunofluorescent microscope and pictures were taken. The slides were stored in a closed holder at 0°C to prevent damage due to UV light.

RESULTS

The immunocytochemical data obtained indicate some inconsistencies in the claims of the DYS Ab supplier, Novocastra. The immunofluorescent photomicrographs for the C57/BL myofibers all show a positive cross-reaction with the DYS1, DYS2, DYS3, and LiAb.

The immunofluorescence data obtained from the mdx mice muscles showed an absolute error with the information supplied by Novocastra. When analyzed, the immunostaining with the DYS1, 2, and 3 mAbs all showed some positive cross-reaction. However, LiAb was the only Ab which showed a strong negative staining of the mdx mouse myofibers.

CONCLUSION

There is an error between the reactivity of the DYS Ab supplied by Novocastra and the actual immunocytochemical results. The discrepancy is that according to the supplier DYS3 is supposed to be specific only for humans. The C57/BL mouse tested positive using a new aliquot of DYS3. This insured that proteases had not degraded the Ab due to age, and that the Ab had not been degraded due to repeated temperature fluctuations as a result of freezing and thawing. DYS1, DYS2, and LiAb were all reliable due to the fact that a positive staining of the dystrophin located on the cell periphery occurred.

The most significant differences were in regard to the immunocytochemical analysis of the mdx myofibers. The only Ab which did not show a positive reaction with the mdx mice was LiAb. DYS1, DYS2, and DYS3 all showed false positives for the reactivity. The mdx mouse does not contain dystrophin; therefore, the cell periphery should not have been stained like the C57/BL samples had been. Novocastra reported that DYS2 was not specific for mdx mice when in fact the myofiber sample stained showed a positive result. Also, DYS3 was supposed to be specific for human samples only.

The contradictions between the results and the literature provided by Novocastra leave many questions as to the validity of the testing conducted by the supplier. DYS1, DYS2, and DYS3 all showed problems and the validity could not be relied upon. LiAb was shown to be very effective in the analysis because it did not show a reactivity for the mdx mouse myofibers. The results show that before any Ab is used in an immunocytochemical test qualitative analysis should be performed on positive and negative samples to insure the validity of the product received. Further work regarding this research needs to be done to increase the sample population size for statistical analysis to test the validity of the data received.

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REFERENCES

1 Koenig, M., Monaco, A.P., and L.M. Kunkel. The complete sequence of dystrophin predicts a rod-shaped cytoskeletal protein. *Cell.* 53(2): 219-26.

2 Hoffman, E.P., Brown, R.H. Jr., and L.M. Kunkel. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell.* 51(6): 919-28.

3 Koenig, M., and L.M. Kunkel. Detailed analysis of the repeat domain of dystrophin reveals four potential hinge segments that may confer flexibility. *J Bio Chem.* 265(8): 4560-66.

4 Fabbrizio, E., Leger, J., Anoal, M., Leger, J.J., and D. Mornet. Monoclonal antibodies targeted against the C-terminal domain of dystrophin or utrophin. *FEBS Lett.* 322(1): 10-14.

5 Hoffman, E.P., Fishbeck, K.H., Brown, R.H., Johnson, M., Medori, R., and et al. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N Engl J Med.* 318(21): 1363-68.

6 Hammmonds, R.G. Jr. Protein sequence of DMD gene is related to actin-binding domain of alpha-actinin. *Cell.* 51(1): 1.

7 *Cell.* 53(2): 219-26.

8 Noegel, A., Witke, W., and M. Schleicher. Calcium-sensitive non-muscle alpha-actinin contains EF-hand structures and highly conserved regions. *FEBS Lett.* 221(2): 391-96.

9 Ervasti, J.M., and K.P. Campbell. Membrane organization of the dystrophin-glycoprotein complex. *Cell.* 66(6): 1121-31.

10 Yoshida, M. and E. Ozawa. Glycoprotein complex anchoring dystrophin to sarcolemma. *J Biochem.* 108(5): 748-52.

11 Ohlendieck, K., Ervasti, J.M., Snook, J.B., and K.P. Campbell. Dystrophin-glycoprotein complex is highly enriched in isolated skeletal muscle sarcolemma. *J Cell Bio.* 112(1): 135-48.

12 Turner, P.R., Fong, P.Y., Denetclaw, W.F., and R.A. Steinhardt. Increased calcium influx in dystrophic muscle. *J Cell Bio.* 115(6): 1701-12.

13 Franco, A. Jr., and J.B. Lansman. Calcium entry through stretch-inactivated ion channels in mdx myotubes. *Nature.* 344(6267): 670-73.

THE EFFECT OF DIFFERENT WAVELENGTHS ON *DAPHNIA* PHOTOTAXIS

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Abstract:

Daphnia is a crustacean water flea which exhibit sensitivity to light. This experiment determines the phototactic reaction of *Daphnia* to varying wavelengths from a horizontal light source. The testing utilized 11 alternating periods of rest and exposure to light. The wavelengths tested were white, red, yellow, green, blue and purple light. The population distribution of the *Daphnia* was measured for each period, and it was observed that *Daphnia* is repelled by lower wavelengths (green, blue, and purple) and white light emitted from a horizontal light source.

Introduction

Daphnia is a crustacean water flea whose sensitivity to light has been studied extensively. Experiments have shown that the*Daphnia* is red-blind and reacts to lower intesities of blue light more than low intensities of green light (Hutchison, 1967). It has also been discovered experimentally that *Daphnia* shows a peak activity (vertical and horizontal movements) response to blue wavelengths, and *Daphnia* also demonstrates a peak swimming velocity when exposed to blue/green wavelengths (Stearns, 1975). In addition, D. C. McNaught (1971) discovered that *Daphnia* from high-blue light environments have even lost their red receptors.

Some discussion has taken place on *Daphnia* sensitivity to different wavelengths of light. The crustacean eye itself may be more sensitive to the shorter wavelengths of the visible spectrum because those are the wavelengths of light that can penetrate to the deepest parts of the animal's habitat (Stearns, 1975). Another study found that cladocera species have four visual pigments which have maximum sensitivity at the wavelengths of ultraviolet, blue, yellow, and red light (McNaught, 1971).

This experiment was executed to determine the phototactic reaction of *Daphnia* to different wavelengths of light. The differentiating factor between this study and earlier findings is that these previous experiments used a vertical light source; however, this study utilizes a horizontal light source. One investigation found that *D. magna* and *D. pulex* are usually negatively phototactic to a horizontal light beam (Hutchison, 1967), but this experiment did not determine the effects of different wavelengths of the horizontal light beam on *Daphnia* phototaxis.

Methods

The experiment used 20 *Daphnia* in a 500mL glass beaker. The beaker was divided into two equal sides by drawing a line across the bottom of the beaker. The horizontal light travelled in a direction perpendicular to this dividing line on the beaker. Gels of various colors were used to convert white light from a desk lamp to light of a specific wavelength.

The *Daphnia* were placed in the beaker and allowed to habituate for 10 minutes. The experiment then followed a pattern of five minute light exposures of different wavelengths with a five minute rest between each exposure. For example, the *Daphnia* were first exposed to white light for five minutes, then a five minute rest (with only flourescent classroom lights on), then five minutes of red light, another five minute rest, etc. until the animals had been exposed to yellow, green, blue, and finally purple light.

Data were collected every minute of each light exposure and rest. These data consisted of a count of how many *Daphnia* were on side A (the side of the beaker closest to the light source). The raw data were then reduced using two statistical tests: the Kruskal-Wallis test on SYSTAT and the sign test.

Results

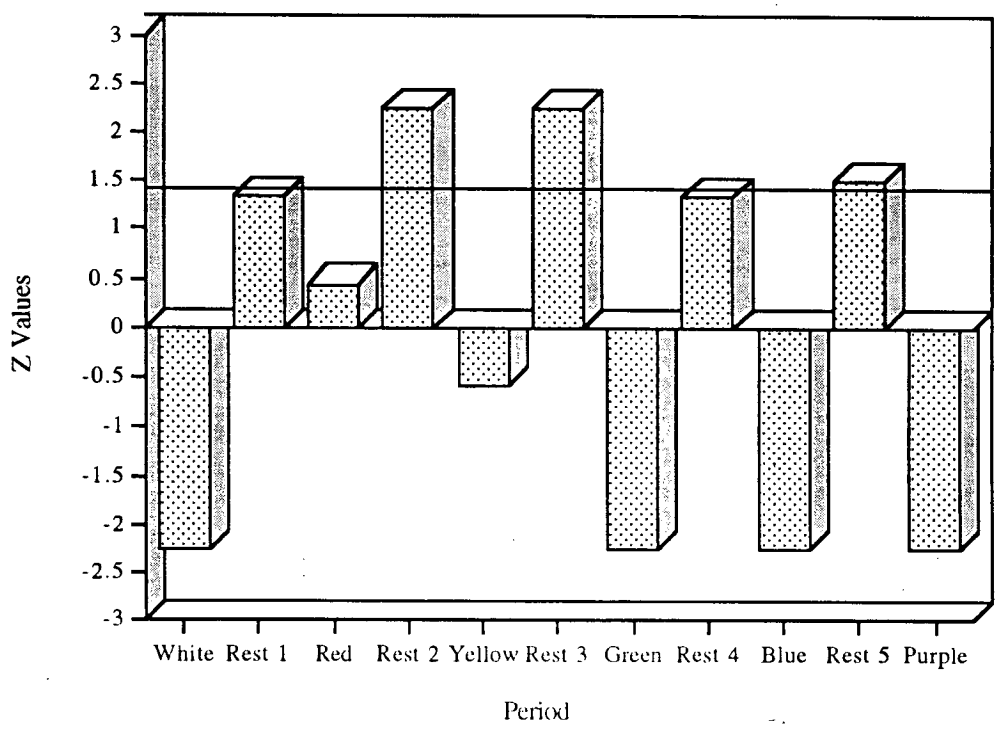
There were five pieces of data for each period (6 light exposures and 5 rests). The Kruskal-Wallis test was used for the entire group of 11 periods of data to determine if the number of *Daphnia* on side A of the beaker does not differ between the periods. The null hypothesis used was that the 11 groups did not differ in the amount of *Daphnia* found on side A. The SYSTAT program calculated the H value: H = 54.00. Since this value is greater than the critical value (H = 5.99), the null hypothesis can be rejected.

The sign test was applied on each individual period's data to determine if the population of *Daphnia* was evenly distributed across the beaker's bottom. The null hypothesis used for this test was that the population of *Daphnia* was distributed evenly between sides A and B during the period of rest or exposure to light. A "z value" of $|z| > 1.96$ is necessary to reject the null hypothesis for that period being analyzed. See the following table and graph for the results of the sign tests on each period. If the z value is negative, the *Daphnia* population was found to be significantly distributed on side B (the side of the beaker farthest away from the light); conversely, if the z value is positive, the *Daphnia* population was found to be significantly distributed on side A.

The Sign Tests

Period and l	Sign Test Value	Reject/Accept Null Hyp
white light	-2.24	reject
rest #1	1.34	accept
red light (7x10-7m)	0.45	accept
rest #2	2.24	reject
yellow light (5.65x10-7m)	-0.58	accept
rest #3	2.24	reject
green light (5.3x10-7m)	-2.24	reject
rest #4	1.34	accept
blue light (4.7x10-7m)	-2.24	reject
rest #5	1.50	accept
purple light (4.0x10-7m)	-2.24	reject

Sign Test Results of Relationship Between *Daphnia* Movement and Light



Discussion

The data of this experiment tell an interesting tale. First, the rejection of the null hypothesis using the Kruskal-Wallis test only suggests that there is some kind of difference between all 11 periods. This factor is why the sign test was used; the sign test is more specific in that it explains which wavelengths of light created an uneven distribution in the *Daphnia* population.

From the resulting z values, it can be seen that white, green, blue, and purple light significantly repelled the *Daphnia* (none of the lights tested significantly attracted the *Daphnia*). The wavelengths of light that repelled the animals are lower than the other two tested (red and yellow). This result is supported by the earlier studies which found most *Daphnia* to be blind to the color red (Hutchison, 1967). The wavelengths that repelled the *Daphnia* are smaller than those that did not and are therefore higher in energy, too. This high amount of energy could also be a factor in repulsion. Also, previous discussion on crustacean eye sensitivity (Hutchison, 1967) correlates well with the findings in that the short wavelengths of the visible spectrum have more energy which in turn enables the light to reach farther depths in water, and it is these penetrating wavelengths to which *Daphnia* would be the most sensitive.

Some intriguing data are the z values of rests 2 and 3. During these periods, the *Daphnia* were significantly attracted to side A (the side closest to the light source) even though the lamp was turned off. There are two possible explanations for these results. In one experiment with *Daphnia*, it was found that the animals were attracted to warmer temperatures: if the bottom of the beaker was cooled, the animals would migrate up (Hutchison, 1967). This result is useful in that

during the periods of light preceding each rest period the light from the lamp may have heated the water. If this is the case, the *Daphnia* would be attracted to the warmer side A (after the dominating light stimulus was turned off). Of course, if the temperature gradient was the only factor affecting *Daphnia* movement during rest periods, one would expect to see the *Daphnia* significantly migrate to side A in every rest period (which was not the case).

A more plausible explanation for the results of rests 2 and 3 comes from an experiment dealing with light intensity. In this earlier study, it was found that a rapid change in light intensity causes a reversal in phototaxis (Hutchison, 1967). This finding can be used for rests 2 and 3 in that they occurred after light exposures that did not repel the *Daphnia* and were lower in energy. Therefore, the stimulus of the previous light eroded faster than during the other rest periods, and the *Daphnia* could respond to the change in light intensity (the light turned off) with a reversed movement that distributed them onto side A. Further experiments for testing this hypothesis may include a longer rest time to determine if the animals eventually migrate back to side A after being exposed to the lower (higher energy) wavelengths.

This experiment achieved two important results. First, and simply, the experiment qualified *Daphnia* phototaxis during exposure to different wavelengths of light. Secondly, it supported the finding that *Daphnia* are usually negatively phototactic to a horizontal light beam (Hutchison, 1967). However, this experiment added another dimension onto the previous "horizontal light beam" study; it discovered a role of wavelength in the phototaxis to a horizontal light beam. When exposed to varying wavelengths from a horizontal light source, *Daphnia* are negatively phototactic to the lower wavelengths.

SOURCES

Hutchison, G.E. 1967. A Treatise on Limnology. Vol 2. p 762-766.

McNaught, D.C. 1971. Plasticity of cladoceran visual systems to environmental changes. Transcript of the American Microscopial Society. 90:113-114.

Stearns, S.C. 1975. Light responses of *Daphnia pulex*. Limnol. Oceangr. 20: 564-570.

POLYMERASE CHAIN REACTIONS AND THE DETECTION OF HUMAN HERPESVIRUS 6

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Human herpesvirus 6 (HHV-6) is the etiologic agent of roseola. Roseola is a childhood infection usually characterized by rapidly rising fever persisting for 3-5 days followed by rash persisting for as long as four days. Little information concerning other effects of this recently discovered virus on the human body is available. Serologic studies may have linked HHV-6 to adult hepatitis cases and to various lymphoproliferative disorders, but the data are inconclusive. Nothing is known of its effects in immunocompromised hosts. HHV-6 is currently detected through indirect serological techniques. We have studied the feasibility of a direct method for detection by utilizing polymerase chain reaction (PCR). Blood samples were collected from students attending LeMoyne-Owen and Rhodes Colleges in the fall of 1991. They were then tested in our laboratory using PCR and at the Centers for Disease Control and Prevention using enzyme immunoassays. The PCR reactions were conducted using HHV-6 specific primers and primers for beta-globin as an internal control, and the fragments were analyzed and identified using gel electrophoresis and radiolabeled probes specific to the human herpesvirus 6, strain Z29 (but with cross-reactivity to other HHV-6 strains). Results from the PCR indicated that 37.2% of the samples were positive for HHV-6 in contrast to the 96.2% seropositivity for HHV-6. The discrepancy may be due to student error.

INTRODUCTION

The human herpesvirus 6 (HHV-6) was recently discovered in 1986. The discovery was heralded as one of the most rapid characterizations of a virus since the human immune deficiency syndrome (HIV). It has been distinguished from other human herpesviruses by immunology and molecular analysis (Josephs et al. 1986, Lopez et al. 1988, Salahuddin et al. 1986).

HHV-6 genome consists of linear double-stranded DNA that has a G+C composition of 43% (Lawrence et al. 1990, Lindquester and Pellett 1991). It averages around 161 to 170 kb in length (Lindquester and Pellett 1991, Martin et al. 1991, Pellett et al. 1990). Approximately 90% of the genome has been cloned in plasmids, cosmids, and bacteriophage and sites for several restriction enzymes have been generated (Lindquester et al. 1993, Martin et al. 1991).

HHV-6 is the causative agent of exanthema subitum (roseola) (Yamanishi et al. 1988). Roseola is a childhood disease that primarily affects infants under the age of two (Bernstein 1991). The disease is characterized by a rapid onset of fever lasting 3 to 4 days, followed by a rash that may persist up to 4 days. Serological assays have also linked HHV-6 to adult hepatitis (Asano et al. 1990) and several other lymphoproliferative disorders (Pellett, Black, and Yamamoto 1992). But as of yet, no causative role has been directly attributed to HHV-6. This is partially due to a 95% seroprevalency in the adult population (Balachandra et al. 1989, Briggs, Fox, and Tedder 1988, Brown et al. 1988, Chou and Scott 1990, Knowles and Gardner 1988, Okuno et al. 1989, Pruksananonda et al. 1992, Tedder et al. 1987), and because no site of latency has been determined . It is widely assumed that HHV-6 establishes a lifelong latent infection in its host as do other human herpesviruses. This assumption is based on observations of increased antibody titers in previously seropositive patients. Therefore, it is possible that many of the etiological

effects of the virus are still unknown. These unknown variables increase the need for a direct assay to detect the virus. As with other human herpesviruses such as herpes simplex, cytomegalovirus, and herpes zoster the effects of HHV-6 in reactivation could prove fatal especially in post-transplant immunosuppressed patients.

HYPOTHESIS

We hypothesized that polymerase chain reaction (PCR)analysis of blood lymphocyte samples would provide us with a more direct method of detection for HHV-6. Current serologic techniques evaluate on the basis of a dilution method where the highest dilution at which the antibody can be detected is referred to as the antibody titer. This is an indirect measurement. We have proposed a more direct method by going to the lymphocytes themselves. Laboratory tests have shown that the virus grows primarily in T lymphocyte cultures. Newly isolated strains are being cultured in cord blood lymphocytes and peripheral blood lymphocytes, and a few have been successful in a variety of T cell lines (Ablashi et al. 1988, Lusso et al. 1988, Tedder et al. 1987).

Polymerase chain reaction

Polymerase chain reaction (PCR) is a relatively new technique that allows for the amplification of small amounts of DNA for the detection by traditional means. This can be attributed to two factors: 1) the availability of purified, thermostable DNA polymerase and 2) chemically synthesized DNA oligonucleotides. PCR is extremely sensitive and can detect a single DNA molecule in a sample, but part of the designated region's nucleotide sequence must be known in order to synthesize the oligonucleotides that complement each end of the double helix. The oligonucleotides act as primers for in vitro DNA synthesis using the thermostable DNA polymerase. This process is conducted under a varying set of temperatures to allow for the denaturing of the DNA, the annealing of the primers, and the cloning of the fragments. Amplification generally requires 20 to 30 cycles. For effective amplification we applied 35 cycles to the HHV-6 DNA.

MATERIALS AND METHODS

Sample collection-- Students from LeMoyne-Owen and Rhodes College were approached to donate blood samples (5ml) for the experiment. A certified nurse was provided to collect the samples. The samples were drawn in 15 ml. heparinized tubes and immediately refrigerated. The student volunteers were also asked to fill out an anonymous questionnaire. The protocol was approved by the Human Subjects Committee at Rhodes College.

Preparation-- Within 24 hours the blood was processed. This phase of the experiment was conducted by Kathleen Donnelly. The plasma was separated and the lymphocytes were extracted following centrifugation in a lymphocyte separation medium. Then the lymphocytes were resuspended in a lysis buffer containing Proteinase K and SDS. The samples were heated at 65 degrees Celsius for two hours for the optimum enzyme activity. Then the enzyme was denatured at 98 degrees Celsius over a ten minute interval. The lymphocyte lysates were frozen at -70 degrees Celsius. The plasma samples were sent to the Centers for Disease Control and Prevention for serological evaluation of HHV-6 and, incidentally, human herpesvirus 7 (HHV-7). PCR reactions were utilized at Rhodes to test the lymphocytes for the presence of HHV-6 DNA.

PCR analysis-- The PCR analysis was conducted using ten microliters of lymphocyte lysates under the reaction condition recommended by Perkin Elmer Cetus with the Gene Amp PCR kit. The oligonucleotides primers synthesized were 5'-ACATGAAAGCCGAGAGATCC and 5'-GTGATGGAGTTATTGGACCAC and were obtained from the molecular resource center at St. Judes Children's Research Hospital. The primer set generated an amplimer that was used to detect

the HHV-6A and HHV-6B. A beta-globin set that generated another amplimer was also used as an internal control to detect KM38 and TCO3.

Gel electrophoresis-- To enhance resolution, the gels were comprised of 1.5% Synergel and 0.7% agarose. The HHV-6 and beta-globin amplimers from the sample blood were loaded side by side.

RESULTS

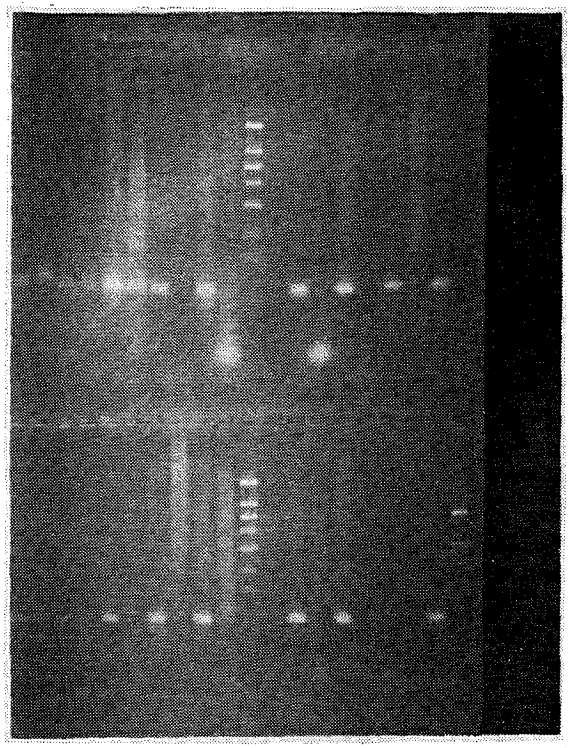
In Figure 1, ethidium bromide stains revealed varying levels of intensity for amplified bands of DNA. Accuracy of these results would be inhanced if the fragments were transferred via Southern blot to nylon membranes and probed with a labeled oligonucleotide complementary to the amplified fragment. The increase in the sensitivity of this method would probably increase the numbers of positive PCR-positive samples. Nevertheless, of the 43 samples which gave positive results with the beta-globin control, 37.2% were positive for HHV-6. According to the serology test results from 53 samples tested by the Centers for Disease Control and Prevention, 96.2% tested positive for HHV-6, and 3.8% tested negative for HHV-6. Incidentally, 96.2% also tested positive for HHV-7, and 3.8 % tested negative for HHV-7. These results are listed in Figure 2. The amount of antibody detected for HHV-6 and HHV-7 is listed in the farthest left hand column. The samples that did not contain any antibody were labeled negative. HHV-6 antibody titers were lower than HHV-6 titers.

All of the PCR-positive samples were also serologically positive for HHV-6. Sample 9a was the only one of the PCR-positive samples to test negative for HHV-7. The remainder of the positive PCR samples were also seriologically positive for HHV-7. Sample 28 was the only one of the samples to test negative for HHV-7 and HHV-6 according to the seroligical results.

DISCUSSION

There is a difference of 59.0% between the seroligical results and the PCR results. This may be explained in a variety of ways. Although we know that HHV-6 grows on T-lymphocytes in culture, we still have no reason to assume that lymphocytes serve as the primary site of infection or latency in vivo. The discrepancy could also be due to error in the processing of the blood samples or/and error in conducting the polymerase chain reactions. Follow up research might analyze the lymphocyte samples for HHV-7 using PCR.

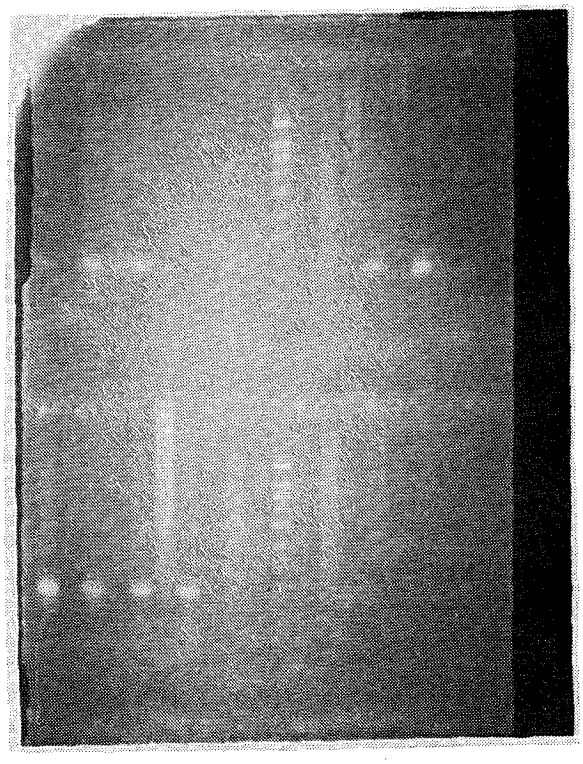
a)



b)

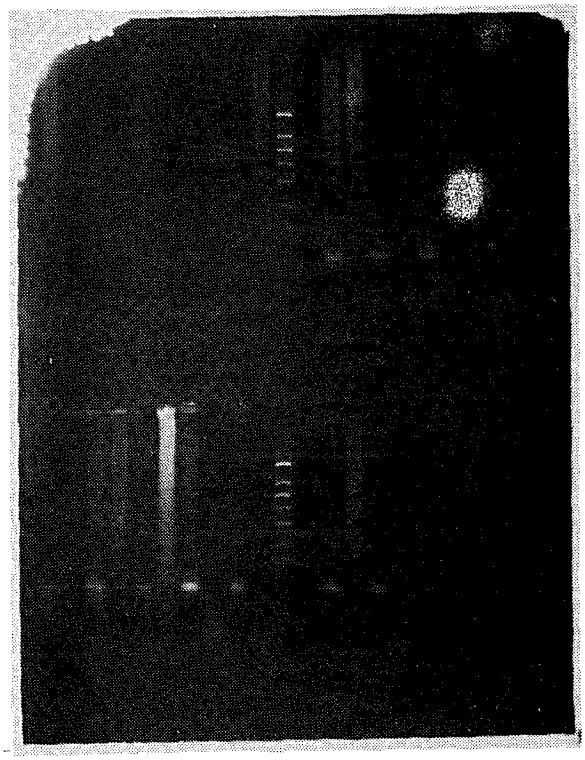
Figure 1-- The electrophoresis gels were run with two primer sets. The beta-globin was used as an internal control. HHV-6A and HHV-6B were used to detect human herpesvirus 6. The two tests were run side by side with the beta-globin on the left. A DNA standard was used as a well as blue juice. The 20 microliters of each sample were tested on the following gels: a) controls, 1a, 2a, 3a, 4a, DNA, blue juice, 5a, 6a, 7a, 8a b) 9a, 10a, 11a, 12a, 13a, DNA, blue juice, 14a, 15a, blanks, 55 c) 47, 42, 41, 40, DNA, blue juice, 39, 38, 37, 35 d) 34, 33, 32, 31, 15, DNA, blue juice, 14, 13, 12, 11 e) 10, 9, 8, 7, 6, DNA, blue juice, 5, 4, 3, 2 f) 54, 53, 52, 51, 50, DNA, blue juice, 49, 44, 48, 45.

c)



d)

e)



f)

58 Figure II

04-19-1993

NEW ANTIBODY FORMULA

GARY LINDQUISTER

	HHV-6 EIA			HHV-7 EIA		
PT #	CAL OD	Ratio	ABY	CAL OD	Ratio	ABY
P1	0.497	5.85	2780	0.228	4.30	290
P2	0.411	4.67	1350	0.208	5.57	250
P3	0.358	4.43	870	0.230	4.09	300
P4	0.256	2.36	370	0.139	2.25	140
P5	0.293	3.54	510	0.154	4.30	160
P6	0.430	4.29	1590	0.098	1.74	100
P7	0.210	1.47	250	0.365	3.62	920
P8	0.428	3.14	1570	0.382	8.01	1060
P9	0.275	3.42	430	0.217	4.91	270
P10	0.478	6.28	2370	0.233	8.77	300
P11	0.447	4.62	1830	0.230	4.96	300
P12	0.304	1.97	550	0.357	3.06	860
P13	0.276	3.66	440	0.271	7.78	420
P14	0.399	2.89	1220	0.201	2.86	230
P15	0.359	4.28	870	0.255	4.83	370
P16	0.415	3.57	1400	0.345	6.95	780
P17	0.468	6.43	2180	0.226	4.83	290
P18	0.373	6.14	980	0.231	6.76	300
P19	0.367	5.73	930	0.351	8.39	820
P20	0.518	5.70	3310	0.196	4.62	220
P21	0.627	6.68	8250	0.204	4.04	240
P22	0.428	2.15	1560	0.268	8.34	410
P23	0.513	5.67	3180	0.135	2.68	130
P24	0.548	8.76	4240	0.168	5.94	180
P25	0.385	3.00	1080	0.263	2.40	390
P26	0.272	4.87	420	0.152	4.19	150
P27	0.319	4.21	620	0.131	4.39	130
P28	-0.015	0.79	NEG	0.029	1.32	NEG
P29	0.504	8.32	2940	0.165	3.55	170
P30	0.417	6.31	1420	0.160	2.97	160
P31	0.337	4.04	730	0.136	2.35	140
P32	0.303	3.61	550	0.169	5.45	180
P33	0.676	5.00	12420	0.233	2.47	300
P34	0.465	6.51	2130	0.153	4.33	160
P35	0.385	3.93	1080	0.232	3.70	300
P36	0.655	8.96	10460	0.128	4.61	130
P37	0.061	1.31	70	0.279	2.60	450
P38	0.477	5.75	2350	0.174	3.32	190
P39	0.290	5.25	490	0.111	4.11	110
P40	0.400	3.23	1230	0.196	2.40	220
P41	0.347	2.87	790	0.236	3.22	310
P42	0.138	1.94	140	0.294	3.97	510
P43	0.353	4.86	830	0.117	4.45	120
P44	0.440	5.86	1730	0.093	3.17	90
P45	0.454	5.65	1930	0.149	2.36	150
BLANK	-0.006	1.00	NEG	-0.008	3.17	NEG
P47	0.475	4.79	2310	0.209	5.75	250
P48	0.437	4.78	1680	0.195	6.45	220
P49	-0.014	0.94	NEG	0.251	5.05	350

PT #	HHV-6 EIA CAL OD	RATIO	ABY	HHV-7 EIA CAL OD	RATIO	ABY
P50	0.374	5.22	990	0.136	3.33	140
P51	-0.013	0.94	NEG	0.247	3.43	340
P52	0.388	3.01	1110	0.323	2.84	650
P53	0.257	3.77	370	0.152	5.33	150
P54	0.271	2.14	420	0.130	1.81	130
P55	0.582	5.91	5640	0.042	2.51	NEG
P1a [redacted]	0.346	2.77	790	0.260	4.02	380
P2a [redacted]	0.403	5.77	1260	0.193	4.99	220
P3a [redacted]	0.139	2.30	140	0.180	2.99	190
P4a [redacted]	0.418	4.41	1430	0.170	3.08	180
P5a [redacted]	0.087	1.85	90	0.204	3.82	240
P6a [redacted]	0.198	2.00	230	0.175	2.16	190
P7A	0.467	3.81	2160	0.121	3.30	120
P8A	0.255	3.40	370	0.055	2.15	70
P9A	0.068	1.61	80	0.038	1.89	NEG
P10A	0.155	2.14	160	0.103	2.15	100
P11A	0.224	2.90	280	0.214	4.19	260
P12A	0.276	3.58	440	0.199	4.75	230
P13A	0.346	4.39	780	0.263	5.17	390
P14A	0.227	3.28	290	0.217	3.69	270
P15A	0.513	6.86	3170	0.178	14.15	190

cut off 0.60

Ratio of ≥ 1.5 good

LITERATURE CITED

Ablashi, D.V., P. Lusso, C.L. Hung, S.Z. Salahuddin, S.F. Josephs, T. Llana, B. Kramarsky, P. Biberfeld, P.D. Markham, and R.C. Gallo. 1988. Utilization of human hematopoietic cell lines for the propagation and characterization of HBLV (human herpesvirus 6). Int J Cancer 15 : 787-791.

Asada, H., S. Yalcin, K. Balachandra, K. Higashi, and K. Yamanishi. 1989. Establishment of titration system for human herpesvirus 6 and evaluation of neutralizing antibody response to the virus. J Clin Microbiol 27 : 2204-2207.

Asano, Y., T. Hoshikawa, S. Suga, T. Yazaki, K Kondo, and K. Yamanishi. 1990. Fatal fulminant hepatitis in an infant with human herpesvirus-6 infection. Lancet 335 : 862-863.

Aubin, J.T., H. Collandre, D. Candotti, D. Ingrand, C. Rouzioux, M. Burgard, S. Richard, J.M. Huraux, and H. Agut. 1991. Several groups among human herpesvirus 6 strains can be distinguished by Southern blotting and polymerase chain reaction. J Clin Microbiol 29 : 367-372.

Balachandra, K., P.I. Ayuthaya, W. Auwanit, C. Jayavasu, T. Okuno, K. Yamanishi, and M. Takahashi. 1989. Prevalence of antibody to human herpesvirus 6 in women and children. Microbiol -Immunol 33 : 515-518.

Bernstein, D.I. 1991. Human herpesvirus-6 and exanthem subitum. Adv. Pediatr. Infect. Dis. 6 : 179-192.

Black, J.B., C. Lopez, and P.E. Pellett. 1992. Induction of host cell protein synthesis by human herpesvirus 6. Virus Res 22 : 13-23.

Briggs, M., J. Fox, and R.S. Tedder. 1988. Age prevalence of antibody to human herpesvirus 6. Lancet i : 1058-1059.

Brown, N.A., A. Kovacs, C.R. Lui, C. Hur, J.A. Zaia, and J.W. Mosley. 1988. Prevalence of antibody to human herpesvirus 6 among blood donors infected with HIV. Lancet i : 217.

Chou, S.W., and K.M. Scott. 1990. Rises in antibody to human herpesvirus 6 detected by enzyme immunoassay in transplant recipients with primary cytomegalovirus infection. J Clin Microbiol 8 : 851-854.

Josephs, S.F., S.Z Salahuddin, D.V. Ablashi, F. Schachter, F. Wong-Staal, and R.C. Gallo. 1986. Genomic Analysis of the human B-lymphotropic virus (HBLV). Science 234 : 600-603.

Knowles, W.A., and S.D. Gardner. 1988. High prevalence of antibody to human herpesvirus-6 and seroconversion associated with rash in two infants. Lancet ii : 912-913.

Lawrence, G.L., M. Chee, M.A. Craxton, U.A. Gompels, R.W. Honess, and B.G. Barrell. 1990. Human herpesvirus 6 is closely related to human cytomegalovirus. J Virol 64 : 287-299.

Lindquester, G.J., R.D. Allen, J.W. Castelli, R. Glenn, F.R. Stamey, T.R. Dambaugh, J.J. O'Brian, R.M. Danovitch, N. Frenkel, and P.E. Pellett. 1993. Restriction endonuclease mapping and cloning of the human herpesvirus 6 strain Z29 genome, a prototype of one variety of human herpesvirus 6 strains. in preparation

Lindquester, G.J., and P.E. Pellett. 1991. Properties of the human herpesvirus 6 strain Z29 genome: G + C content, length, and presence of variable-length directly repeated terminal sequence elements. Virology 182 : 102-110.

Lopez, C., P. Pellett, J. Stewart, C. Goldsmith, K. Sanderlin, J. Black, D. Warfield, and P. Feorino. 1988. Characteristics of human herpesvirus-6. J Infect Dis 157 : 1271-1273.

Martin, M.E., B.J. Thomson, R.W. Honess, M.A. Craxton, U.A. Gompels, M.Y. Liu, E. Littler, J.R. Arrand, I. Teo, and M.D. Jones. 1991. The genome of human herpesvirus 6: maps of unit-length and concatemeric genomes for nine restriction endonucleases. J Gen Virol 72 : 157-168.

Okuno, T., K. Takahashi, K. Balachandra, K. Shiraki, K. Yamanishi, M. Takahashi, and K. Baba. 1989. Seroepidemiology of human herpesvirus 6 infection in normal children and adults. J Clin Microbiol 27 : 651-653.

Pellett, P., G. Lindquester, P. Feorino, and C. Lopez. 1990. Genomic heterogeneity of human herpesvirus 6 isolates. In Immunobiology and Prophylaxis of Human Herpesvirus Infections. Edited by C. Lopez, R. Mori, B. Roizman and R. W. Whitley. New York: Plenum.

Pellett, P.E., J.B. Black, and M. Yamamoto. 1992. Human herpesvirus 6: The virus and the search for its role as a human pathogen. Adv in Virus Res 41 : 1-52.

Pruksananonda, P., C.B. Hall, R.A. Insel, K. McIntyre, P.E. Pellett, C.E. Long, K.C. Schnabel, P.H. Pincus, F.R. Stamey, T.R. Dambaugh, and J.A. Stewart. 1992. Primary human herpesvirus 6 infection in young children. N-Engl-J-Med 326 : 1445-1450.

Salahuddin, S.Z., D.V. Ablashi, R.D. Markham, S.F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B Kramarsky, and R.C. Gallo. 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science 234 : 596-600.

Tedder, R.S., M. Briggs, C.H. Cameron, R. Honess, D. Robertson, and H. Whittle. 1987. A novel lymphotropic herpesvirus. Lancet ii : 390-392.

Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata. 1988. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1 : 1065-1067.

SIGNIFICANTLY DIMINISHED SERUM COPPER AND ZINC CONCENTRATIONS IN PATIENTS WITH SEVERE THERMAL INJURY.

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ABSTRACT

Because of the paucity of available literature concerning copper (Cu) and zinc (Zn) requirements in patients with severe thermal injury, this study analyzed clinical records from 73 patients having more than 85% total burn surface area (TBSA) for Cu and Zn losses in the first serum samples taken after the burn. In this prospective study, the research revealed that in patients with severe thermal trauma the serum Cu and Zn levels were significantly diminished ($p < 0.0001$). These observations are in good agreement with those of Berger et al. (2), Bhattacharya et al. (4), Boosalis et al. (5,6), and Shewmake et al. (11).

INTRODUCTION

Cu and Zn are essential micronutrients for the normal function of the cell. Cu is important as a component to numerous enzyme systems, such as ceruloplasmin as reported by Aspin and Sass-Kortsak (1) and Prasad (8), as well as lysyl oxidase from Harris et al. (7). Diminished Cu levels are known to occur in neutropenia, leukopenia, bone demineralization, and impaired immune function as reported by the USRDA (9). Zn is required for the synthesis of RNA and DNA. It is also involved in the function of over 200 metalloenzymes according to Prasad (8) and Sandstead (10). There have been numerous conflicting reports regarding Cu status in patients with thermal injury as stated by Boosalis et al. (5). Also since alterations in mineral metabolism and nutritional requirements may occur during severe thermal injury-response as shown by Bhattacharya et al. (4), serum Cu and Zn concentrations were studied retro-spectively in 73 patients with severe thermal injury. The serum specimens tested in this investigation were the first ones collected post-burn to study the initial status of serum Cu and Zn concentrations in this patient population.

HYPOTHESIS

A. Since Cu and Zn are essential micronutrients, and because this demand increases during tissue repair, it is hypothesized that the serum Cu and Zn levels may diminish significantly following severe thermal injury.

B. Therefore, the validity of this hypothesis was tested in a prospective clinical study using the data collected from 73 patients with severe thermal injury with more than 85% TBSA burn.

MATERIALS AND METHODS

The materials needed for this research project consisted of the records of the clinical tests performed on patients, from the Burn Center at the Elvis Presley Memorial Trauma Center of the Regional Medical Center, Memphis, by The Chemistry and Nutrient Data Output Laboratory (CAN-DO LAB), University of Tennessee, Memphis. Flame Atomic Absorption Spectroscopy (AAS) was used for the elemental analysis in serum along with the dual Cu/Zn standards prepared and correlated with those from the US National Bureau of Standards (NBS).

The clinical records from the CAN-DO LAB were studied retrospectively from 1988 to present (July 1993). From these records, the results of serum Cu and Zn levels from the burn patients with more than 85% TBSA were recorded. Only the first serum samples collected and analyzed from each patient were used for this study. After computing the clinical records, seventy-three samples were recorded and applied toward this research. These samples were tested using the Varian 1275-Model Double Beam Atomic Absorption Spectrophotometer with deuterium background correction (Varian Techtron Ltd., Melbourne, Australia) according to Bhattacharya et

al. (3). The dual Cu/Zn standards used were at concentrations of 0.100, 0.200, 0.300, 0.400, and 0.500 µg/ml. These standards were prepared from stock solutions obtained from Varian Techtron (Palo Alto, California) containing 1,000 µg/ml, Cu and Zn, respectively. The serum specimens were prepared in a 1:5 dilution using double distilled water. The serum Cu and Zn concentrations were obtained in µg/ml and were then expressed in µg/dl. These experiment values obtained from the burn patients were then compared with the normal values of 105.0 + 3.0 (Mean + SEM) and 102.0 + 3.0 (Mean + SEM) for Cu and Zn, respectively, which were established at the CAN-DO LAB, and reported by Bhattacharya et al. (4).

RESULTS

The data collected in this prospective study support the hypothesis of this study. The mean serum values for Cu and Zn were diminished ($p < 0.0001$) by 45% and 70%, respectively, compared to the normal values.

When the Cu value of the serum was tested, 67 of the 73 patients (92%) showed values which were below the normal values of 105.0 + 3.0 (Mean + SEM). A mean of 57.51 µg/dl with a SEM of 3.15 was obtained for the first Cu serum samples tested following the burn. The results for the Cu findings are presented in Table 1. This table shows the marked decrease of serum Cu in burn patients compared to that of normal subjects. Figure 1 shows the distribution pattern and large decrease from the mean Cu concentrations of the normal subjects.

For the entire burn patient population serum Zn levels decreased to below 40 µg/dl. The normal reported Zn value being 102.0 + 3.0 (Mean + SEM). The data for the serum Zn values is presented in Table 1 and Figure 2. Table 1 shows the Zn values for the burn patients with a comparison to the normal values. For the burn patients, a mean value of 30.29 µg/dl was obtained with a SEM of only 0.71. Figure 2 illustrates the reduction and distribution of Zn concentrations in the first serum samples taken following the burn.

CONCLUSIONS

In conclusion, it has been successfully demonstrated that serum Cu and Zn levels are significantly diminished immediately following severe thermal injury ($p < 0.0001$). It appears that Zn is one of the most important and most significantly diminished elements associated with severe thermal injury. However, the serum Cu level, although significantly depressed when compared to normal subjects, does not diminish as much as the serum Zn level does in burn patients.

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REFERENCES

1. Aspin, N. and A. Sass-Kortsak. "Copper" in Disorders of Mineral Metabolism, Vol. 1. New York: Academic Press, 1981.

2. Berger, M. M., C. Cavadini, A. Bart, R. Mansourian, S. Guinchard, I. Bartholdi, A. Vandervale, S. Krupp, R. Chioléro, J. Freeman, and H. Dirren. Cutaneous Copper and Zinc Losses in Burns. Burns. 18(5): 373-380, 1992.

3. Bhattacharya, S. K., T. G. Goodwin, and A. J. Crawford. Submicro Determination of Copper and Zinc in Needle Biopsy-Sized Cardiac and Skeletal Muscles by Atomic Absorption Spectroscopy Using Stoichiometric Air-Acetylene Flame. Analytical Letters. 17(B14): 1567-1591, 1984.

4. Bhattacharya, S. K., W. L. Hickerson, S. L. Colgin, P. L. Johnson, S. King, and A. R. Whitney. Significantly Altered Zinc and Copper Levels in Serum and Urine From Burn Patients and the Role of Aggressive Hyperalimentation. J. Am. Coll. Nutr. 8(5): 429, 1989.

5. Boosalis, M. G., J. T. McCall, L. D. Solem, D. H. Ahrenholz, and C. J. McClain. Serum Copper and Ceruloplasmin Levels and Urinary Copper Excretion in Thermal Injury. Am. J. Clin. Nutr. 44: 899-906, 1986.

6. Boosalis, M. G., L. D. Solem, J. T. McCall, D. H. Ahrenholz, and C. J. McClain. Serum Zinc Response in Thermal Injury. J. Am. Coll. Nutr. 7(1): 69-76, 1988.

7. Harris, E. D., W. A. Gonnerman, J. E. Savage, B. O'Dell. Connective Tissue Amine Oxidase. II Purification and Partial Characterization of Lysyl Oxidase From Chick Aorta. Biochim. Biophys. Acta. 341: 332-344, 1974.

8. Prasad, A. S., Ed. Trace Elements in Human Health and Disease, Vol. 1. New York: Academic Press, 1976.

9. Recommended Dietary Allowances, Committee on Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council. 9th ed. Washington D.C. : National Academy Press, 1980.

10. Sandstead, H. H. "Zinc in Human Nutrition" in Disorders of Mineral Metabolism, Vol. 1. New York: Academic Press, 1981.

11. Shewmake, K. B., G. E. Talbert, B. H. Bowser-Wallace, F. T. Caldwell Jr., and J. B. Cone. Alterations in Plasma Copper, Zinc, and Ceruloplasmin Levels in Patients With Thermal Trauma. J. Burn Care Rehab. 9(1): 13-17, 1988.

TABLE 1. Serum Copper and Zinc Levels in Normal Subjects and Burn Patients

GROUP	Normal*	Burn Patients (N)
Cu (μg/dl)	105.0 ± 3.0	57.51 ± 3.15 (73)
Zn (μg/dl)	102.0 ± 3.0	30.29 ± 0.71 (73)

Figure in parenthesis shows the number of individuals studied.
*The normal serum values were established in the UT CAN-DO Laboratory.

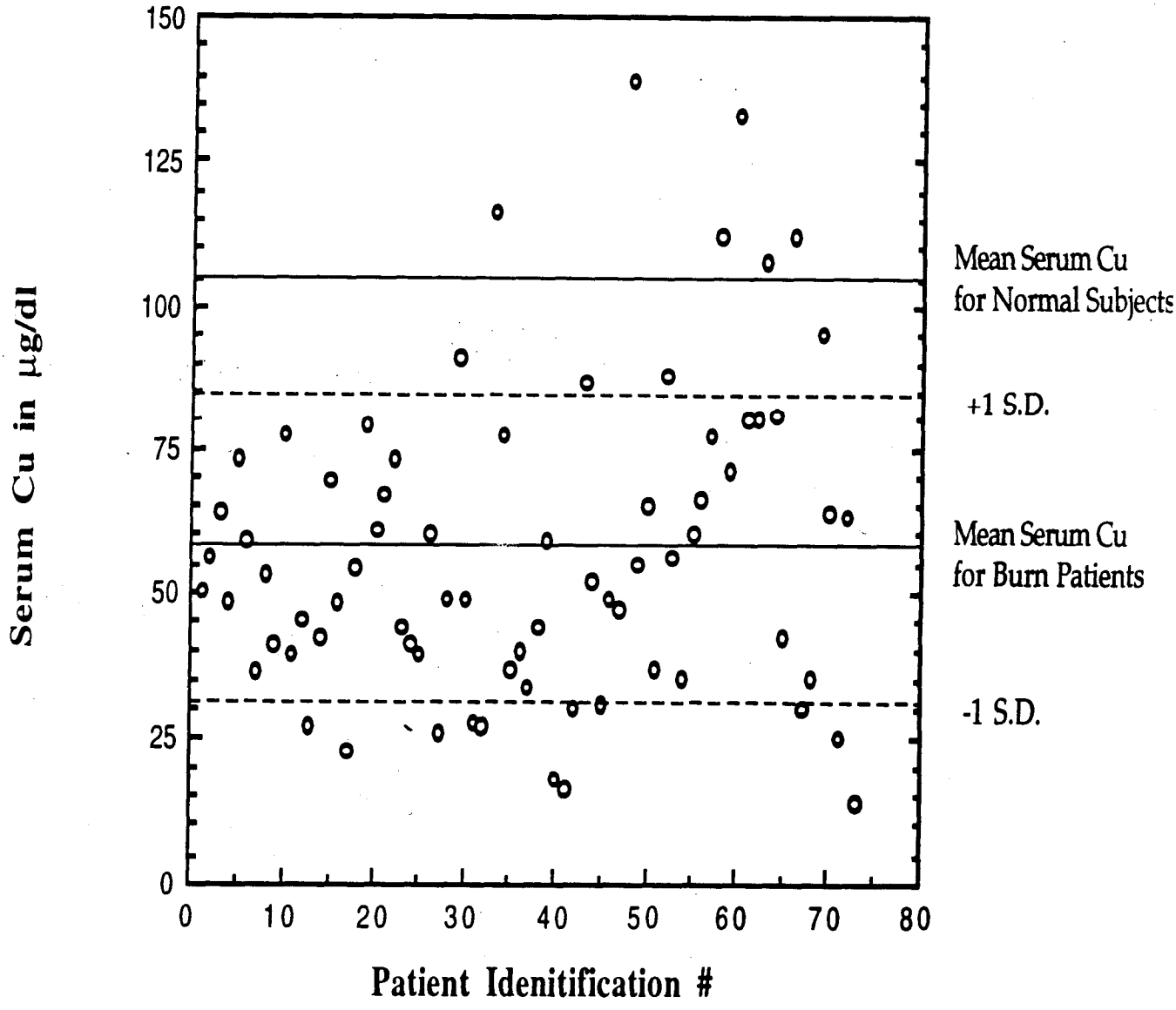

Figure 1. Distribution of Serum Copper in Burn Patients

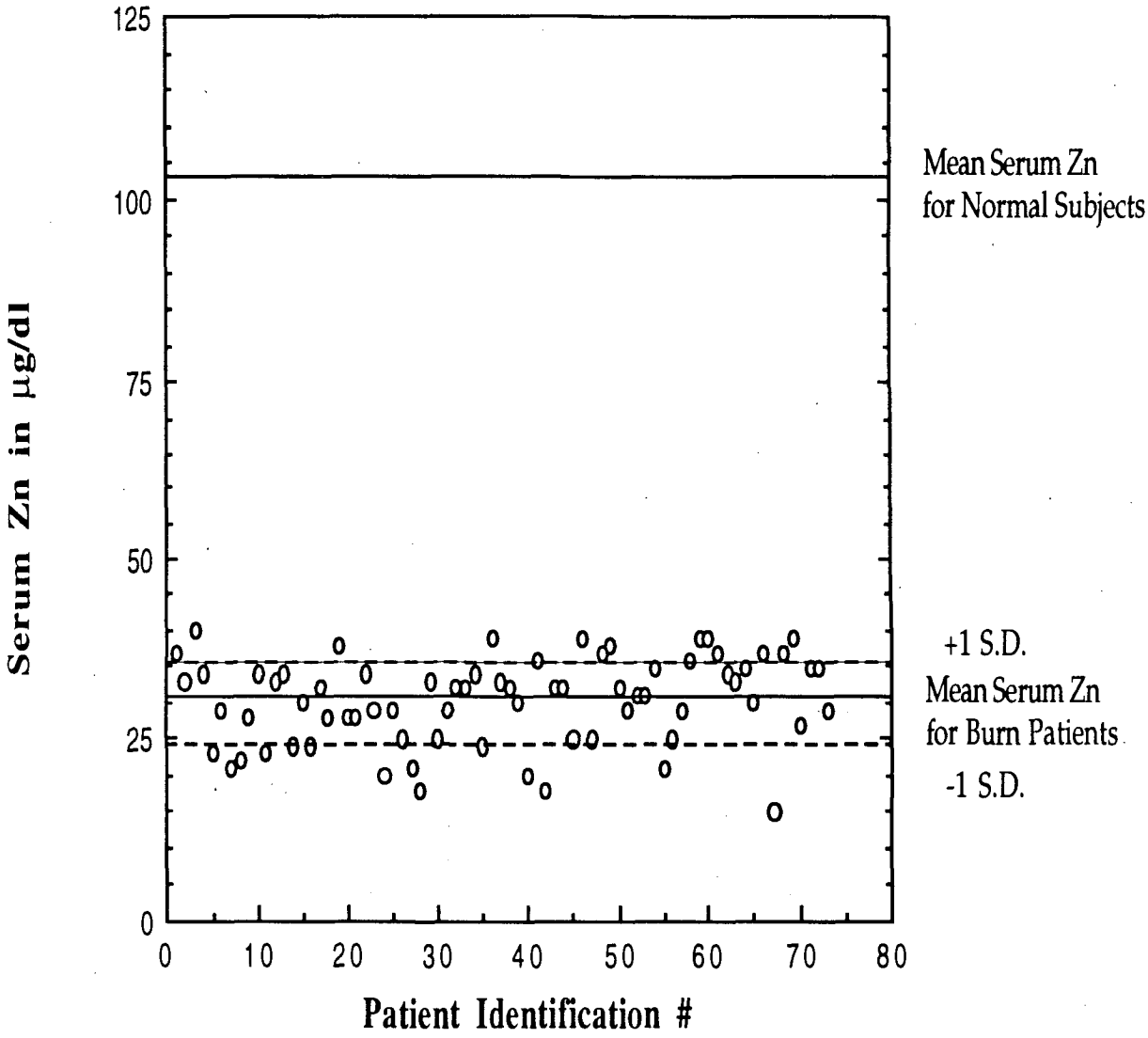

Figure 2. Distribution of Serum Zinc in Burn Patients

The Effects of Group Size on Foraging and Predator Scanning in Ground Feeding Birds

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Abstract:

Group feeding is a common phenomenon among many socially feeding vertebrates which is generally considered to confer increased protection from predators (Lima 1995). Members of a group would consequently be afforded increased protection from possible predatory attack due to the increased number of other individuals scanning for predators. I have proposed that this increased protection afforded to individuals in progressively larger groups will result in progressively larger proportions of time allocated for foraging. To test this hypothesis I measured the effects of the distance of ground feeding groups from cover and of the position of individual birds within a group, whether in the center or on the edge, on foraging (recorded in terms of peck rate). For the seven species tested, significant support was found in favor of group size affecting peck rate. Neither the effects of distance of foraging groups from cover or the position of individuals within those groups had significant effects on peck rate. The intuitive notion that there exist species-specific norms for ground-foraging rates (i.e., not all species forage at the same rate) also recieved strong support from these experimental trials.

Introduction:

Linked with increasing group size in birds and other mammals is a trend towards "...a decrease in individual levels of anti-predatory vigilance..." (Lima 1995). The explanation behind this trend is that as group size increases, each individual within that group no longer needs to spend as much of its time scanning for predators (i.e., decreased vigilance) due to the presence of many more individuals on the look-out; this has been termed as the "many-eyes" hypothesis (Lima 1990). I have used the number of pecks over a 30sec-time period as an expression of the amount of time an individual allocates to feeding. Thus, as group size increases, I expect the number of pecks to increase along with it.

In addition to measuring the hypothesized effects of group size on the number of pecks made by individual birds within a feeding group, the effects of the position of individual birds within a feeding group and the distance of individuals from cover were also tested. The idea that the position of individuals within a group affects the number of pecks made by that individual argues that the closer a bird is to the edge of a group, the more time it will spend scanning for predators. The bird is at the perimiter acting like a first line of defense or early warning lookout, which, through no conscious intention of the bird's, would not only benefit the group but would fulfill it's own self-preservation instinct since it would be closest to any would-be predators. Conversely, a bird in the center of a large group would not have to spend as much time scanning for predators since it is surrounded by so many other birds that are vigilantly scanning.

The argument for the distance of a bird from cover having an effect on the number of pecks it makes is that the further an individual is from cover, the more vigilant it will need to be since it will need the earliest warning possible to be able to get back to cover before a predator is upon it. Thus birds in groups far away from cover will be more vigilant, and thus make fewer pecks over a 30sec-time period, than birds which are in groups which are closer to cover.

There were seven species of birds used in the final calculations of this experiment: the American Robin (*Turdus migratorious*), Brewer's Blackbird (*Euphagus cyanocephalus*), House Sparrow (*Passer domesticus*), Northern Cardinal (*Cardinalis cardinalis*), Rusty Blackbird (*Euphagus carolinus*), Slate-colored Junco (*Junco hyemalis*), and White-throated Sparrow (*Zonotrichia albicollis*).

Methods:

I conducted this experiment exclusively on the campus of Rhodes College in Memphis, TN, from November 1994 to January 1995. Each trial contained the following observations: the

species of the bird, the size of the group, a foraging quantification (the number of pecks over a 30sec. time period), the distance of the bird from cover, and the position of the bird in the group.

The methodology for conducting a single trial was as follows. First, an individual or individual in a group of birds was identified and the date, time, and location of the trial period were recorded. Next, the size of that group was recorded. I randomly selected an individual either in the middle or on the outer edge of a group and recorded the number of pecks it made, and the distance of that individual from cover. If the bird was not on the outside edge or periphery of a group it was subsequently recorded as being in the center. I repeated this process for other individuals in that group or in other groups altogether.

The species of each bird was identified with the help of Peterson (1980). Birds were recorded according to their common names rather than by their Latin designation for ease of recording. The number of birds in a specific ground feeding group were counted. In the case that a particular group was extremely large (i.e., above 30 birds), a designation of "30+" or "50+" was assigned which is accurate between the range of 30-40, or 50-60, respectively. A single individual was counted as a group of size one. A ground feeding group, for the purposes of this experiment, is defined as any group of birds foraging on the ground in which the individuals of that group are relatively close to one another (i.e., no individual could be more than five to ten feet from another member of the group and still be considered a member of that group) and who respond to stimuli as a unified body. For example, a group of 40 Brewer's Blackbirds might be spread across 50 square feet but the individual members of that group would be no more than a few feet from other individuals; furthermore, this group would react, more or less, as a single body to the approach of a predator and alight into the trees almost simultaneously.

Some exceptions to the spacing of individuals in a group exist, as can be seen with American Robins who often are more than 10 to 15 feet from other members of the same foraging group. An example of a situation in which different species of birds feeding in the same area might be considered part of the same group would exist if the different species of birds responded together to stimuli; conversely, if two different species of birds were foraging in the same area but did not respond to stimuli as a group (i.e., all fly away at the same time), then I did not consider them members of the same group.

The number of pecks made by an individual bird over a 30sec-period was used to quantify the frequency of the amount of time the individual spent foraging versus the amount of time it spent scanning for predators. The distance of individual birds within a group from cover was recorded in feet. Once the distance exceeded 15 feet, distances were recorded in increments of five. What was considered cover varied from species to species. For example, while for most smaller species (i.e., sparrows, juncos, cardinals...) brush as well as trees would qualify as cover, many larger species (i.e., blackbirds, grackles,...) utilize only trees as cover.

Each trial was recorded only if the individual bird remained on the ground for the entire 30-sec. period. No artificial means whatsoever were used to attract birds; the entire study was conducted in a natural environment.

Results & Discussion:

As stated earlier, there were seven species of birds used in the final calculations of this experiment: the American Robin, Brewer's Blackbird, House Sparrow, Northern Cardinal, Rusty Blackbird, Slate-colored Junco, and White-throated Sparrow (see Appendix 2 for raw data). Eight other species were thrown out on the basis that they possessed too few trials (below ten). A ninth species' data, the yellow-shafted flicker—a woodpecker, was discarded on the basis that the time it took to peck seemed faster, due to the woodpecker's physiology which intrinsically makes it peck faster than the other species. All of the other species appeared to peck at about the same speed. Furthermore, a number of trials (approximately the first three days' data) were discarded from the beginning of the survey to decrease any prejudices that might arise over time due an increase in my efficiency and skill in recording and observing trials or finding better viewing locations, etc.

For the purposes of SYSTAT, which I used for calculation of all of the statistics in this experiment, an adjustment was made to large sized group data (i.e., greater than 30). For

example, the groups for which size was recorded as "30+" and "50+," were entered into SYSTAT as "35" and "55," respectively.

Some measure of support for the existence of species-specific norms for the number of pecks made by birds of a certain species within an allotted time frame, for distances from cover, and for normal group sizes, as opposed to random, similar norms for all birds was justified by the Kruskal-Wallis (H) test which concluded that neither the means for the number of pecks (H = 148.6740), the distances (H = 110.7602), or the position values (H = 151.4112) for each of the species were equal at the alpha = 0.005 level of significance. The means (x) used for the previous calculations were as follows in Table 1 (GS = group size; NP = number of pecks; DIST = distance; SE = standard error):

Table 1.

species (no. of trials)	x̄ (GS)±SE	x̄ (NP)±SE	x̄ (DIST)±SE
American Robin (57)	3.7±0.34	3.2±0.41	19.5±1.56
Brewer's Blackbird (26)	37.6±5.07	18.7±1.06	13.3±0.63
House Sparrow (12)	7.1±1.52	13.3±2.44	5.1±0.99
Northern Cardinal (33)	1.7±0.11	6.0±0.54	7.2±0.72
Rusty Blackbird (11)	36.9±6.90	16.8±2.00	11.4±1.36
Slate-colored Junco (31)	7.5±0.75	15.6±0.75	9.4±0.76
White-throated Sparrow (80)	6.4±4.40	13.1±0.65	3.1±0.37

These averages were further used to determine if there was an overall correlation, using all seven species, between group size and number of pecks. This correlation (Spearman's correlation, $r_s = 0.9643$) was statistically significant at the alpha 0.05 level (Spearman's correlation analysis, $z = 2.3620$), supporting a direct correlation between group size and number of pecks as is evidenced in the graph of the seven species' means for group size and number of pecks in Fig. 1. When I did this same correlation for distance ($r_s = 0.1429$, $z = 0.3500$), it was not significantly correlated to the number of pecks at the alpha 0.05 level.

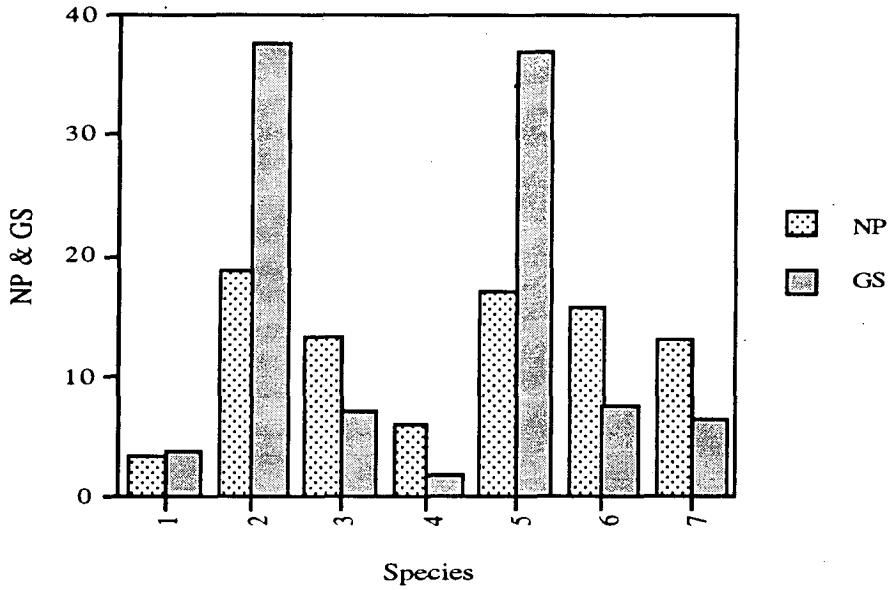


Fig. 1. This figure shows the relationship between the seven species x (in no particular order) for group size (GS) and number of pecks (NP). Generally, as GS increases, NP increases also, and thus is illustrative of the correlation between the two factors.

Additionaly, I did Spearman's correlations for each of the species individually by looking at all of the trials for a species. Out of seven species, only three correlations proved significant at the alpha 0.05 level (House Sparrow, $r_s = 0.7161$, $z = 2.375$; Rusty Blackbird, $r_s = 0.6732$, $z = 2.129$; and White-throated Sparrow, $r_s = 0.4057$, $z = 3.6059$).

I performed similar correlation analyses within each species to determine whether the number of pecks correlated either to distance or the position of individuals within a group; only one out of seven species showed significant correlation (alpha = 0.05) between the aforementioned factors and the number of pecks made by an individual bird. Thus, it seems unlikely that either of these factors has a significant effect on the foraging of individual birds.

The precise effects of group size (predicted in Fig. 2), distance from cover, and position within a group on the foraging quantification, the number of pecks per 30sec. time period, were modeled with multiple regression in SYSTAT. The resulting models (as seen in Appendix 1) predict the effects of each of these factors on the number of pecks made by an individual over a 30sec period.

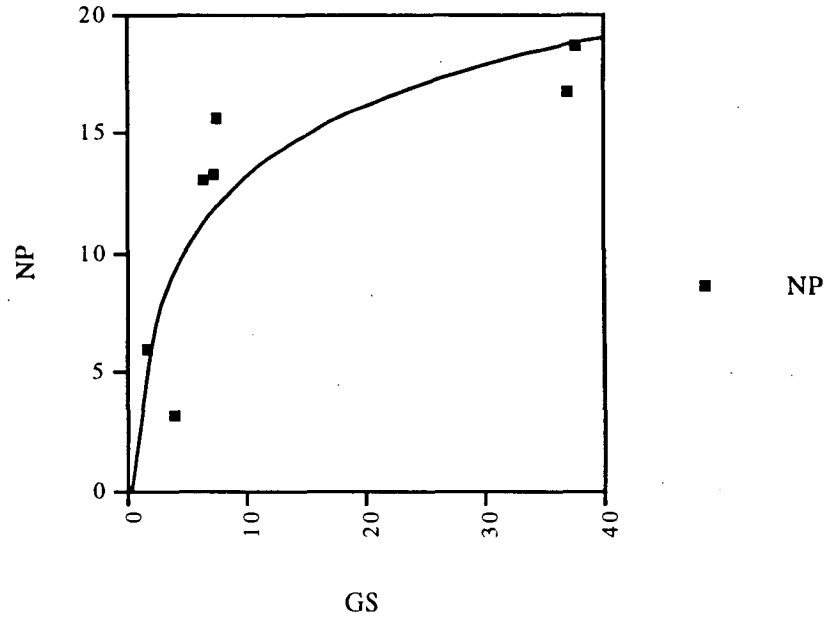


Fig. 2 This curve is a model (y=9.731LOG(x) + 3.408) which predicts the relationship between group size (GS) and number of pecks (NP) for the combined $\bar{x}$ of the seven species. NP increases with increasing GS, however, as GS increases it has a diminishing effect on the NP made by an individual.

Some of the problems that might be inherent to this experiment follow. A greater number of trials for each species will ellucidate some of the results in terms of which factors effect the number of pecks an individual makes. Also, there may have been some variation within each species due to the length of time that the experiment took place, and the resulting changes due to seasonal variation (from fall to winter) such as a decrease in the total amount of cover available to birds due to defoliation and a decrease in the total biomass available to ground foragers for feeding. Variation in group size and the number of pecks within each species from before and after the first of January were tested with the Mann-Whitney (U) test; for each of the factors, only two out of seven species displayed a significant change. I feel that this does not strongly support the claim of variation within the trials due to seasonal changes.

In conclusion, as a group, the correlation between group size and number of pecks was indeed significant (as stated above), supporting their hypothesized relationship. Neither the distance of birds from cover nor the position of birds within a group was significantly correlated to the number of pecks for the group of species. Furthermore, with each species taken individually, three out of seven of the species studied support the correlation between group size and number of pecks. The correlation between group size and the number of pecks in the bird species within this survey is similar to findings in the behaviors of other group feeders (i.e., mammals) and further supports that this correlation is common to many group feeding animals (Lima 1995).

Literature Cited:

Gadboi, S. 1993. Elementary Probability and Statistics. pp. 356.

Lima, S. L. 1990. The influence of models on the interpretation of vigilance. Interpretation and Explanation in the Study of Animal Behavior: Vol. 2. Explanation, Evolution and Adaption (Ed. by M. Bekoff & D. Jamieson), pp. 246-267. Boulder, Colorado: Westview Press.

Lima, S. L. 1995. Back to the basics of anti-predatory vigilance: the group size effect. Animal Behavior 49:11-20.

Peterson, R. T. 1980. A Field Guide to the Birds of Eastern and Central North America. Publ., Houghton Mifflin Company, Boston. pp. 384.

Appendix 1. The following multiple regression models (where C0, C1, C2, and C3 are constants; NP = number of pecks, GS = group size, DIST = distance from cover, and PSN = position within the group) predict the effects of each of these factors on the number of pecks made by an individual over a 30sec period. These models may be of use in further study of the effects of these factors on foraging.

species	**model**
[general model:	NP= C0 + C1(GS) + C2(DIST) + C3(PSN)]
American Robin	NP= 1.9848 + .0251(GS) - .0659(DIST) + 2.1876(PSN)
Brewer's Blackbird	NP= 12.2387 + .0581(GS) + .3955(DIST) - .6231(PSN)
House Sparrow	NP=-3.7557 + 1.2977(GS) + .8494(DIST) + 2.5265(PSN)
Northern Cardinal	NP= 2.9336 + .6843(GS) + .2651(DIST)
Rusty Blackbird	NP= 28.4449 - .0142(GS) - 1.2642(DIST) + 2.1125(PSN)
Slate-colored Junco	NP= 13.1234 + .1198(GS) - .1962(DIST) + 2.5037(PSN)
White-throated Sparrow	NP= 9.4345 + .5153(GS) - .3457(DIST) + 1.1406(PSN)

Appendix 2. Trial data.

date/time/loc	**species of indiv**	**group size**	**#pecks/30sec**	**dist from cover**	**position in group**
10/19/94	American Robin	4	1	5	E
1630	"	4	7	"	E
front 40	"	7	8	"	E
	"	7	9	"	E
	Rusty Blackbird	25	18	10	C
	"	50	17	"	E
	"	"	17	"	C
	"	"	11	15	E
	"	"	16	15	C
	"	60	8	15	E
	"	"	30	5	C
	"	"	19	10	E
	"	"	24	5	C
1800	"	"	18	10	C
10/28/94	Rock Dove	4	13	15	E
1415	"	"	10	15	E
fisher gardens	"	"	9	15	E
front 40	"	"	6	20	E
	"	"	11	25	E
	"	"	31	25	E
1515	"	"	11	25	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
10/30/94	Blue Jay	1	18	2	E
1220	Song Sparrow	1	25	3	E
fisher gardens	"	1	25	3	E
	Wt-thr Sparrow	3	12	1	E
	Song Sparrow	1	18	4	E
	N. Mockingbird	1	28	1	E
	Wt-thr Sparrow	4	11	3	E
	"	5	11	4	C
	"	6	0	2	E
1330	"	6	6	3	E
11/2/94	Wt-thr Sparrow	3	14	2	E
1515	"	5	16	2	E
fisher gardens	"	7	10	2	E
	"	7	9	3	E
1620	"	5	14	3	C
11/6/94	American Robin	1	4	20	E
0700	"	3	0	"	E
front 40	"	3	2	"	C
	"	4	8	25	E
	"	4	3	"	E
	"	4	2	"	E
	"	4	1	"	E
	"	4	0	"	E
	"	3	0	"	E
	"	3	9	"	E
0800	"	4	9	"	E
0830	Wt-thr Sparrow	1	11	2	E
fisher gardens					
1000					
11/7/94	Wt-thr Sparrow	8	9	2	E
1600	"	14	18	3	C
fisher gardens	"	2	12	3	E
	"	5	9	3	E
	"	4	15	1	E
	"	4	10	2	C
	"	6	14	2	E
	"	17	16	1	C
	"	17	17	2	C
	"	14	12	2	E
	"	17	16	1	C
1700	"	17	18	3	C
11/14/94	Wt-thr Sparrow	1	11	2	E
1500	"	2	7	3	E
fisher gardens	House Sparrow	15	21	3	E
frat houses	"	"	14	5	E
	"	"	30	5	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
1630	Wt-thr Sparrow	12	28	4	E
11/16/94	Wt-thr Sparrow	1	8	4	E
1600	"	1	11	4	E
fisher gardens	"	1	12	5	E
1700	N. cardinal	2	8	6	E
11/17/94	Wt-thr Sparrow	4	10	5	E
1300	Slate-col Junco	4	15	4	E
front 40	"	3	11	5	E
fisher gardens	Ylw-shft Flicker	1	31	30	E
	Brewer's Blkbrd	8	25	10	E
	"	6	12	10	E
	Rusty Blkbrd	1	7	20	E
	Common Grackle	2	17	5	C
	"	40	17	7	E
	"	10	16	15	C
	"	10	15	5	C
	American Robin	7	0	15	E
	"	"	4	15	C
	"	"	11	20	C
	"	"	7	10	E
	"	"	1	10	C
	Brewer's Blkbrd	20	18	10	C
	"	"	22	10	C
	"	"	18	10	C
	Wt-thr Sparrow	6	6	1	E
	"	4	18	1	C
	"	6	22	1	C
	"	6	7	3	E
	"	6	8	2	E
	"	5	15	2	E
	N. Cardinal	1	9	15	E
	Ylw-shft Flicker	2	35	20	E
	"	2	42	15	E
	"	2	42	30	E
	American Robin	1	0	20	E
	Ylw-shft Flicker	2	0	20	E
	Wt-thr Sparrow	8	13	8	C
	"	"	13	8	C
	N. Cardinal	1	5	5	E
1630	Wt-thr Sparrow	1	7	10	E
11/20/94	Slate-col Junco	10	11	1	E
gymnasium	"	14	19	"	C
	Wt-thr Sparrow	1	9	1	E
	"	1	9	"	E
	"	1	8	"	E
	"	1	6	"	E
1530	Slate-col Junco	4	6	2	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
1/7/94	Brewer's Blkbrd		60+	23 15	E
1130	"	30	17	"	C
fisher gardens	"	60+	15	"	C
front 40	"	"	22	"	E
	N. Cardinal	1	5	5	E
	"	"	1	"	E
	"	"	3	"	E
	"	"	3	"	E
	"	2	4	"	E
	"	"	4	"	E
	"	"	2	"	E
	"	"	0	7	E
	"	"	2	7	E
	"	"	8	5	E
	"	"	4	2	E
	Brewer's Blkbrd		60+	20 15	E
	Wt-thr Sparrow	4	17	1	E
	"	5	24	1	E
	N. Cardinal	2	5	15	E
	"	2	9	15	E
	"	3	10	15	E
	N. Mockingbird	1	0	10	E
	N. Cardinal	3	6	8	E
	Wt-thr Sparrow	4	12	1	E
	Brewer's Blkbrd	60+	20	15	C
	Wt-thr Sparrow	8	16	1	E
	"	8	18	1	C
	"	14	10	10	E
	"	"	23	10	C
	"	"	11	15	E
	"	"	12	15	C
	"	"	12	15	C
1700	"	"	18	10	E
1/8/95	N. Cardinal	1	4	10	E
1100	"	1	12	15	E
fisher gardens	"	1	5	10	E
front 40	Wt-thr Sparrow	6	19	5	E
	"	"	18	1	E
	"	"	10	1	C
	"	"	25	1	E
	N. Cardinal	1	7	10	E
	Brewer's Blkbrd	60+	29	15	C
	"	"	17	15	C
1200	Wt-thr Sparrow	6	14	3	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
1400	Wt-thr Sparrow	7	11	1	E
palmer	Slate-col Junco	9	10	12	E
	"	5	12	10	C
	"	8	16	7	C
	"	8	19	"	C
	"	8	16	"	C
	Wt-thr Sparrow	8	25	5	C
	Slate-col Junco	2	17	1	E
	"	2	20	2	E
	"	7	19	5	C
	"	8	12	12	E
	"	8	16	12	C
	"	2	14	10	E
	Wt-thr Sparrow	2	13	3	E
back 40	"	1	9	3	E
gymnasium	N. Mockingbird	1	7	8	E
	American Robin	1	3	20	E
	"	3	3	20	E
	Wt-thr Sparrow	1	2	1	E
	Ylw-shft Flicker	2	36	15	E
	"	1	45	15	E
	"	1	52	15	E
	"	1	46	30	E
	Mourning Dove	5	5	15	E
	"	5	13	15	E
	American Robin	2	3	15	E
	"	2	7	10	E
	"	2	1	10	E
	"	2	5	15	E
	Ylw-shft Flicker	1	40	15	E
	American Robin	2	3	20	E
1700	"	1	3	10	E
1/10/95	American Robin	1	0	15	E
1430	"	1	4	15	E
frat houses	House Sparrow	6	13	2	E
palmer	American robin	1	6	4	E
	House Sparrow	4	14	4	C
	"	5	23	12	C
	"	4	13	12	C
	American Robin	1	0	5	E
1630	"	11	6	15	E
1/11/95	Wt-thr Sparrow	5	3	1	E
1500	"	5	9	1	C
fisher gardens	"	3	9	1	E
palmer	"	4	16	1	C
	"	4	6	4	E
	Slate-col Junco	15	15	8	E
	American Robin	3	8	20	E
	Mourning Dove	1	20	10	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
	American Robin	13	3	15	C
	Brn-hdd Cowbird	5	10	15	E
1600	"	20	17	15	C
1/12/95	Slate-col Junco	10	20	5	E
1600	"	10	11	15	C
fisher gardens	"	10	18	10	C
palmer	"	4	16	15	E
	"	10	19	12	C
1730	"	5	9	15	E
1/15/95	Brewer's Blkbrd		60+	17 12	C
1100	"	"	21	12	C
fisher gardens	Slate-col Junco	8	17	15	C
front 40	"	8	24	5	C
	"	8	10	15	E
	N. Cardinal	1	9	1	E
	Wt-thr Sparrow	6	4	1	E
	"	10	16	1	E
	Slate-col Junco	22	17	10	E
	Brewer's Blkbrd	60+	24	12	C
	Common Grackl	"	14	12	C
1400	American Robin	2	1	50+	E
1/16/95	Wt-thr Sparrow	1	17	1	E
1330	"	5	9	1	C
fisher gardens	"	5	13	1	C
	"	5	27	1	C
	"	8	23	1	E
	"	8	17	2	E
	"	8	24	1	E
	"	8	18	2	E
	"	8	10	2	E
frat houses	House Sparrow	1	2	6	E
	"	8	13	3	C
	"	8	10	3	C
	N. Cardinal	1	3	6	E
1620	House Sparrow	3	7	3	E
1/17/95	American Robin	1	2	3	E
1530	"	1	8	4	E
frat houses	House Sparrow	1	0	3	E
back 40	American Robin	1	3	12	E
1630	Brewer's Blkbrd	2	17	12	E
1/21/95	N. Cardinal	2	6	3	E
1300	American Robin	6	0	50	E
fisher gardens	"	6	2	40	E
back 40	"	7	6	40	E
	"	8	0	25	E
	"	8	1	20	E

date/time/loc	species of indiv	group size	#pecks/30sec	dist from cover	position in group
back 40	Brewer's Blkbrd	1	23	12	E
	American Robin	5	1	40	E
	"	1	2	25	E
	Brewer's Blkbrd	9	19	15	C
	N. Cardinal	1	8	15	E
	Tufted Titmouse	2	4	5	E
1530	"	2	6	4	E
1/22/95	Brewer's Blkbrd	15	8	10	C
1000	"	15	13	12	C
fisher gardens	"	13	10	10	E
	"	13	10	10	C
	American Robin	1	0	50	E
	"	6	0	25	E
1130	"	5	4	40	C
1145	Brewer's Blkbrd		40+	23 20	E
fisher gardens	N. Cardinal	2	8	5	E
front 40	"	2	7	6	E
	"	2	6	6	E
	"	2	7	6	E
	"	2	11	5	E
	"	2	12	5	E
	N. Cardinal	2	4	3	E
	American Robin	2	0	15	E
1300	"	1	0	12	E
1/30/95	Slate-col Junco	5	16	12	E
1545	"	5	19	15	E
fisher gardens	"	5	19	12	C
front 40	"	5	22	12	E
	Common Grackle	50+	18	20	E
	Brewer's Blkbrd		"	15 20	E
	"	"	28	20	C
	American Robin	4	4	15	E
	"	4	5	12	E
	"	4	0	15	E
	"	4	3	15	E
	"	5	0	20	E
1645	Common Grackle	30+	15	20	E

Water Potential Gradients in Plants

Andy Russell, Tara Atwood, and Crystall Spence

Abstract

The water potential in most plants exists as a gradient and is a function of the height of the sample taken. For example, in most plants, the water potential will be the most negative at the very top of the plant and will decrease as you move down the plant toward the soil. The water potential should be more negative at the top of the tree in order for the water to be drawn upward in the tree from the soil and the lower reaches of the plants. The potential in the roots should be more negative than that of the soil in order for the water to move from the soil and into the roots, and not in the opposite direction. This trend continues up the plant so that water is continuously drawn toward the lowest water potential, which, theoretically, should be near the highest point of the tree. In this experiment, the water potential gradient was examined in several trees. The trees were divided into eight sections by height, with each section being of equal height. Several branches were taken from each section and placed in a Plant Water Status Console, which gave the average water potential for each sample. The values for each sample of each section were compiled to give the average water potential for the entire section of the tree. By comparing these values to one another, the gradient in water potential can be seen. This experiment was designed to show the water potential gradients that exist in plants, with the values tending to decrease with increasing in the tree; the results that were gained were in agreement with our hypothesis and the vertical water potential gradient was indeed found to exist within the sample plants.

Introduction

The method by which plants take in water through their roots from the soil is osmosis. In order for this to occur, the water potential of the roots must be more negative than the potential of the soil, since water always moves to an area of lower water potential. **Water potential** is defined as *"the chemical potential of water in a system or part of a system, expressed in units of pressure and compared to the chemical potential of pure water at atmospheric pressure and at the same temperature,"* [4]. The water potential for pure water was arbitrarily set at 0. The water potential scale starts at 0 and then as the water potential increases the numbers become more negative. This explains how the water initially moves into the plant, but not how the water moves to the various parts and areas of the plant. As the water moves into the roots, and from there into the xylem, the pressure builds up until the water is forced upward through the xylem. An opposite force is also in effect, and that is the force of gravity. As more and more water enters this column of water in the xylem, it becomes heavier, resulting in a need for more water to enter in order for the water to move up the plant. This system of transport works in short plants or in the lower few feet of trees because of the increasing force of gravity. In order to draw the water up to the highest points, however, the transpiration cohesion system is used. Transpiration is occurring in the leaves, allowing water to move out of the plant. Since water is highly cohesive, when water exits the leaves by transpiration, the contents in the xylem column move up the plant and does not break due to the cohesion between the water molecules. The driving force in moving the water up through the xylem is water potential; the water in the leaves wants to exit into the air because the potential of the air is more negative than the potential of the leaves. Since the water exits the mesophyll cells, they now pull water up from the xylem (which has a less negative water potential) to replace the water that they have just lost to the atmosphere. Therefore, if this hypothesis is true, water potential must be maintained in a gradient which decreases as the height of the plant increases, in order to allow for the continual upward movement of water through the tree. The relative humidity is also an important factor influencing the plant in this transpiration cohesion theory. As previously stated, water is drawn into the mesophyll cells in the leaves from the xylem. These cells have thin walls and those cells on the upper leaves are exposed to much light. This light evaporates the water in the cells and makes the relative humidity in the cells higher than that of the outside. This allows the water to follow the osmotic gradient and the steam exits the leaf through the stomata. As the steam exits, water is pulled up through the xylem to take its place. As

this process takes place in all of the leaves large amounts of water are pulled up the tree. The higher up the leaf is, the more negative the water potential needs to be.

Water does not enter the roots in sufficient amounts, however, to simply overcome the gravitational barrier to water distribution throughout the plant. In order for gravity to be overpowered, the water potential must be lower in the higher regions of the plant in order for the water to move to the higher reaches. Nicanor, et al, proved that the potential will be more negative in the higher regions, as well as the regions that were more distant from the roots. They state that "measurements obtained using leaf segments were 0.05 to 0.06MPa higher than those obtained from leaf tips,"[3]. Therefore, as stated before, we expect the water potential to become more and more negative as the height of the tree increases.

Many factors influence the water potential of the plant. Temperature, amount of sunlight, and the relative humidity all affect this value. The humidity of the environment was found to play a determining role in the determination of water potential by Heike and Hortge. They found that humidity and water potential gradients are connected; at a constant humidity, the water lost by the tree increases with an increasing mean water potential gradient. As humidity increases, the amount of water lost to transpiration decreased, so water potential increases,[2].

How do the upper reaches of the plant come to gain this decreased water potential? These top branches will be exposed to more sunlight than the lower branches; this allows for more transpiration to occur. With much water leaving due to the transpiration, the water potential of these upper leaves will become more negative. Since these are now at a lower water potential than the leaves immediately below them, they will draw the water up out of these lower leaves. The middle-level leaves are now giving up water to the branches above them, so the potential of these middle branches and leaves are now more negative than the branches immediately beneath them. Water will now flow into these middle branches from the lower branches, causing the potential of the lower branches to becomes more negative. This continuum of branches drawing water from the branches immediately below them, extends up and down the height of the tree.

In order to test this hypothesis about water potential gradients, portions of the plants were removed and placed in a pressure chamber to determine the potential of each segment. This method was shown to be very effective in this form of investigation; "the protocol proved to be an accurate and reliable way of measuring water potential...using the pressure chamber technique," [3]. This method was chosen instead of another method which installs tensiometers in the soil flush with the soil's surface. This technique is used primarily in determining the potential of grasses and very short, non-leafy plants,[1].

Materials and Methods

The trees that we did our investigation with were holly trees, which had vertical growth patterns, and were fully in leaf. A yardstick was needed to measure the height of each plant and to measure the heights of each section. A roll of masking tape was also needed in order to demarcate the individual sections. Small scissors and a razor blade were needed for cutting the individual branches. A Plant Water Status Console Model 3000 was also used; a nitrogen tank was needed for the operation of this apparatus. A Sartorius balance was used to weigh the material in the determination of the fresh and dry weights. In order to find the dry weights, an oven was needed to heat and dry out the leaf material.

After the plants were chosen, the trees were measured and then each tree was divided into 8 equal sections, beginning at the uppermost branch on the tree and ending with the branch closest to the soil.

Starting with the top section of the plant, branches were removed and were placed in the pressure chamber one branch at a time. The instructions for operation of this machine can be found in the lab handout. Several samples from each section were tested and the water potential values for the branches in each section were averaged; this gave the average water potential for the section. This protocol was followed in examining all of the trees. The average values for the top sections of all trees were taken, as well as for the second through the final section.

The leaves from the sample branches in each section were then removed from the branches and weighed, collectively. This weight is called the fresh weight of the material, (FW). The leaves were then placed in an open bag and placed in the oven for 48 hours. After this time, the leaves were weighed again, yielding the dry weight of the material, (DW).

Results

The data shows the average water potentials of each of the eight sections. The average water potentials of these sections were as follows: section 1 was -14.6 MPa; section 2 was -13.89; Section 3, -12.92; section 4, -12.28; section 5 was -12.53; section 6 was -11.6; section 7 was -10.68; and section 8 was -10.2 MPa. These sections were numbered according to their heights on the trees. Section 1 was the highest section of the tree, and section 8 was the very bottom of the tree. This data is shown in **Figure 1.**

Figures 2 and 3 show the compared wet and dry weights of each of the measured sections of the tree as a function of height and water potential. The average wet and dry weight for section 1 was 21.84g and 17.23g, respectively. Section 2 was 59.21 and 47.96g. Section 3 was 32.28 and 26.77g. Section 4 was 34.5 and 25.14g. Section 5 was 40.4 and 30.97g. Section 6 was 82.32 and 64.29g. Section 7 was 84.91 and 66.91g. Section 8 was 23.62 and 21.15 g. All of this information can be found in Table 1.

Discussion

In this experiment, we did find a gradient in water potential that decreased as the height of the section increased; however two sections did not completely conform with this gradient. They were only slightly deviant from the linear relationship that exists between the potential and height of each individual section. The slope of the line that runs through our data points has a correlation coefficient of 0.965, which leads us to believe that this slight disruption in the gradient is not of great importance. We are attributing this shift from the linear progression to the shape of the tree. The size and shape of the tree play an important role in the determination of the water potential because of the amount of material to which the water must travel; the data in Table 1 shows that Section 4 has the lowest dry weight of all of the sections, excluding the very top and the very bottom sections. Since this portion of the tree had such little leaf material present, not as much water was required for the survival of this section and, hence, the water potential value for this section was less negative.

The hourglass figure of the holly trees might also have contributed to the shift in the water potential because of differing light levels that reached the various parts of the plants. The bottom parts of the plants were exposed to more light and wind-driven water loss; both of these factors would cause a decrease in the water potential readings.

Another possible explanation for the deviant values would be inconsistencies in the sample size. By using a large sample branch which contains much leaf material, a greater pressure would be needed to pull the sap out of the xylem, and a more negative water potential reading would be the result.

Overall, the deviation from the actual gradient that exists is very small, and the discrepancy that was found is negligible, considering the many possible sources of error. We believe that the small deviation from the existing gradient is due to our error, and it does not truly exist within the trees under examination.

Acknowledgments

We would like to thank all of the 1995 Plant Physiology class for their participation in this experiment and for cooperation. We would also like to thank Dr. Stinemetz for his help and instruction.

Table 1			
Section	Water Potential (MPa)	Fresh Weight (g)	Dry Weight (g)
1	14.6	21.84	17.23
2	13.89	59.21	47.96
3	12.92	32.28	26.77
4	12.28	34.5	25.14
5	12.53	40.4	30.97
6	11.6	82.32	64.29
7	10.68	84.91	66.91
8	10.2	23.62	21.15

Figure 1
Water Potential as a Function of Height

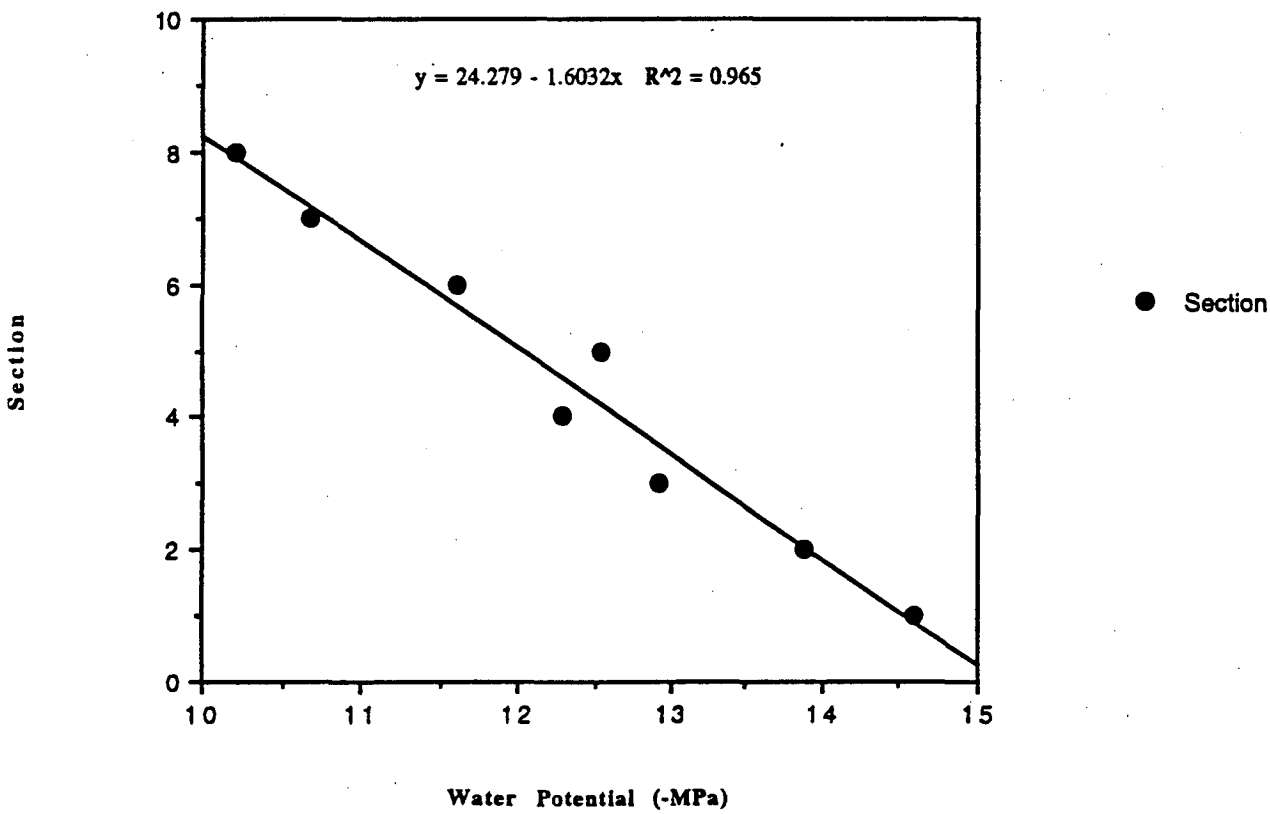


Figure 2
Fresh and Dry Weights as a Function of Water Potential

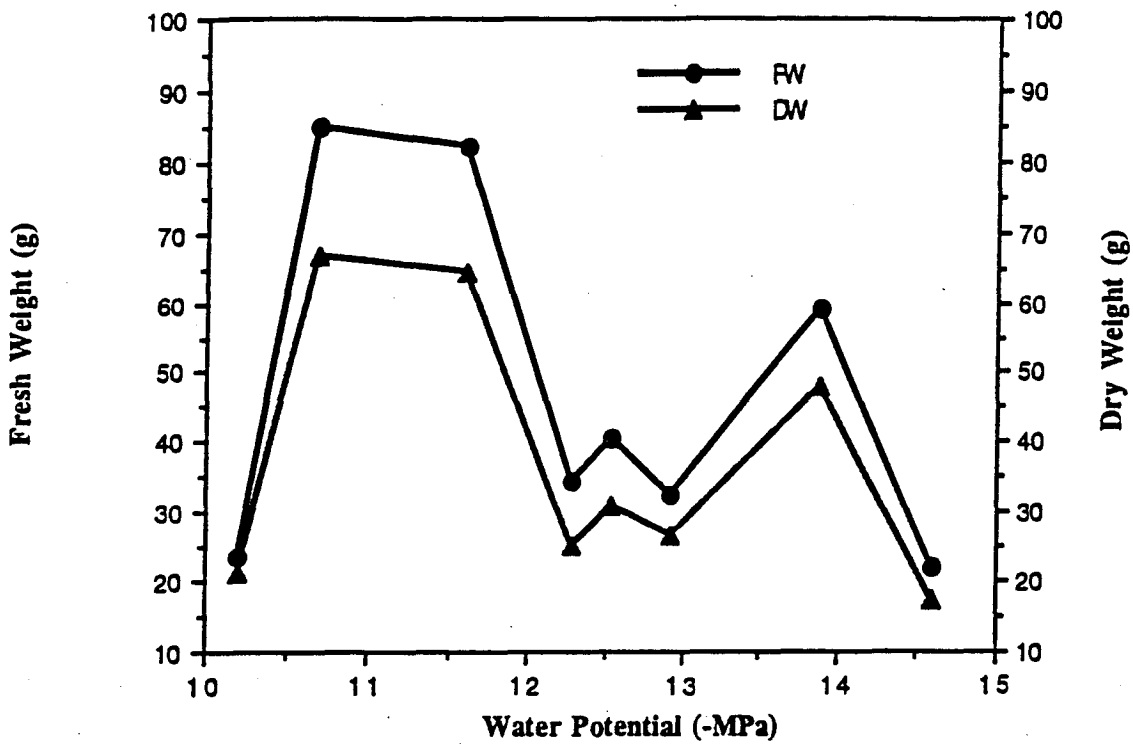
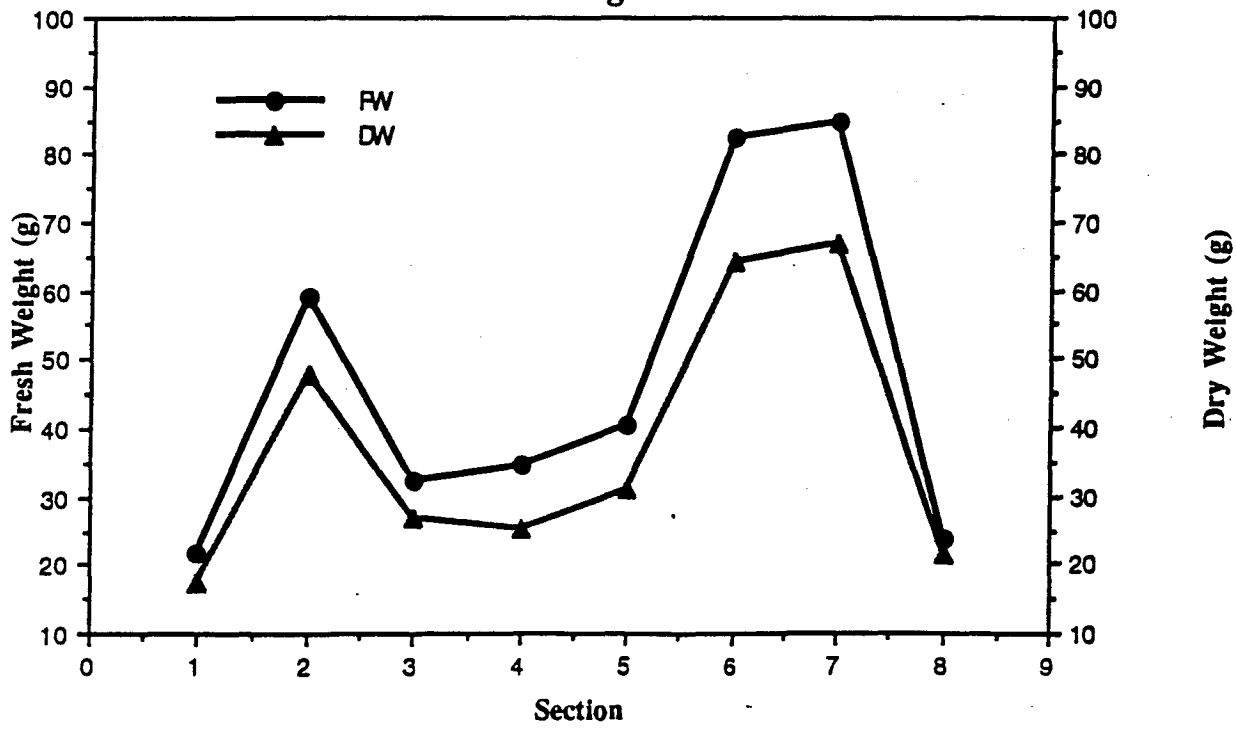


Figure 3
Fresh Weight and Dry Weight as a Function of Height



Interviews

The folllowing interviews were conducted in order to find out what some recent graduates are doing with their degree out in the real world, and what advice they might have for those of us who are still up and coming. Here's what they had to say.

Nancy Turner, originally from Houston, TX, graduated from Rhodes in 1994 with a B.S. in biology. While at Rhodes, nancy was active in Alpha Omicron Pi sorority, serving as Philanthropy Chair and Rush Chair. She also acted as editor of the *Rhodes Science Journal* and as secretary of Beta Beta Beta, the biological honor society. Her senior year, Nancy served as president of the Rhodes Student Assembly and was a member of Omicron Delta Kappa, Mortar Board, and Order of Omega honor societies.

Question: What do you recall as your most memorable or significant experience during your science training at Rhodes?

Answer: Molecular Biology class; being able to do independent study research as a freshman.

Question: What aspect of your training do you utilize most in your career/present job, and what would you recommend to students as "dos" and "don'ts"?

Answer: At present, I am not using my science background in my job but I may use it in future occupations. I would recommend that science students at Rhodes take a wide variety of liberal arts courses(which they really have to anyway) to prepare them for the "outside" world. Although science is wonderful, the world doesn't run on it alone and you need to have solid foundations in other disciplines as well.

Question: How has your science training specifically fit into your career?

Answer: Right now my biology training does not fit into my career.

Question: Is there any course yuo would like to take over, and why?

Answer: My favorite biology courses were Molecular Biology, Virology/Immunology, and Genetics. It might be nice to review these courses again after having completed them because that might allow me to view each of these topics in their entirety instead of concentrating on the particular facts for each unit of study within each of these courses.

Question: What aspects of the science program at Rhodes would you change or leave the same?

Answer: I would like to see more non-academic activities provided for science students and faculty to get to know each other better. I think the science departments should "bond" more!

Libby McCann received a master's degree in Natural Resource Policy and Administration with an emphasis on Advocacy and Environmental Education from the University of Michigan where she taught courses in Natural Resources and Biology. After graduate school she spent five months cycling New Zealand and working on Organic farms in that country. Currently, she coordinates an environmental education program called Adopt-A-Lake which is part of the Wisconsin Lakes Partnership.

Question: What do you recall as your most memorable or significant experience during your science training at Rhodes?

Answer: There was a relatively small number of senior biology students the year I graduated from Rhodes which enabled us to become close friends. One memorable experience is the Senior Seminar course. Senior Seminar was the first opportunity for the seniors and professors to share their experiences and what they saw as challenges in the scientific field. Discovering that we all had some similar concerns and interests was somehow comforting in the light of the seniors imminent departure into the "real world" of professional scientific pursuits.

One of the more positive aspects of attending a liberal arts college like Rhodes is the small class size and student-teacher ratio. I believe the mutual respect professors and students shared fostered a more positive learning environment.

Question: What aspect of your training do you utilize most in your career, and what would you recommend to students as "dos" and "don'ts"?

Answer: The science courses(as well as courses in other disciplines) challenged me both intellectually and personally to look at issues from a variety of perspectives. I developed these critical thinking skills not only in terms of the "scientific method" of research, but also from a more social and political perspective. Such courses such as Ecology and Evolution introduced me to various theories and issues which I hadn't questioned in the past; the intellectual environment at Rhodes fostered in me the interest and desire to pursue these issues in greater depth. A good measure of how invaluable my experiences at Rhodes truly were came to me when I entered the graduate program in Natural Resources at the University of Michigan- Ann Arbor. I quickly realized the skills and knowledge I acquired at Rhodes had (thankfully) prepared me incredibly well for the rigors of graduate school.

I recommend that students don't sweat the little stuff. It is easy in an academic environment to be consumed over measures of performance such as grades, getting into graduate school, medical school, etc. While those elements have their place there are other less tangible measures of success which are of critical importance- the lifelong friends you make, and the personal and intellectual challenges you undertake are among a long list of items you may never put on a resume, but which are likely to be important in the scheme of things.

Question: What aspects of the science program at Rhodes would you change or leave the same?

Answer: I would have enjoyed more opportunities for discussion of current scientific issues in a larger social context among my peers and professors. It would have been helpful to address such subjects as gender issues, scientific research in a political context, scientific ethics, etc. in perhaps a more structured, yet open, setting at Rhodes. Also, as is true in many disciplines, it can be fairly easy to isolate ourselves, particularly intellectually, from the larger social context of our academic pursuits. We need avenues to develop the kind of critical thinking skills necessary to effectively pursue scientific endeavors in the current social and political climate, recognizing the need for interdisciplinary learning. I think Rhodes could be at the forefront of building linkages among various disciplines, looking at an array of issues which cut across strict disciplinary lines.

Julie Henderson, who graduated in 1994, was a chenistry major at Rhodes with an emphasis in the boiochemistry tract. She was involved in Rhodes College Singers for two years, and was a member of Alpha Omicron Pi sorority, in which she served as Treasurer, Sr. Panhellenic Delegate, and Rho Chi. She participated in an internship her senior year for Flavorite Laboratories, Inc. in Horn Lake, MS and also worked in the chemistry storeroom for two years. She is currently working for Valvoline Car Care Products as a lab technician in Hernando, MS and lives in Memphis, TN.

Question: What do you recall as your most memorable or significant experience during your science training at Rhodes?

Answer: The personal help the professors could give you. They all had a way of making you feel that they truly cared about what you learned from them.

Question: What aspect of your training do you utilize most in your career/present job, and what would you recommend as "dos" and "don'ts"?

Answer: I use some of my instrumental and chemical knowledge but not a whole lot. I would say I don't expect too much from your forst job-- you may not use many of your science skills. I recommend you learn all that you can because you never know when you will need certain skills.

Question: How has your science training specifically fit into your career?

Answer: As a whole my science training doesn't fit into my job. I was hired because I have a chemistry degree, but at present I am unable to do all of the "chemistry things" they would like me to do. Even those things include only a fraction of the training I had.

Question: Is there any course you would like to take over, and why?

Answer: There is no course I would like to take over again, but since I was biochemistry track I didn't get the opportunity to take Instrumental Analysis or Advanced Inorganic, which are two courses I would have liked to have taken.

Question: What aspects of the science program at Rhodes would you change or leave the same?

Answer: I would recommend that anyone plannng on going into the working world of science should take Instrumental Analysis and some courses that have you interact with other people. Since the science program seems like it is made for those who will continue in school, I would add some things to help those planning on a career immediately following their training at Rhodes.

Renee Pardieck attended Rhodes from 1989 to 1993, and received a B.S. in biology. She is presently working on her master's in marine science at Virginia Institute of Marine Science, William and Mary.

Question: What do you recall as your most memorable or significant experience during your science training at Rhodes?

Answer: I really enjoyed field trips in Ecology class. We conducted experiments in lakes and rivers and got a taste of field research, learning different methods of studying water chemistry, and plankton and benthic sampling. I have encountered many of these methods in marine science. I alos love to work in the field. This is one of the reasons I am studying marine science.

Question: What aspect of your training do you utilize most in your career/present job, and what would you recommend as "dos" and "don'ts"?

Answer: My reaearch involves statistics, experimental design, field work, background literature research, and scientific writing. Some advice for anyone pursuing a master's or PhD degree in science: Make sure you are truly interested in the research topic you choose. You will work in this topic for several years in completing a degree. Even the simplest question takes an enormous amount of work to answer. Some of the work is tedious. Last year, I greatly enjoyed conducting my experiment in the field; I also spent several months sorting samples and counting blue crab post larvae. The reward comes with anticipating results and discovering something new. If you are going into ecology, learn as much about experimental design and statistics as possible.

Question: How has your science training specifically fit into your career?

Answer: My training at Rhodes has led me in differnt directions of research and provided a framework from which to understand the marine system. Many of the concepts presented in graduate classes were variations on concepts that I learned at Rhodes.

Question: Is there any course you would like to take over, and why?

Answer: I learned a lot of biological facts at Rhodes - processes of photosynthesis, evolution, physiology, etc. I wish I had taken more math and physics. Marine science is a highly interdisciplinary field requiring some understanding of biology, physics, and chemistry. You can imagine how marine organisms are affected by tides, storms and currents, changes in salinity and water chemistry. The interactions of different forces are fascinating. My recent research has focused on how blue crab postlarvae sense flood tide near estuaries by changes in pressure and chemical cues. It is probable that physical forces strongly influence blue crab distributions. I have learned a lot about physics since I have been here, but a basic background in physics would have helped me along.

Question: What aspects of the science program at Rhodes would you change or leave the same?

Answer: Senior Seminar was a good experience for me. We wrote a grant proposal and presented our project at the end of the year. I became very interested in designing my experiment. For research oriented people, however, I think more formal and methodical training in experimental design, scientific thinking, and statistics is needed. Concepts such as multiple working hypotheses, experimental planning, use of controls, "strong inference", and replication are very important.

Research Information in Biology

Dr. Gary Lindquester: I have been working on the molecular biology of human herpesvirus 6(HHV-6) for the past 8 years since the virus's discovery. HHV-6 is the etiologic agent of roseola, a mild childhood disease characterized by a fever and rash of short duration. Recently, some exciting results have been presented suggesting a possible correlation between HHV-6 and multiple sclerosis. My work with HHV-6 has involved the cloning, mapping and characterization of the double-stranded DNA genome and studies on the sequences involved in the initiation of DNA replication.

During the 1995-1996 year I will be on sabbatical leave pursuing research on a group of herpesviruses which infect horses. I will be involved in the cloning and characterization of genes which express surface glycoproteins on these viruses. Besides telling us about their structure, function and evolution, this project will yield viral proteins which could serve as antigen for use in the development of vaccines.

Most recently, I have entered into collaboration with Dr. Hill with the goal of isolating and studying the cellulase gene from *Achlya ambisexualis* which he hypothesizes is involved in the process of branching growth. Students have participated in many aspects of the research I have described, and I am always happy to talk with students about the possibility of working in my lab.

Dr. Alan Jaslow: My research interests are in the areas of vertebrate functional morphology and animal behavior. Past student projects include studies of mammal hair ultrastructure, mimicry, acoustic behavior, size and shape relationships in vertebrae, and scaling relationships in leg bones of ungulate mammals and ratite birds. I am working with several students and I keep a wait list.

Dr. Terry Hill: My research deals with the mechanisms of growth of microorganisms, especially of fungi and fungus-like protists. I've been investigating the mechanisms of secretion of enzymes involved in nutrition and morphogenesis- in the latter case, those that restructure the cell wall, permitting growth. The methods employed are those of traditional enzymology and protein chemistry(spectrophotometric enxyme assays, ultracentrifugation, gel exclusion and ion exchange chromotography, and electrophoresis), and more recently I've begun to employ immunochemical methods - i.e., those involving antibodies to locate and characterize the proteins of interest. For about the past year, I've been collaborating with Dr. Loprete in Chemistry on some of these projects, and we've shared direction of some students working on the characterization of cellulase enzymes in the protist *Achlya ambisexualis*..

In recent years, students have been involved in all aspects of the work, from enzyme isolation to organelle isolation to raising monoclonal antibodies against some of the proteins important to fungal growth. This term, students have also begun a project aimed at isolating the messenger RNAs produced during fungal growth, mainly under the direction of Dr. Lindquester. There is plenty of opportunity for students to approach the work from a variety of angles. If we find room in our academic calendar to teach the Electron microscopy course again, I'd also like to get students involved in some ultrastructural studies.

Dr. Carolyn Jaslow: My primary research interests are in the areas of biomechanics, functional morphology, and the development of bone and other mineralized tissues. Biomechanics is the application of physical and engineering laws to the study of biological materials: for instance, can we predict the design of a tooth or leg bone based on the forces and physical demands that structure will experiance? Biomechanics is part of the study of functional morphology, which seeks to correlate an organism's form(morphology) with its functional or ecological demands. I am willing to take on students for projects in these areas depending on their previous course experiences and their interest.

Dr. David Kesler: My current research deals with freshwater mussels. These threatened species have dwindled due to degredation of water quality, habitat loss, and invasion of exotic species. I am interested, among other things, in the rate at which these organisms grow and for how long they live. I have measured internal rings as indicators of age and growth rate. I have also marked, measured, and annually sampled mussels. These two estimates of growth rate do not agree, and I am in the process of figuring out which one is correct. While this research is not appropriate for students, I will support a wide range of products dealing with freshwater ecology. For example, lately students have conducted research on the role of predation on larval dragonfly community structure, limnological characteristics of an area lake, and sedimant load carried by the wolf river.

While I might seem more enthusiastic if student research dealt with my new interest in dragonfly biology, or the distribution of the threatened mussel found in the Wolf River, or other such invertebrate ecological topic, I have a higher priority. I am of the philosophy that "finding the problem" is important. If a student is interested in a realistic problem, I'll support it.

Dr. John Olsen: My current interests revolve around two different kinds of technology and the use of data generated by these technologies to answer evolutionary questions. I have had students using the scanning electron microscope(SEM) to address two different kinds of questions. On the one hand, we have been looking at ultrastructural data that may be useful in the investigation of systematic questions in flowering plants, primarily in the sunflower family. The other SEM focus has been on the structure of various fibers. Weavers have long known that different fibers (from different plants, animals, and also synthetics) have differnt properties. I have had students working on the SEM analysis of natural and synthetic fibers froma purely descriptive point of view.

The other technology I employ is high performance liquid chromatography (HPLC). In this regard I am developing baseline data for the use of HPLC in the separation and identification of secondary plant compounds(flavonoids). Students interested in this would be expected to learn the operation of HPLC, they would be running various standards, both singly and in combinations, and they would be testing different solvent gradient protocols for their efficiency in separating the flavonoids. I am certainly willing to sponsor student research projects.

Dr. Bob Jones: My research is on the genetics of esterase enzymes in the Cotton Boll Weevil. Weevils from the Gast Boll Weevil Rearing Laboratory and weevils collected from cotton fields in Shelby County are examined for the frequency of various esterases. Wild weevils are crossed with laboratory ones to culture genes for other studies.

Research in Chemistry

Dr. Helmuth Gilow's research concerns the investigation of the chemistry of (RS02)2CH2 and similarly reactive methylenes. He is especially interested in : 1) minimizing the steric effects of the bulky sulfonyl groups, 2)reactions that will proceed rapidly under mild conditions and in high yield, 3) synthesis of heterocyclic systems, 4) synthesis of some new polymers and 5) conversion of the disulfonyl substitutions to other functional groups. Students are welcome to participate in this research as much as they want to.

Dr. Bradford Pendley's work involves the characterization and use of ultramicroelectrodes. An ultramicroelectrode is a micrometer sized wire encased in glass with one end polished to expose a disk and the other end used to make electrical contact with an instrument. These devices can be used to study electrochemical reactions in atypical media. Dr. Pendley plans on working with at least one student next year and is always open to having students do research with him throughout the year, including the summer.