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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 five 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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## SOCIAL BEHAVIOR OF ORANGUTANS

Anne Marie Basarrate†

### ABSTRACT

*Behavioral studies have shown that captive primates exhibit social behaviors, and by virtue of the individual selectionist approach, these behaviors will increase the reproductive success and personal or inclusive fitness of the individual. Female primates form a dominance hierarchy whereby rank is inherited from mother to daughter. It was found that a mother orangutan keeps her baby in direct contact more frequently when a non-related female is present.*

### INTRODUCTION

Through a plethora of behavioral studies on captive primates, it has been found that there do exist certain conditions that precipitate the evolution of particular patterns of social behavior through natural selection. In accordance with the individual selectionist approach to natural selection, these behaviors must increase the reproductive success and personal fitness of the individual, or, if the behavior is costly, the reproductive success of close relatives enough to offset the cost to personal fitness. Because of the relatively long life span expected for primates, forty + years for orangutans, it is difficult to directly correlate the effects of a specific behavior on individual fitness; it is thus better to examine the effects of the behavior that are likely to be correlated with fitness—a decrease in infant mortality (Cheney *et al.* 1986). Cheney *et al.* (1980) also report that primates exhibit complex social relationships that lead to increased individual reproductive success, and that these social relationships may be based on a sophisticated, cognitive mechanism of kin recognition.

The structure of these social relationships seen in groups of female primates living together, whether naturally or in captivity, is based on a dominance hierarchy whereby kin are treated preferentially. In primates, female rank tends to be inherited across generations; thus, mothers pass dominance rank to their daughters and tend to keep close ties with them throughout their lives. In addition, the daughter's rank is found to be adjacent to that of the mother. In fact, a study of free ranging yellow baboons, *P. cynocephalus*, found that during the first ten years few changes in dominance were seen (other than by death or maturation of the young females). At the end of the ten-year period, most of the adult females in the group occupied ranks that conformed to the positions occupied by members of their matriline a decade earlier (Hausfater *et al.* 1982 in Samuels *et al.* 1987). It was also found that mothers preferred associating with their daughters: they approached adult daughters more often than other adult females. In addition, females without mothers still alive experienced a much higher infant mortality rate (72% as opposed to the average with mothers of 32%) and lower fecundity. Many of these females did not even achieve their birth rank. And the higher ranking females had priority access to scarce resources and were thus more reproductively successful themselves. Therefore, matrilinear rank inheritance

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appears to be an adaptation to a particular set of ecological and social circumstances that require relatively large groups of related and unrelated females to live together in an area where there exists a positive association between rank and reproductive success (Fairbanks *et al.* 1986).

Parker (1974) proposed a "game theory model" to predict the relationships of rank with age. He believed that the females with less to gain (a smaller "fitness budget") should withdraw from an aggressive situation before the contest escalates. Hence, Parker hypothesized that as female grows older and her reproductive value declines, she should be less likely to initiate confrontations and more likely to submit when challenged (Parker 1974 in Fairbanks *et al.* 1986). However, the theory that dominance is inherited and that ranking is dependent of relatives helps explain why older females actually continue engaging in agonistic behaviors. Fairbanks and McGuire (1986) reported that adult females do not reduce their involvement in aggression and other dominance-related behaviors, even as they get older and their reproductive value declines. This personal decline in reproductive value is countered by the possible gain in reproductive success of present and future relatives. If the older female were to passively submit her rank when challenged, she would be reducing her inclusive fitness because a shift in rank of one female will subsequently effect the rank of closely related females, as well as that of her daughters and future granddaughter. If a female improves her rank, she effects both her possible personal and inclusive fitness. And if the mother must make a choice between helping two offspring, the tendency (as seen among macaques) is for the mother to increase her inclusive fitness by helping the daughter with the highest reproductive gains, i.e. the younger one. Furthermore, females consistently support their female relatives during agonistic encounters with members of other geneologies. Thus, when two females meet in an agonistic contest, the outcome is influenced not only by individual attributes, but also by the degree of support each female receives from her close relatives. And this observation that kin support each other is central to the concept of inclusive fitness. In fact, studies of Japanese and pigtail macaques show that these females not only associate with kin preferentially, but, within kin class, they formed alliances more often with closer relatives—siblings as opposed to cousins (Chapus and Schulman 1980 in Fairbanks *et al.* 1986; Cheney *et al.* 1986; Cheney 1977, 1983 in Fairbanks *et al.* 1986).

This theory does not, however, imply that changes in the dominance ranking never occur. On the contrary, changes do occur, although what causes changes in rank to occur or what causes an acceleration of this rate are not always clearly understood. Researchers have found long periods of stability in dominance hierarchies punctuated by short periods of instability and change; these were then followed by another long period of stability. Silk and Boyd (1983) have proposed that the change may be due to changes in the relative size of matriline; these size fluctuations may be due to the death or ill health of the matriarch (Silk and Boyd 1983 in Samuels *et al.* 1987).

Another behavior seen in dominance-oriented social groups is that of manipulation and tolerance. Powel (1971) defines manipulation as a relatively stylized set of behaviors of a lower ranking female to a higher ranking one include hands-on-head, hand-on-shoulders, and embrace-from-the-front. Fairbanks *et al.* (1986) found that lower ranking vervet monkey females are attracted to the high ranking females, and they will compete among

themselves for the chance to sit near and groom the dominant females. In addition, they groomed females higher ranked than they were more often than they groomed lower ranked females. While manipulative behaviors may be returned by the recipient with aggression if the initiator is a closely ranked female, the lower ranking females are often rewarded for their manipulative behavior. By being associated with a higher ranked female, the lower female get the benefits of increased tolerance at food sites and reduced harrassment from others in the group. However, David MacDonald argues that tolerance is most often seen among kin. He states that "to an extent which depends on the interloper's degree of relationship, the net cost of tolerance to the occupier's fitness is diminished in proportion to the benefit accruing to the interloper, if they are related" (MacDonald 1983). This theory was tested by DeWaal (1986) when he denied Rhesus monkeys water for three hours. He then put the monkeys into an area where only a small percentage (the more dominant ones) could get at the water. DeWaal found that social tolerance in drinking was highest among kin (DeWaal 1986; Fairbanks 1986; MacDonald 1983).

In orangutans, social dominance is readily established in captivity (Nader and Tilford in Maples 1980). Maples (1980) reports that expressions of dominance are predominant among non-related females living together in zoos. Furthermore, the presence of a newborn effects dominance rank, the female with the infant being dominant over the others. Females in zoos were also found to be dominant regardless of which home cage was used; "territory" was not an advantage. The subordinate females remained suspended from cage bars for longer periods than did their more dominant cagemates.

In the wild, female orangutans have relatively small home ranges, approximately 0.65 km (Rodman 1973, Horr 1972 in Maples 1980). These females also exercised a considerable degree of mobility and, thus, overlap of home ranges ensued (MacKinnon 1974 in Maples 1980). This overlap of home ranges in the wild gives one the impression of extensive tolerance among orangutan females. Maples (1980) believes that this impression is partly incorrect; he states that tolerance exists, but the actual amount of tolerance is obscured because related older offspring are more likely to live in close proximity to their mothers and siblings.

The purpose of my experiment was to determine whether or not the presence of Chickie, a nonrelated female orangutan, effected the proximity of Sally to her fifteen-month-old daughter. It was my hypothesis that Sally and her baby would be in direct contact, as opposed to being within one arm's reach or further than one arm's reach, more often with Chickie present than would be randomly expected.

## METHODS

Each individual observation period was one hour and was made at approximately three o'clock in the afternoon. During this hour, I would look at Sally and her baby every 2 minutes ( During the first two observation periods, observations were made every five minutes) and score their behavior on two points: Sally's proximity to her baby and the presence or absence of Chickie. Thus, I would score them in the following way:

- 1 = Baby and Sally in direct contact
- 2 = Baby within one (1) arm's reach of Sally
- 3 = Baby outside of one arm's reach

P = Chickie present

A = Chickie absent

## RESULTS

Table 1 shows the various observations for each day:

**Table 1. Orangutan Observations.**

DAY	1P	1A	2P	2A	3P	3A
1	5	2	1	4	0	0
2	3	1	0	5	0	3
3	10	16	0	3	0	0
4	10	3	0	9	0	8
5	0	17	0	10	0	3
6	24	6	0	0	0	0
7	30	0	0	0	0	0
7	82	45	1	31	0	14

Total Present = 83 and Total Absent = 90.

Total Observations = 173.

Total Category 1 Behaviors = 127.

Total Category 2 Behaviors = 32.

Total Category 3 Behaviors = 14.

## DISCUSSION

In order to see if there was a significance between the number of "Direct Contact/Chickie Present" and "Direct/Chickie Absent" observations recorded, I decided to use a Chi-square test. Because over twenty percent of my data contained less than five observations for a given behavior on a certain day and several behaviors contained zero observations, I had to collapse the data in order to get a valid test. I also had to correct for the proportion of observations made where Chickie was present or absent because Chickie was not present in fifty percent of the observations. To do this I totaled all the "Presents" and "Absents," (P = 83 and A = 90). Thus, out of the 173 total observations recorded, Chickie was present for 83 of them and she was absent for 90. The total number of Category 1 behaviors was 127 (includes absents (45) and presents (82)). One would thus expect  $83/173 \times 127$  to be 1P behaviors, and  $90/173 \times 127$  to be 1A. Hence, the following would be expected:

Direct Contact/Present:  $83/173 \times 127 = 60.9$

Direct Contact/Absent:  $90/173 \times 127 = 66.1$

A Chi-square test of (observed - expected)/(expected) was performed.

The following shows my results:

$$(82 - 60.9)/(60.9) + (45 - 66.1)/(66.1) = \\ 7.31 + 7.74 = 14.05.$$

Using a Chi-square table with 1 degree of freedom, it is found that the probability at the .005 level is less than 7.879. Therefore, 14.05 is greater than 7.879 and the data are significant. From this, I concluded that the presence of Chickie did have a significant effect on the proximity of Sally towards her baby.

Upon reviewing the category 2 and 3 behaviors, I found that Sally allowed her baby to be further away from her direct contact when Chickie was absent. Sally allowed the baby to go within one arm's reach 31 times when Chickie was absent, as opposed to one time when Chickie was present. In addition, Sally allowed her baby to wander out of her reach fourteen times when Chickie was absent, and zero times when Chickie was present. Thus, there appears to be a definite correlation between the presence/absence of Chickie and Sally's proximity to her baby. I also found in my daily observations that if Sally and her baby were not within arm's reach and Chickie entered the cage, Sally would immediately position herself between Chickie and the baby. Although I saw no signs of direct aggression, in the presence of Chickie, Sally would watch both the baby and Chickie closely.

There are a variety of ways that one could explain Sally's behavior in the presence of Chickie, the simplest being a mother's "natural instinct" to protect her child. But I believe that Sally's behavior can also be explained by an ultimate function, and it can be related to the overall patterns of behavior seen among groups of female primates living together. Although Sally's protection and care for her child benefits the child itself, Sally is not being altruistic—she too receives rewards from her behavior. By providing for her baby she is helping guarantee an increase in her Darwinian fitness. It is essential to recall that dominance is passed down from mother to daughter and that adult females occupy the rank that conforms to the positions occupied by members of their matriline (Hausfater *et al.* 1982 in Samuels *et al.* 1987). Sally has gained nothing if the child does not survive to carry on her genes. Sally's fitness will also be raised if future generations (i.e. granddaughters) are also reproductively successful.

Seyfarth's (1977) studies of vervet monkeys concluded that mothers prefer to associate with adult daughters more than with other adult females (Seyfarth 1977 in Fairbanks 1986). based on his results, I believe that Sally will continue this close kinship bond with her daughter throughout their lives. Again, keeping close ties with her daughter is advantageous to Sally. Vast amounts of observations have found that kin bonds are very close in female primate groups. Kin groups join together in aggressive conflicts with other, nonkin groups because a shift in dominance rank of one female can effect the rank of all her other relatives. If Sally continues to remain close to her daughter, the probability of Chickie being able to successfully challenge and subsequently displace the daughter would be rather small. If Sally were not to retain close kinship bonds with her daughter and her daughter was displaced by Chickie, the reproductive success of Sally's future generations would be decreased because lower ranking females have a lower rate of reproductive success and a higher infant mortality rate (Fairbanks *et al.* 1986). Any decline in dominance rank of Sally or her close kin would be a definite loss because, according to Maples (1980), female orangutans living with non-related females in zoos have a higher dominance rank

if they have an infant. Maples also found that subordinate orangutans tend to remain suspended from cage bars for much longer periods than do the dominant ones. During all my observations, I never saw Sally hanging from the bars, but Chickie could occasionally be seen hanging or swinging from the cage bars. This does not mean that Chickie's subordination is the only factor that influenced her behavior; this observation could be explained by Chickie's younger age or Sally's obesity. Furthermore, Maples has found that dominant females were dominant regardless of which cage they were placed in. My results concur with Maples's observation that "territory" does not effect dominance: I saw no changes in behavior when Sally and her baby were in different cages.

One question still remains unanswered. I can not conclude from my study whether Sally's proximity to her baby in Chickie's presence is due to Sally's own movements, or if the baby is moving towards Sally.

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## A NEW RECIPE FOR PI

Anne Junkin,†

### ABSTRACT

*In this paper we investigate the asymptotic behavior of a non-linear difference equation. Given various initial conditions we show geometrically that the asymptotic limit of the solution is a rational multiple of  $\pi$  and that there is a relation between the starting point and the rational multiple. Thus, we have a new method for computing the value of  $\pi$ .*

### PAPER

We will examine the asymptotic behavior of the sequence

$$g_n = 2^n \sqrt{2 - \sqrt{2 + \sqrt{2 + \sqrt{2 + \dots + f_0}}}}$$

Let  $f_n = \sqrt{2 + \sqrt{2 + \sqrt{2 + \dots + f_0}}}$  or, written as a non-linear difference equation,  $f_n = \sqrt{2 + f_{n-1}}$ . This means that  $g_n = 2^n \sqrt{2 - f_n}$ . We are interested in this particular sequence because, as we will prove geometrically,  $\lim_{n \rightarrow \infty} g_n = \pi/6$  with the initial value  $f_0 = \sqrt{3}$ .

Examining the coupled equations,  $f_n = \sqrt{2 + f_{n-1}}$  and  $g_n = 2^n \sqrt{2 - f_n}$ , we first wanted to combine these into one difference equation. In order to do that we define  $h_n = (g_n)^2$ . So,  $f_n$  and  $f_{n-1}$  in terms of  $h_n$  and  $h_{n-1}$ :

$$f_n = \frac{-h_n}{2^{2n}} + 2$$

and

$$f_{n-1} = \frac{-h_{n-1}}{2^{2(n-1)}} + 2$$

so from squaring  $f_n = \sqrt{2 + f_{n-1}}$  and putting the above values of  $f_n$  and  $f_{n-1}$  written in terms of  $h_n$  and  $h_{n-1}$ ,

$$\left(\frac{-h_n}{2^{2n}} + 2\right)^2 = 2 + \frac{-h_{n-1}}{2^{2n-2}} + 2$$

which yields

$$h_n^2 - 2^{2n+2}(h_n - h_{n-1}) = 0$$

This is the difference equation on which we will focus our attention, remembering that  $g_n = \sqrt{h_n}$ .

**Theorem 1:** Given the difference equation  $f_n = \sqrt{2 + f_{n-1}}$ , where  $0 < f_0 < 2$ , and the sequence  $h = 2^{2n}(2 - f_{n-1})$ , the limit of  $h_n$  as  $n$  approaches infinity is approximately equal to  $12h_0/(12 - h_0)$ , where  $h_0 = 2 - f_0$ .

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Proof: Defining  $\Delta h = h_n - h_{n-1}$ :

$$\frac{1}{2^{2n+2}} = \frac{\Delta h}{h_n^2}$$

To examine the asymptotic behavior, sum both sides,

$$\sum_{n=1}^{\infty} \frac{1}{2^{2n+2}} = \sum_{n=1}^{\infty} \frac{\Delta h_n}{h_n^2}$$

Note that the left side can be summed exactly as a geometric series:

$$\sum_{n=1}^{\infty} \frac{1}{2^{2n+2}} = \frac{1}{12}$$

Examining the right-hand side, and assuming for the moment that there exists an asymptotic value of  $h_n$ , which we will call  $h_{\infty}$ :

$$\sum_{n=1}^{\infty} \frac{\Delta h_n}{h_n^2} = \int_{h_0}^{h_{\infty}} \frac{dh}{h^2} = \frac{1}{h_0} - \frac{1}{h_{\infty}}$$

Therefore,  $h_{\infty} = \frac{12h_0}{12-h_0}$ .

Note that this is indeed an approximation because it assumes  $\Delta h_n$  to be equal to  $dh$ . Remembering that  $\Delta h_n = h_n - h_{n-1}$  or  $\Delta h = h(n) - h(n-1)$  and  $n = 1, 2, 3, \dots$ ,  $\Delta h = h(n) - h(n - \Delta n)$ . If  $\Delta n \rightarrow dn$ ,  $\Delta h \rightarrow dh$ . However,  $\Delta n$  is, as just indicated above discrete and equal to one.

To evaluate the validity of this approximation, we must examine the properties of  $\Delta h$  in more detail. First of all, reexamining

$$\frac{1}{2^{2n+2}} = \frac{\Delta h_n}{h_n^2}$$

we find  $1/2^{2n+2} > 0$  and that  $h_n^2 > 0$ . Since that is true,  $\Delta h_n$  must be greater than zero. Using that and the fact that  $\Delta h_n = (h_n - h_{n-1})$ , it follows that  $h_n > h_{n-1}$ .

Lemma: The  $\Delta h$ 's decrease as  $n$  gets larger, or  $\Delta h_{n+1} < \Delta h_n$ .

To prove  $\Delta h_{n+1} < \Delta h_n$ , or  $\frac{\Delta h_{n+1}}{\Delta h_n} < 1$ . We will use the following equations from above:

$$\begin{aligned} \Delta h_n &= \frac{h_n^2}{2^{2n+2}} \quad \text{(i)} \\ h_n &= 2^{2n}(2 - f_n) \quad \text{(ii)} \\ f_n &= \sqrt{2 + f_{n-1}} \quad \text{(iii)} \end{aligned}$$

$$\begin{aligned} \text{Examine } \sqrt{\frac{\Delta h_{n+1}}{\Delta h_n}} &= \left( \frac{h_{n+1}}{\text{sqrt}2^{2(n+1)+2}} \right) \left( \frac{\sqrt{2^{2n+2}}}{h_n} \right) \\ &= \left( \frac{h_{n+1}}{2^{n+2}} \right) \left( \frac{2^{n+1}}{h_n} \right) = \left( \frac{1}{2} \right) \left( \frac{h_{n+1}}{h_n} \right) \text{ using (i) above.} \\ &= \frac{1}{2} \left( \frac{2^{2(n+1)}(2 - f_{n+1})}{2^{2n}(2 - f_n)} \right) \text{ by (ii).} \end{aligned}$$

$$\begin{aligned}
&= 2 \frac{(2-f_{n+1})}{(2-f_n)} \\
&= 2 \left( \frac{2-\sqrt{2+f_n}}{2-f_n} \right) \left( \frac{2+\sqrt{2+f_n}}{2+\sqrt{2+f_n}} \right) \text{ from (iii) above.} \\
&= 2 \left( \frac{4-(2+f_n)}{2-f_n} \right) \left( \frac{1}{2+\sqrt{2+f_n}} \right) \\
&= \frac{2}{2+\sqrt{2+f_n}} < 1
\end{aligned}$$

and

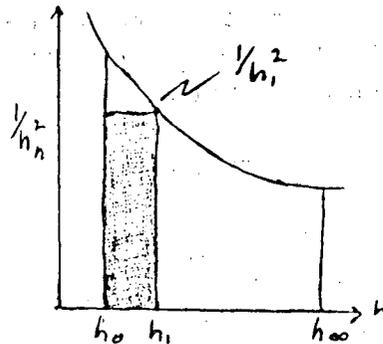
$$\frac{\Delta h_{n+1}}{\Delta h_n} = \left( \frac{2}{2+\sqrt{2+f_n}} \right)^2 < 1^2 < 1.$$

Q.E.D.

Theorem 2: The asymptotic value  $h_\infty$  satisfies  $\frac{12h_1}{12-h_1} < h_\infty < \frac{12h_0}{12-h_0}$ .

Proof: Since,  $h_n$  increases and  $\Delta h_n$  decreases as  $n$  gets larger, a graph of  $1/h_n^2$  versus  $h_n$  yields the following (Fig. 1):

Figure 1



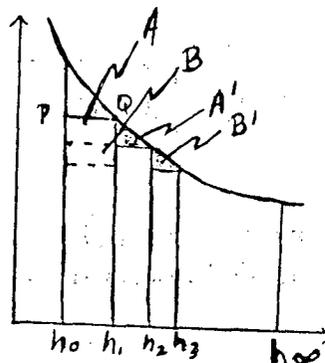
Note that  $\Delta h_1/h_1^2$  is equal to the area of the rectangle shaded in. Thus,  $\sum_{n=1}^{\infty} \frac{\Delta h_n}{h_n^2}$  is equal to the sum of the areas of the discrete triangles. Therefore, by the definition of the integral,

$$\sum_{n=1}^{\infty} \frac{\Delta h_n}{h_n^2} < \int_{h_0}^{h_\infty} \frac{dh}{h^2}$$

This in turn means  $h_\infty < \frac{12h_0}{12-h_0}$ .

If the integral of the above equation is evaluated from  $h_1$  to  $h_\infty$ , this results in an upper bound for  $h_n$ . The reasoning for this begins by looking at Fig. 1 again.

Figure 1(revisited)



First of all, notice that to show this, we must prove the area of the rectangle  $h_0 h_1 P Q$  is greater than the sum of the areas of all the shaded in places. The first important fact used to show this is that each rectangle is shorter than the one to its left; in other words,  $h_{n+1} > h_n$  (as was proved earlier) so  $1/h_n < 1/h_{n+1}$ . Also, an infinite number of rectangles must fit in a finite space while  $\Delta h > 0$  with  $h_n > h_{n+1}$ , so  $\Delta h_n > \Delta h_{n+1}$  (as was proved earlier). This means that area  $A$  of the first rectangle is greater than  $A'$ ,  $B > B'$  and so forth. Thus,

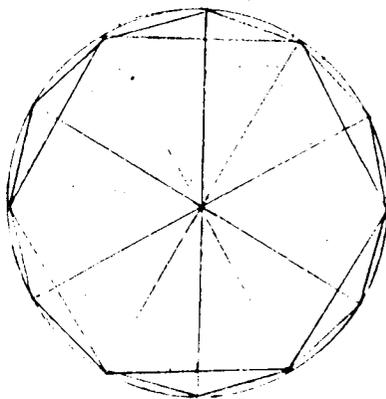
$$\int_{h_1}^{h_\infty} \frac{dh}{h^2} < \sum_{n=1}^{\infty} \frac{\Delta h}{h_n^2}$$

Therefore,  $\frac{12h_1}{12-h_1} < h_\infty < \frac{12h_0}{12-h_0}$ .

Theorem 3: The  $\lim_{n \rightarrow \infty} h_n$  equals  $\pi/6$  given  $f_0 = \sqrt{3}$ , and equals  $\pi/4$  if  $f_0 = \sqrt{2}$

Proof: The reason  $h_n$  is so interesting lies in a geometric proof relating it to pi. Taking a unit circle so that the area of the circle equals pi and inscribing a regular polygon such as a hexagon in it, the area of the hexagon, found using simple geometry, approximates the area of the circle and thus the value of pi.

Figure 2



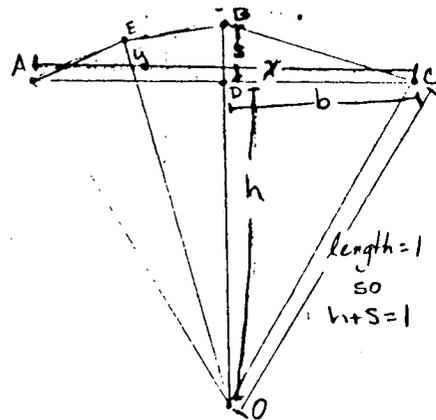
As the hexagon is broken into a 12-gon, 24-gon and etc. with larger divisions (see Fig. 2), the area approaches pi. As the number of sides of the polygon approaches infinity, the inscribed figure becomes the circle itself. The equation describing such a process is as follows:

$$\pi = \lim_{n \rightarrow \infty} 6 \cdot 2^n \sqrt{2 - \sqrt{2 + \sqrt{2 + \dots + \sqrt{3}}}}$$

or  $\sqrt{h_\infty} = \pi/6$ .

To prove this, we must first prove that for a circle of radius one, with three points ( $A, B, C$  in Fig. 3) such that the radius  $OB$  bisects the arc  $AC$ , the quadrilateral formed has area equal to  $(\frac{1}{2})x$ .

Figure 3



The area of the triangle  $OCD = OCA = (\frac{1}{2})bh$  and the area of triangle  $BCD = BAD = (\frac{1}{2})sb$ . The area of the quadrilateral is

$$2(\frac{1}{2}bh) + 2(\frac{1}{2}sb) = b(h + s) = b(1) = \frac{1}{2}x$$

Given  $x$ , to find  $y$  and thus the areas of the two new quadrilaterals, use the Pythagorean theorem,

$$1 = (\frac{1}{2}x)^2 + h^2 \text{ so } h = \sqrt{1 - \frac{1}{4}x^2}$$

$$\text{and } (1 - h)^2 + (\frac{1}{2}x)^2 = y^2$$

$$y = \sqrt{2 - \sqrt{4 - x^2}}$$

Therefore, the area of the new quadrilateral ( $ABEO$ ) would be  $(\frac{1}{2})y$ . For example, with  $x = 1$  (a heagon),

$$y_0 = \sqrt{2 - \sqrt{3}}$$

$$\text{new } x = \sqrt{2 - \sqrt{3}}$$

$$y_1 = \sqrt{2 - \sqrt{2 + \sqrt{3}}}$$

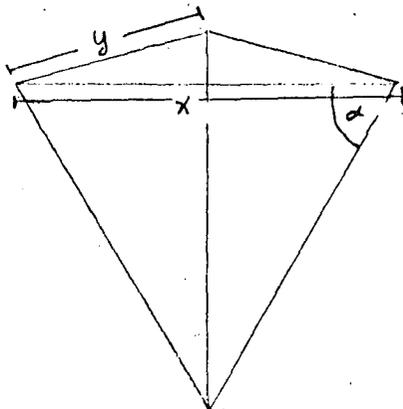
and so forth. For a square,

$$x_0 = \sqrt{4 - (\frac{\sqrt{2}}{2})^2} = \sqrt{2}$$

$$y_0 = \sqrt{2 - \sqrt{2}}$$

and the sequence is  $y_n = \sqrt{2 - \sqrt{2 + \sqrt{2 + \dots + \sqrt{2}}}}$ . Note that with each division, the number of quadrilaterals that make up the polygon doubles which explains the  $2^n$  term.

Figure 3 (revisited)



Finally, we have,

$$\frac{\pi}{6} = \lim_{n \rightarrow \infty} 2^n \sqrt{2 - \sqrt{2 + \sqrt{2 + \dots + \sqrt{3}}}}$$

$$\frac{\pi}{4} = \lim_{n \rightarrow \infty} 2^n \sqrt{2 - \sqrt{2 + \sqrt{2 + \dots + \sqrt{2}}}}$$

The first is  $\pi/6$  since the hexagon initially contains six of these quadrilaterals and  $\pi/4$  for the second since the square has four such sections.

Just to see how close the approximation is with  $f_0 = \sqrt{3}$ :

$$3.1410 < 6\sqrt{h_\infty} < 3.1688$$

The approximation can always be improved by actually summing  $\sum_{n=1}^k \frac{\Delta h}{h_n^2}$  and then evaluating  $\sum_{n=k}^{\infty} \frac{dh}{h_n^2}$  by approximating it to its corresponding integral and realizing,

$$\sum_{n=1}^k \frac{\Delta h}{h_n^2} + \int_{h_{k-1}}^{h_\infty} \frac{dh}{h^2} < h_\infty < \sum_{n=1}^k \frac{\Delta h}{h_n^2} + \int_{h_k}^{h_\infty} \frac{dh}{h^2}$$

Of course as  $h \rightarrow \infty$ ,  $h_\infty$  approaches the exact value.

Although this method of obtaining pi using inscribed polygons has been used before such as Archimedes method, as far as we know, no sequence such as the one above has been found to evaluate pi (Borwein, 113). This sequence  $f_{n+1} = \sqrt{2 + f_n}$  has been examined with different initial conditions by others, but they dismissed the initial value of say  $f_0 = \sqrt{3}$  as trivial (see "Problems ...", 789-93). This was because the  $\lim_{n \rightarrow \infty} f_n$  is equal to 2 (see proof in Appendix). In our case, this means that  $\lim_{n \rightarrow \infty} \sqrt{2 - f_n}$  equals zero if  $0 < f_0 < 2$  and  $\lim_{n \rightarrow \infty} 2^n$  equals infinity. However, the product of the two is a multiple of pi. Also, since  $\lim_{n \rightarrow \infty} \sqrt{2 - f_n} = 0$  with  $0 < f_0 < 2$ , the value of  $f_0$  within those limits, it seems, would make no difference. However, we prove geometrically that is not the case. To obtain a general relationship between the value of  $f_0$  and the multiple of pi, we look again at the relationship of  $x$  to  $y$  in Fig. 3. Let  $K$  be the number of sides of the original polygon.

From this figure,  $\cos\alpha = \frac{\pi}{2}$  so  $x = 2\cos\alpha$ . Letting  $x_1 = 2\cos\alpha$ ,

$$y_1 = \sqrt{2 - \sqrt{4 - 4\cos 2\alpha}} = \sqrt{2 - 2\sin\alpha}$$

$$x_2 = \sqrt{2 - 2\sin\alpha}$$

$$y_2 = \sqrt{2 - \sqrt{2 + 2\sin\alpha}}$$

Therefore, generally,

$$\pi = \lim_{n \rightarrow \infty} K \cdot 2^n \sqrt{2 - \sqrt{2 + \sqrt{2 + \dots + \sqrt{2 + 2\sin\alpha}}}}$$

However, for any integer  $K > 1$ ,  $\alpha = \frac{1}{2}(\frac{360^\circ}{K}) = \frac{180^\circ}{K}$  So,  $f_n = \sqrt{2 + f_{n-1}}$  and  $f_0 = \sqrt{2 + 2\sin(\frac{180^\circ}{K})}$ . Therefore,  $\pi = \lim_{n \rightarrow \infty} K \cdot g_n$ , given  $f_0 = \sqrt{2 + 2\sin(\frac{180^\circ}{K})}$ . However, the draw back is that  $\sin(\frac{180^\circ}{K}) = \sin(\frac{\pi}{K})$ , so this is only useful for angles for which the *sine* is already known, such as  $\pi/4$ . Otherwise to evaluate  $f_0$  requires using the value of pi. If pi is known, the influence of  $f_0$ , the starting value, on  $\lim_{n \rightarrow \infty} h_n$  can be seen. This relationship is that  $\lim_{n \rightarrow \infty} h_n = \pi/K$  when  $f_0 = \sqrt{2 + 2\sin(\pi/K)}$ .

Therefore, geometrically, we illustrated that the starting point affects the value of the multiple of pi. In investigating the asymptotic behavior of the difference equation

$$h_n = 2^{2n+2}(h_n - h_{n-1}) \text{ or } \frac{1}{2^{2n+2}} = \frac{\Delta h}{h_n^2}$$

a non-linear difference equation, and interpreting it geometrically, we have found a new method for calculating pi.

## APPENDIX

Prove:  $\sqrt{2 + \sqrt{2 + \sqrt{2 + \dots}}} = 2$

Proof: Since,

$$\sqrt{2} < 2$$

$$\sqrt{2 + \sqrt{2}} < \sqrt{2 + 2} = 2$$

$$\sqrt{2 + \sqrt{2 + \sqrt{2}}} < \sqrt{2 + 2} = 2,$$

then we can let  $x = \sqrt{2 + \sqrt{2 + \sqrt{2 + \dots}}}$  because the sequence is bounded. With  $x$  defined as above, we square  $x$ :

$$(x = \sqrt{2 + \sqrt{2 + \sqrt{2 \dots}}})^2$$

$$x^2 = 2 + \sqrt{2 + \sqrt{2 + \sqrt{2 \dots}}}$$

$$x^2 = 2 + x$$

$$(x - 2)(x + 1) = 0$$

$$x = 2 \text{ or } x = -1 \text{ (which we drop, since } x > 0)$$

Q.E.D.

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## EXTERNAL MORPHOGENETIC MOVEMENT DURING GASTRULATION OF *Rana pipiens*

Harold Wright,† William Holden,††

### ABSTRACT

*Ever since Vogt's first experiments with amphibian eggs (Vogt, 1929), fate maps have been a vital tool to biologists in studying the external and internal morphogenetic movements of different germplasma layers. It was the purpose of this experiment to observe external morphogenetic movement between the early gastrula (dorsal lip) and neural plate stages of development in the frog, Rana pipiens. Rather than using the common practice of vital staining, a laser was employed to mark designated regions on the surface of the frog embryos. The information gathered from this experiment was then correlated with published data concerning the gastrula stage of development to determine accuracy of this new laser fate-mapping procedure.*

### MATERIALS AND METHODS

#### Materials

- 1) 20 + frog embryos at the early gastrula (dorsal lip) stage of development.
- 2) 20 biologically clean stender dishes with tops.
- 3) 1 stereomicroscope with a 7-fold power of magnification.
- 4) 5 wax operating dishes with a 5mm depression in the center.
- 5) 2 pipets.
- 6) 1 fine forcep.
- 7) 1 thermometer.
- 8) 2 large culture dishes.

#### The Laser Microscope

Lasers are used in many areas of science today. The quality of lasers that was most useful for this experiment is their emission of coherent, monochromatic light. This quality allowed us to precisely mark specific blastomeres of the embryos. The laser utilized for this experiment was a Hadron Model 513 ruby biolaser mounted on a Wild Heerbrugg compound microscope. The laser was powered by a Hadron Model 103A power supply unit with a power input of 230 joules (max) and a power output between 0 and 800 millijoules. The laser had a pulse length of 150 microseconds with an effective beam diameter of 5mm (pulse rate of 1 every 2 minutes). The wavelength of the light was  $694.3\mu\text{m}$ . By utilizing a 12.5x ocular and a 4x objective (n.a. 0.10), a marking of approximately 0.1mm in diameter

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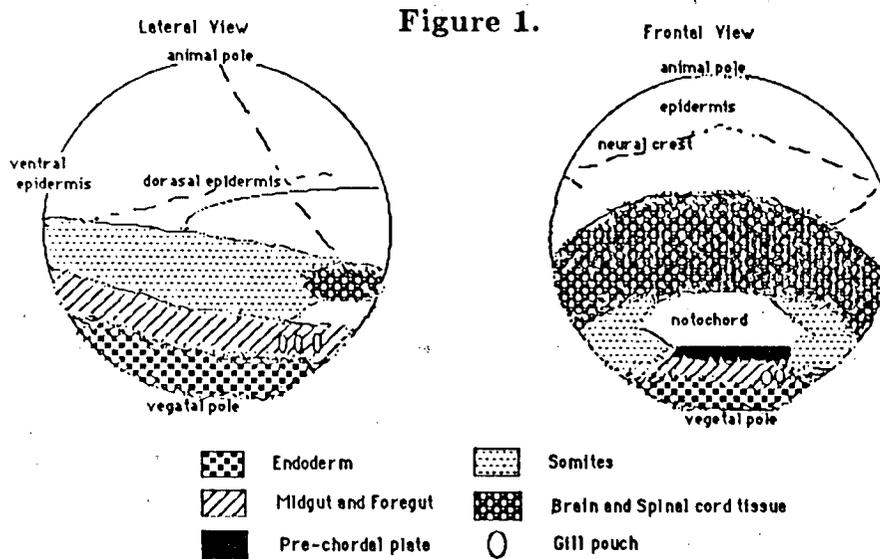
was made. A number of unfertilized embryos were test marked and it seems that 140 joules (the minimum amount of energy needed to discern a mark on the surface) would be used for these particular embryos.

**Procedure**

The embryos used in this experiment were isolated from a Wisconsin variety of *Rana pipiens* previously injected with six anterior pituitary lobes. This injection induced premature ovulation. Paired testis of four frogs of the same variety were then removed and cut into separate clean culture dishes containing 10ml of spring water. Next, into each of these dishes were added the frog eggs, which were gently squeezed from the female's uteri. The dishes were then rotated to evenly distribute the eggs among the sperm. After three minutes, 200ml of spring water was added to each dish (this was marked as the time of fertilization). These now fertilized embryos were allowed to develop until the dorsal lip stage at 25°C (19 to 20 hours).

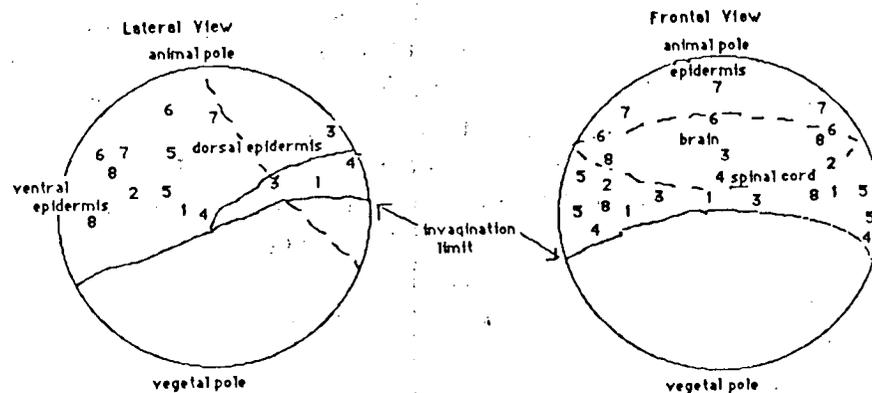
**RESULTS**

All eight of the embryos studied survived the laser markings. Aside from the necessary destruction of a few blastomeres in the lased area, development proceeded normally throughout the stages that were observed. Figure 1 denotes the movements of the marked areas (beginning at the dorsal lip stage) up to the formation of the neural plate. After the formation of the neural plate, the marked blastomere regions became detached from the embryos. This action caused no visible deformations in the continued development of the embryos observed up to the operculum stage. Figure 2 shows the approximate germ layer area of each lased area.



Lateral and frontal view of fate maps by vital staining techniques, depicting the external germ layers of the frog. (Matthews)

Figure 2.



Schematic representation of the blastomere regions lased in this experiment.

## DISCUSSION

The results of this experiment confirmed the previous published data by Vogt and, as shown in Figure 1, by Matthews concerning the external movement of blastomeres during gastrulation in *Anurans*. In this experiment two fundamentally important paths of morphogenetic movement were observed. The first of these was invagination. The lased blastomeres closest to the chorda-mesodermal field (dorsal lip) were observed to move inside the dorsal lip (and eventual blastopore) region. These lased blastomeres were killed. Although their movement continued (the viable surrounding blastomeres contributed to this), they would eventually slough off with no detrimental effect to the embryo. This observed phenomena may be explained as a regulatory device of embryos at this stage of development. Furthermore, since no visible defects were observed in the later stages of development, one can assume that the embryos reorganized their germ layers to compensate for those few blastomeres that were lost (approximately four for every mark). In fact, this type of "light primary damage," as seen in experiments by Storb in which equally susceptible mitochondria exhibited visible damage in the surrounding cells and nuclei when using "low-level" radiation like that used in our study.

The second path witnessed was the epibolical movement of neural ectoderm during the formation of the blastopore. Those blastomeres marked further towards the animal pole in the ectodermal region never made actual contact with the dorsal lip region. Instead, the blastomeres lying distally moved forward and curled around the edges of the embryo until the yolk plug was completely surrounded by ectoderm. The dead blastomeres also became detached from the embryo once they had arrived at the neural plate stage. The remaining blastomeres might have eventually entered up in the neural tube of the frog.

Although one cannot actually say that specific blastomeres will form particular structures, it is possible to predict the fate of certain regions of blastomeres. Techniques using

methods such as vital stains (dyes) and peroxidase cell injections have led to the development of detailed fate maps similar to the one digrammed in figure 1. Each region depicts (a) certain germ layer(s) through which such morphogenetic processes as invagination, involution, and epiboly will form specific sections in the adult *Rana pipiens* (Cooke). Some general, simple relationships have been drawn. One such example, as related by Spratt, correlates the chorda-mesodermal region with different inductive capacities. For example, mesodermal tissue distal to notochord tissue is responsible for inducing the structures of the forebrain while the posterior brain structures are induced by the parachordal and chordal areas. Furthermore, Spratt elaborates on the inductive relationship between the two germ layer regions caused the induction of the posterior brain region while shorter contact resulted in the formation of the anterior structures of the brain (Johnen).

In conclusion, the following points have been deduced from the results obtained in performing the experiment:

- 1) Morphogenetic movement (invagination and epiboly) is observable externally when utilizing such techniques as laser marking and vital staining.
- 2) Embryos, in the gastrula stage of development, possess some regulatory ability in maintaining normal morphogenetic movement of their germ layers.
- 3) Low-level use of laser radiation does not invoke lethal morphological damage in the overall development of the embryos.
- 4) Given information regarding external morphogenetic movement, more detailed fate maps may be developed to indicate the germ layer regions which form germ layers in the adult in the future.

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## SLURRY METHOD FOR ELEMENTAL ANALYSIS OF COAL BY PLASMA EMISSION SPECTROSCOPY

Teresa Burnst†

### ABSTRACT

*This research was carried out during a summer undergraduate research program at the University of Alabama (Tuscaloosa). The goal of this research was to continue the studies on a new method of solid sample introduction into a plasma emission spectrophotometer. Normally solid samples require days or weeks of acid digestion before they can be introduced. The proposed method of grinding, in conjunction with the appropriate spray chamber, requires only minutes. The results of this study using ICP spectroscopy are being compared to a more extensive research of the same problem using DCP spectroscopy.*

### INTRODUCTION

Plasma emission spectroscopy has become increasingly important in industry for trace elemental analysis. Plasma methods are superior to atomic absorption because they allow quick multi-element analysis. There are three types of plasma: direct current plasma (DCP), inductively coupled plasma (ICP), and microwave induced plasma (MIP). The DCP and ICP are most widely used. The DCP has electrodes that maintain the plasma while the ICP uses a magnetic field induced by a radio-frequency coil. Both operate at approximately the same gas temperature (6000-9000K). The ICP consumes more argon than the DCP, using approximately 17 l/min compared to about 8 l/min for the DCP. The main difference between the two is the analyte residence time due to the way the sample is introduced into the plasma.

Sample introduced into DCP enters at the fold in the plasma between the electrodes and tends to skirt around the plasma because of magnetic and thermal barriers. Increasing the injection velocity of the sample helps to lower this problem by breaking the sample through these barriers. Also, there is only a limited region in the plasma where measurements can be made, otherwise the continuum is too great. Because of this the residence time for DCP is an order of magnitude lower than for ICP where the sample is brought up through the center of the concentric quartz tubes carrying the argon, allowing an 8-10 msec residence time. This is important because there is longer sample/plasma contact giving greater opportunity for ionized argon atoms to excite the sample so that it can emit radiation as it returns to the ground state. This also allows more time to break apart refractory materials that might be present in a sample.

The traditional method of sample preparation has been by acid digestion. A slurry method would be preferred because it is much quicker to prepare and this is crucial in industry. It also eliminates the need to use strong acids. There has been some success in

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slurry methods since the development of the Babington nebulizer which allows for clog-free operation. Also the micronizing mill allows for samples to be ground to about 10 micrometers in diameter.

Previous research has been aimed at finding a suitable slurry method of preparation for the elemental analysis of coal, using the DCP. The recoveries of the slurry samples compared to the digestion samples were good, but they were not consistent for one element, or for one coal to the next. Also aluminum exhibited low recoveries that were believed to be due to the presence of refractory materials. It is hoped that aluminum recoveries will improve with analysis via ICP because of the longer residence time in the plasma. For the same reason the overall results for the slurry should improve because the suspended coal particles will have a longer time to break down.

## MATERIALS AND METHODS

### A. Apparatus

Grinding Mills:	Bell Art Products Micromill for large pieces of coal; McCrone Associates Micronizing Mill used to prepare slurries
Excitation Source:	Perkin-Elmer ICP/5500 with full quartz torch; argon flow rates - plasma 14 l/min, nebulizer 0.77 l/min, aux 0 l/min while running samples
Nebulizer:	Babington teflon nebulizer, 0.006 inches in diameter, with carrier flow of 0.77 l/min and solution flow of 1.5 ml/min
Monochromator:	Perkin-Elmer ICP/5500; slit width 0.02nm Czery-Turner design, UV grating: 2880 lines/mm; visible grating: 1440 lines/mm
Readout Systems:	Cole-Parmer Model 8373-10 recorder

### B. Reagents

Concentrated nitric acid was distilled in the lab. All dilutions and suspensions were made with 3 M nitric acid made from the distilled acid. The Standard Reference Materials (SRM) were ordered from the National Bureau of Standards; SRM 1635 is a subbituminous coal and SRM 1632b is a bituminous coal. Another soft coal was from Brookwood, Alabama, and the anthracite was from Scranton, Pennsylvania.

### C. Procedure

Samples weighing 1.00 grams were placed in a clean grinding container with agate grinding elements and 5ml of distilled, deionized water was used to rinse the coal into the elements. The coal was ground in the micronizing mill for seven minutes, which has been shown to be the optimum grinding time. Ten additional milliliters of water were added and the container was shaken to obtain a consistent mixture. The first sample ground was thrown out to prevent contamination from particles of a previously ground material. The

following replicates were combined in a clean beaker and put in the oven to dry. After drying the coal was ready to be weighed out and diluted with the required amount of 3 M nitric acid to make a 2% slurry. Emission measurements were made for about one minute, and the average signal was determined by alternating samples with blank and measuring the difference between the two positions.

#### D. Digestion Procedure

Coal to be used in digestion was weighed out to 1.000 grams and placed in a clean teflon beaker, with 20ml of purified nitric acid and 5ml of concentrated hydrofluoric acid. The beaker was covered with a teflon lid and heated overnight (15 hours.) Then, 10ml of ultrapure perchloric acid was added and the solution was evaporated to near dryness (1-2ml.) If necessary, digestions were continued by adding 10ml each of hydrofluoric and perchloric acids and heating for an additional 3-5 hours and then evaporating. This was repeated until the digestion was completed. After evaporating to near dryness for the final time the walls and lid of the beaker were washed with 3 M nitric acid and the contents transferred quantitatively to a 50ml volumetric flask. The nitric acid was warmed in the beaker to facilitate the transfer. The solution was diluted to the mark to obtain a 2% solution. Two to four replicates were made for each coal type.

## RESULTS

The concentration of six elements in each coal sample was determined from a calibration curve of each element, and the percent recovery was calculated. The recovery of each element was determined by its signal found by using a slurry sample compared to the signal found using a digestion of the same sample. Since the digestion method is accepted as being correct, the percent recovery is a measure of how successful the slurry sample was for the element and the sample. Table 1 is a compilation of the averaged data for the SRM 1635 coal; Table 2 are the results for the Brookwood coal.

Table 1. SRM Coal Data

2% SRM 1635 Coal				
Element (nm)	Slurry	Digestion	Certified	% Recovery
Al (396.0)	0.30%	0.37%	0.32%	80.9
Cr (205.4)	16ppm	6ppm	0.3ppm	297
Cu (219.9)	4.9ppm	5.5ppm	0.3ppm	81.9
Fe (238.1)	0.235%	0.240%	0.005%	95.8
Mg (279.5)	0.100%	0.095%		105
Mn (257.4)	24ppm	22ppm	1.5ppm	104

**Table 2. Brookwood Coal Data**

2% Brookwood Coal			
Element (nm)	Slurry	Digestion	% Recovery
Al (396.0)	0.545%	1.175%	47.0
Cr (205.4)	3.5ppm	12ppm	21.8
Cu (219.9)	25ppm	45ppm	56.7
Fe (238.1)	0.255%	0.395%	64.7
Mg (279.5)	130ppm	345ppm	39.5
Mn (257.4)	12ppm	17ppm	70.5

### DISCUSSION

The results for the SRM 1635 using the ICP were similar to those obtained with the DCP with the exception of chromium, which suffered severe contamination. The copper values were also slightly elevated. It is possible this contamination occurred from flaking of stainless steel particles while the sample was drying in the oven or from a bottle that was contaminated.

The results of the Brookwood coal were not as good. The percent recoveries ranged from 20 to 70%, and the expected increase for aluminum was not seen. Measuring at different heights in the plasma did not change the recovery. The question was raised as to whether the aluminum was being preferentially selected going to the plasma from the spray chamber, so the chamber was cleaned out and the runoff was collected from a sample of Brookwood slurry. The sample was digested and analyzed for the six elements. The results seem to suggest that the aluminum is being selectively held back in the spray chamber, probably because it is in the form of a mineral occlusion.

In general, these results are only early suggestions of what to expect for data by ICP methods. It seems to compare favorably with the results already obtained by DCP. Further testing will probably improve the recoveries for the Brookwood coal and surely eliminate the source of chromium contamination. Further investigation of aluminum recoveries will most likely show the aluminum to be involved in mineral occlusion, the extent of which varying with the type of coal. Despite difficulties, the slurry method still has potential as a method for solids introduction, requiring only 15 minutes sample preparation time, compared with over 48 hours that are required for the traditional method of acid digestion.

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## CHEMICAL COMMUNICATION AMONG FIVE SOUTHERN APPALACHIAN SALAMANDER SPECIES

Ara Hanissian†

### ABSTRACT

*Chemical communication was examined in five Southern Appalachian salamander species. Substrate choice tests revealed that a chemical communication network does exist between D. monticola and D. ochrophaeus which may allow the latter species to avoid the larger (potentially predatory) species, D. monticola.*

### INTRODUCTION

As many as six of the eleven species currently recognized as members of the salamander genus *Desmognathus* may exist in sympatry (Southerland, 1986). Southern Appalachian streamside salamander communities commonly consist of large numbers of four *Desmognathus* species: *D. ochrophaeus*, *D. monticola*, *D. quadramaculatus*, and *D. fuscus*. In addition, a fourth species, *Pseudotriton ruber* is often found in these communities.

The study of the interactions among the Southern Appalachian populations of the above species has recently been the object of much attention from a number of herpetologists (Hairston 1980, Roudebush and Taylor 1986, Southerland 1986). Southerland (1986) found that the afore-mentioned four sympatric *Desmognathus* species preferred different substrates in experimental trials. The study also revealed that the choices were affected by the presence of other salamanders. The cause of this alteration of behavior has been postulated either to be influenced by competition-based or predation-based interactions (Roudebush and Taylor 1987, Southerland 1986, Nishikawa 1987). Southerland (1986) tested for the presence of such interactions and found that avoidance of other salamanders is the segregating force among individuals and species. He further notes that this avoidance is probably due to the severe risk of predation of small salamanders. The findings of Roudebush and Taylor (1987) are in disagreement with those of Southerland. Roudebush and Taylor contend that interspecific interactions are chiefly determined by species and only secondarily by size. Moreover, they postulate the existence of a pheromone communication system (used by *D. monticola*) as a means of avoiding risk of predation by decreasing its contact with a potential predator (*D. quadramaculatus*).

Chemical (pheromonal) cues are thought to play decisive role in a variety of salamander activities. Ability to locate home ranges (Madison 1972), prey-catching behavior (Lindquist and Bachmann 1972, Hanissian et al, 1987), ability to find nest sites (Forester 1976), and ability to identify the sex and species of potential mates (Dawley 1984, Jaeger and Gergits 1979, Tristram 1977) are merely a few of an entire array of behaviors that have been either partially or wholly controlled by the olfactory system of salamanders.

It was the purpose of this experiment to determine if there indeed exists a chemical communication system among the four *Desmognathus* species and *Pseudotriton ruber*. It

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was hypothesized that this system may offer information leading to the preferential avoidance by the salamanders of potential competitors and/or predators. The nature of the responses (viz. competitor or predator) was not investigated, however.

This study also investigated the effect of sex on the substrate choice of the salamanders.

## MATERIALS AND METHODS

The salamanders used in the study: *D. ochrophaeus*, *D. quadramaculatus*, *D. monticola*, *D. fuscus*, and *Pseudotriton ruber*, (snout to vent lengths >35mm, >75, >60, >50, >65) were collected in the late spring and early summer of 1987 from the environs of Appalachian State University. Laboratory experiments were performed in the summer, autumn, and winter of 1987. Salamanders were kept in clear plastic shoe boxes (15 x 30 x 9cm) which were lined with damp paper towels upon which were placed 15cm diameter filter papers. These filter papers remained in the test chambers for a ten day period so presumably to be sufficiently marked by the salamanders' odor. *D. ochrophaeus* salamanders were separated according to sex (immediately before the time necessary to mark the filter papers for the sex choice experiments) whereas the other specimens were housed in non-sex discriminated groups of two or more individuals. All of the plastic shoe boxes were maintained in a controlled temperature chamber at 15°C with a 12 hour:12 hour light:dark cycle. The animals were fed *Drosophila melanogaster* (apterous) each week.

Substrate choice tests were conducted in transparent plastic petri dishes with diameter of 15cm, 2.5cm depth). Filter papers (assumed to have been marked by the animals in whose cages they were kept) were cut in half and placed in the dishes in such a manner that each specimen was faced with a choice between two differently marked semicircles.

The *D. ochrophaeus* salamanders, the specimens used in all of the choice tests, were allowed to habituate in the test dishes (under a 9cm dish) for a 15 minute period at the beginning of which the light in the laboratory was dimmed (by turning off half of the room's lights). This alteration of lighting allowed greater similarity to the light conditions of the time of the animals' peak activity. This dimming also putatively reduced the likelihood of "light-bias" which may affect the salamanders' behavior.

Upon the completion of this fifteen minute habituation period, the smaller dish was lifted and the substrate on which the salamander's snout rested was recorded each minute for one hour. This resting of the animals' snout was construed as a "choice" in light of the findings of Tristram (1977) who showed that the nasolabial grooves are involved in substrate sampling.

To lower the probability of a side bias, the substrate positions were alternated in each dish.

## RESULTS

### *D. monticola* vs. *D. ochrophaeus*

A t-value of 8.58 indicates our ability to reject the null-hypothesis that the specimens would spend equal time on each substrate. The *D. ochrophaeus* specimens showed a statistically significant preference for the substrates marked by conspecific animals over those marked by *D. monticola*.

*Pseudotriton ruber* vs. *D. ochrophaeus*

*D. ochrophaeus* showed no statistically significant preference for conspecific marked substrates over those marked by *P. ruber*. This is indicated by a t-value of 0.70 which is insufficiently high in order to reject the null hypothesis.

*D. quadramaculatus* vs. *D. ochrophaeus*

*D. ochrophaeus* did not show statistically significant preference for conspecific marked substrates, as reflected by a t-value of 0.20. This value does not allow rejection of the null hypothesis.

*D. fuscus* vs. *D. ochrophaeus*

A t-value of 1.12 is insufficient to reject the null hypothesis that the salamander *D. ochrophaeus* would spend equal time on the substrates marked by conspecifics and by *D. fuscus*.

*P. ruber* vs. *D. monticola*

A t-value of 1.22 again indicates an insufficient value to reject the null hypothesis of equal time spent by *D. ochrophaeus* on the two substrates.

*D. quadramaculatus* vs. *D. monticola*

*D. ochrophaeus* does not show a statistically significant preference for either substrate, as indicated by a t-value of 1.24.

*D. ochrophaeus*: Male Sex Test

Male *D. ochrophaeus* were exposed to conspecific male and female substrates. A t-value of 0.62 indicates that the specimens showed no significant preference for either substrate.

*D. ochrophaeus*: Female Sex Test

Female *D. ochrophaeus* were allowed to choose between substrates marked by male and female conspecifics. A t-value of 0.84 was insufficient to reject the null-hypothesis.

## DISCUSSION

## Sex Tests

The t-values obtained in the substrate choice test with male *D. ochrophaeus* exposed to substrates marked by male and female conspecifics and the corresponding test performed using a female specimen were insufficiently high to allow rejection of the null hypothesis (0.62 and 0.84, respectively;  $\mu=30$  with  $\alpha=0.05$ ;  $df=19, 17$ ). The experiment allowed the test animals to choose between substrates marked by conspecifics of the same and opposite sex (Dawley 1984). Results were in accord with the findings of Dawley (1984) who also found that salamanders showed no significant preference for either side. In her paper, Dawley suggests that if the chemicals that convey information of sex are volatile, then substrates marked by the animals may no longer convey any information. This is indeed a plausible hypothesis for the explanation of the inconclusive results.

Perhaps another tenable explanation exists in consideration of the timing of the experiment. The tests were performed in the late autumn of 1987, well in the midst of the

mating season of *D. ochrophaeus*. The mating cycle of this species follows an annual cycle with females emerging from brooding in late August. The ensuing spermatogenic cycle commences in May (Huheey and Brandon 1972) At the time of testing the salamanders may no longer have been "primed" to detect cues which would be of little importance in the temporal context. This hypothesis is corroborated by Madison (1975) who found in *P. cinereus* a change in substrate preference during the non-breeding season of the animal. Madison found that test salamanders did show preference of neighboring conspecifics of the same sex before the breeding season. This preference was not found during the mating season, however. It is thus evident that the equivocal nature of these findings may even be expected in light of the temporal change in sexual behavior of the animals.

### Olfactory Detection of Congeneric Salamanders

#### *D. monticola* vs. *D. ochrophaeus*

*D. ochrophaeus* statistically significantly preferred the substrates marked by conspecifics over those marked by *D. monticola*. We may conclude from the data that the salamanders left some discernible species-specific odor which allowed the detection and consequent avoidance of congeneric animals. A chemical communication system does therefore appear to exist between *D. ochrophaeus* and *D. monticola*, at least to the extent that *D. ochrophaeus* receives cues from *D. monticola* ( not necessarily vice versa ). The possession of such an ability to detect the presence of *D. monticola* may be adaptive for *D. ochrophaeus*. The system may be advantageous in its provision of a means of avoidance of *D. monticola* which may presumably decrease its chance of being eaten. Southerland (1986) notes the possibility that *D. monticola* is a predator upon *D. ochrophaeus*. This mere mention of a probability of the predatory nature of *monticola* upon *ochrophaeus* may be developed further upon additional consideration of Southerland's findings. Southerland postulated that interspecific interactions depend primarily on size ( thus larger *Desmognathus* salamanders would prey on smaller congenics ). Roudebush and Taylor (1987) deemphasize the importance of size in these interactions, claiming that species is the primary determinant of such interactions. One can attain a theoretical compromise in hypothesizing that species specific information in the form of olfactory cues may convey size ( viz. average species size ) information, thus reconciling two ostensibly incompatible hypotheses.

It is also plausible to suggest that the ochrophaeus chose the substrate marked by conspecifics only because of the familiarity of the odor of those animals. The ability to detect conspecifics offers a selective advantage in that it decreases the possibility of mating with non species members thus decreasing the likelihood of the reproductively costly formation of inviable hybrids.

*D. ochrophaeus* salamanders were neither separated according to sex in this nor in any other of the congeneric tests. This lack of separation is appropriate in consideration of the finding of insignificance in preference between male and female ochrophaeus in the sex tests that were performed.

*P. ruber* vs. *D. ochrophaeus*

In substrate choice tests, *D. ochrophaeus* did not statistically significantly prefer either substrate ( $t=0.70$ ). This value indicates that there is no chemical communication network in operation between these two species. This may be due to the absence of a selective advantage offered by the existence of such a system. *D. ochrophaeus* may not have come in contact with *P. ruber* to the extent that the evolution of an energetically costly olfactory system would have been unlikely as the costs would outweigh the benefits. It is more likely that experimental errors due to improper medium for olfactory cues which are potentially volatile. Little is known about the olfactory system of *P. ruber* and one cannot be sure of the non-volatile nature of its olfactory cues. In contrast, non-volatility of pheromonal cues has been demonstrated (Roudebush and Taylor 1987) in *Desmognathus* salamanders (*quadramaculatus* and *monticola*). The pheromonal markers (Jaeger and Gergits 1979) that may have been deposited could possibly have been of insufficient concentration to allow detection by the test animals. The *P. rubers* which marked the substrate may have been too low in number (2) to mark the substrate adequately. This hypothesis concerning the cause of inconclusion appears likely as low numbers of *ochrophaeus* were used as well to mark these substrates. All tests conducted before the reception of a large shipment of *D. ochrophaeus* were conducted using no more than three animals per

box to mark the substrate. The tests involving sex discrimination, and choice between *D. ochrophaeus* and *D. monticola* were conducted with 8-15 salamanders per identically sized box. This increase in number of animals which could potentially deposit their odors on the substrate may have contributed to the attainment of conclusive results in that experiment. The temporal confounding of the sex tests disqualifies them from disproving the inadequate scenting hypothesis for the attaining of inconclusive evidence.

*D. quadramaculatus* vs. *D. ochrophaeus* and *D. fuscus* vs. *D. ochrophaeus*

Again in these two experiments, inconclusive results were obtained. One may postulate as an explanation for these findings: (a) the existence of volatile pheromonal markers (b) inadequate marking by too few salamanders to mark the substrate or (c) the non-existence of such a communication system. *Quadramaculatus* is a known predator of *Desmognathus* (Southerland 1986, Roudebush and Taylor 1987). It is thus likely, for the same reason suggested in the discussion of *D. monticola* vs. *ochrophaeus* (adaptive advantage) that such a system exists. Thus (a) and (b) appear more plausible explanations for the findings.

*P. ruber* vs. *D. monticola* and *D. quadramaculatus* vs. *D. monticola*

From these two tests again inconclusive results were obtained. One may speculate here that inadequate scenting was responsible for inconclusive results as a low number of potential scenting animals were unavailable for the entire duration of the experiment. Also, one may conjecture that the *ochrophaeus* test animals were faced with a choice between two "evils" in both tests. All of the above animals have been implicated as potential predators of smaller *Desmognathus* (Roudebush and Taylor 1987, Southerland 1986). Thus the *ochrophaeus* may have been faced with no "choice" at all.

It has thus been shown that *D. ochrophaeus* can detect odors from *D. monticola* which may enable them to avoid potentially harmful interactions with this larger animal.

All other tests proved inconclusive due possibly to a variety of reasons discussed above.

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## GENDER DIFFERENCES IN CLASSROOM SPEECH BEHAVIOR: AN OBSERVATIONAL RESEARCH STUDY

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### ABSTRACT

*The classroom speech behaviors of male and female students at Rhodes College were compared to see if gender differences similar to those reported by the Association of American Colleges Project on the Status and Education of Women would also be found at Rhodes. The study was conducted by students in Psychology 306 (Language and Communication) during Term II of 1987, under the supervision of Dr. Marsha Walton. Twelve discussion oriented classes ranging in size from eight to twenty-two were selected from three divisions of the college, Natural Sciences, Social Sciences and Humanities. A unique pair of observers attended each class session and independently coded classroom speech behavior. Males were found to volunteer at a rate that was more than twice that of females. The percentage of males in each class who contributed at least one speech act was found to be greater than the percentage of females who did. No significant differences were found in the types of speech acts employed by men and women. These and other findings indicated that further research should be done to assure that the educational environment at Rhodes be equally comfortable for women and men.*

### INTRODUCTION

A summary of research, published by the Association of American Colleges Project on the Status and Education of Women found that there are substantial gender differences in the amount and style of class participation. In colleges and universities across the nation men have been found to talk more frequently than women, and to take longer speaking turns. Men interrupt others much more frequently than women do, and women are most likely to be interrupted. These findings led the Association of American Colleges (AAC) to conclude that women students are faced with a "chilly climate" in the classroom that could limit their educational opportunities. An observational study at Rhodes College was designed to identify and measure type and frequency of speech acts made by men and women in the classes of the college. The differences found between the two genders were analyzed to determine whether they could potentially contribute to a chilly climate for women on the Rhodes College campus.

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## METHODS

The sample consisted of twelve classes chosen on the basis of class size and of class structure (classes which involved student discussion and input). Class size ranged from eight to twenty-two, with 13.8 as the mean size. The twelve classes consisted of four classes each from three divisions of the college: Social Science, Natural Science, and Humanities. Each of the twelve classes was attended by a unique pair of observers. Observers sat in locations that would optimize their view of class members and would allow observations to be made unobtrusively. Records were made on standardized tally sheets that were constructed to make recording easy, quick, and accurate. Observers made independent recordings of the speech acts using operational definitions that had been agreed upon previously. No communication occurred between the observers during the class. The reliability of the observations was determined by correlating one observer's data with those of the other. Table 1 lists these correlations for each variable. Observers recorded the following information about student speech acts, specifying the gender of the speaker for each speech act recorded. The "number of volunteered speech acts" corresponds to those speech acts which were independently contributed (i.e., not solicited by the professor in any way), did not interrupt, and were not the result of being called upon. The "number of interrupting speech acts" corresponds to those speech acts which interrupted either another student or the professor and gained the floor for the interrupting student. The "number of called upon speech acts" corresponds to those speech acts which were offered in response to being called upon by the professor, not including those where the speaker had expressed a desire to be called upon. The "number of recognized speech acts" corresponds to those speech acts for which a student expressed a desire to speak and was recognized by the professor. [Note: This variable was excluded from data analysis because there was not acceptable reliability in the two observers judgments.]

The previous four speech acts were mutually exclusive. Each was classified in one or more of the following categories.

- |                                      |  |
|--------------------------------------|--|
| 1) number of clarification questions | those questions which sought clarification on the subject being discussed, e.g. definition of a term.                      |
| 2) number of discussion questions    | those questions which conveyed an original, inquisitive remark, e.g. a question relating to previously unrelated concepts. |
| 3) number of solicited remarks       | those remarks which were elicited by a professor's question.   |
| 4) number of unsolicited remarks     | those remarks which were not elicited by a professor's question.   |
| 5) number of opposing remarks        | those remarks which opposed a viewpoint previously expressed.  |

Each speech act was further classified as to whether it was completed or whether it was brought to an end by an interruption, resulting in the following variable:

number of interrupted speech acts	number of speech acts which were not completed because the current speaker lost the floor to an interrupting speaker. The interrupting speaker was identified as either a male student, a female student, or the professor.
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After the class session, observers reviewed their records and tabulated the following measures:

- 1) number of speech acts contributed by the most frequently speaking female.
- 2) number of speech acts contributed by the most frequently speaking male.
- 3) number of females contributing at least one speech act.
- 4) number of males contributing at least one speech act.

For each gender, the absolute number, the rate and the percentage of the above speech acts were computed. The rate for each speech act performed by women was found by dividing the frequency of that speech act by the number of women in the class. The same was done for men. By dividing the frequency of each particular speech act by the total number of speech acts (again, separated by gender) the percentage occurrence of each speech act was determined.

## RESULTS

A series of t-tests was performed comparing the mean number of male speech acts of each type to the number of female speech acts of that type. From these analyses the following results were obtained. In T-tests comparing males and females across absolute number, rate, and percentage of each type of speech act, only two variables showed significant differences. Males volunteered at a rate that was more than twice that of females (females  $\bar{X} = 2.05$ , males  $\bar{X} = 4.68$ ,  $t = -2.43$ ,  $p < .05$ ). The second finding was that the percentage of males in each class who contributed at least one speech act was greater than the percentage of females who did (females  $\bar{X} = .67$ , males  $\bar{X} = .91$ ,  $t = -2.57$ ,  $p < .05$ ).

A second series of T-tests compared the effect of professor gender on the occurrence of speech acts by students. A significant difference was found on only one variable. In classes taught by male professors, the number of speech turns taken by the most frequently speaking female was almost twice that taken by the most frequently speaking female in classes taught by female professors (female professor  $\bar{X} = 6.00$ , male professor  $\bar{X} = 11.56$ ,  $t = -3.11$ ,  $p < .05$ ).

A one-way analysis of variance was performed to determine if division of the college (Natural Sciences, Social Sciences and Humanities) had an effect on the occurrence of speech acts.

The results showed that there was no significant difference in the number of males or females represented in the classes of each division. Males and females were not over or under represented in any of the three divisions studied.

**Table 1. Reliability Coefficients**  
(expressed as correlation coefficients)

SPEECH ACT	INTEROBSERVER RELIABILITY*	PROBABILITY
Number Volunteered Speech Acts	r = .88 (f)	p < .001
	r = .75 (m)	p < .005
Number Interrupting Speech Acts	r = .82 (f)	p < .001
	r = .99 (m)	p < .001
Number Called Upon Speech Acts	r = .98 (f)	p < .001
	r = .71 (m)	p < .005
Number Recognized Speech Acts	r = .25 (f)	p < .220
	r = .39 (m)	p < .104
Number Interruptions Of Females	r = .82 (m)	p < .001
	r = .62 (f)	p < 0.05
	r = .82 (p)	p < .001
Of Males	r = .98 (m)	p < .001
	r = .96 (f)	p < .001
	r = .71 (p)	p < .005
Number Clarification Questions	r = .89 (f)	p < .001
	r = .94 (m)	p < .001
Number Discussion Questions	r = .66 (f)	p < 0.01
	r = .88 (m)	p < .001
Number Solicited Remarks	r = .74 (f)	p < .005
	r = .55 (m)	p < 0.05
Number Unsolicited Remarks	r = .65 (f)	p < 0.05
	r = .92 (m)	p < .001
Number Opposing Remarks	r = .73 (f)	p < .005
	r = .99 (m)	p < .001
Number Speech Acts By Most Frequent	r = .89 (f)	p < .001
	r = .88 (m)	p < .001
Number Of Contributors	r = .97 (f)	p < .001
	r = .97 (m)	p < .001

\* (f = female, m = male, p = professor)

No significant differences were found in the behavior of the professor (i.e. interrupting, calling upon, recognizing) toward either males or females across the divisions.

The number of times females were interrupted was greater in the Humanities ( $\bar{X} = 4.75$ ) than in the Natural Sciences ( $\bar{X} = 1.25$ ),  $F(2,9) = 4.46$ ,  $p = .05$ . The rate at which females were interrupted, however, was **not** significantly different in the three divisions.

Finally, the variables time of day, day of week, number of males, number of females and percentage of females were correlated with all other variables, yielding a 5 X 82 matrix, from which fourteen variables were correlated at the absolute value of  $r = .5$  or higher. Table 2 presents these correlations.

**TABLE 2**

NUMBER OF FEMALES IN CLASS correlated with	
NUMBER OF DISTINCT FEMALE SPEAKERS	$r = .76, p < .005$
NUMBER OF SPEECH TURNS PER FEMALE	$r = -.55, p < .05$
NUMBER OF MALE SPEECH TURNS	$r = -.50, p < .05$
RATE AT WHICH FEMALE SPEECH TURNS WERE INTERRUPTED BY PROF	$r = -.57, p < .05$
PERCENTAGE OF MALE SPEECH TURNS INTERRUPTED BY FEMALES)	$r = .58, p < .05$
PERCENTAGE OF MALE SPEECH TURNS INTERRUPTED BY MALES)	$r = .50, p < .05$
NUMBER OF MALES IN CLASS correlated with	
NUMBER OF DISTINCT MALE SPEAKERS	$r = .90, p < .001$
PERCENTAGE OF MALES correlated with	
NUMBER OF MALE SPEECH TURNS	$r = .63, p < .05$
NUMBER OF DISTINCT FEMALE SPEAKERS	$r = -.66, p < .01$
NUMBER OF DISTINCT MALE SPEAKERS	$r = .69, p < .01$
NUMBER OF MALE SPEECH TURNS INTERRUPTED BY PROF.	$r = .52, p < .05$
PERCENTAGE OF MALE SPEECH TURNS INTERRUPTED BY MALES)	$r = .57, p < .05$
PERCENTAGE OF FEMALE OPPOSING SPEECH TURNS	$r = .55, p < .05$
NUMBER OF SPEECH TURNS BY MOST FREQUENTLY SPEAKING FEMALE	$r = .52, p < .05$

As the percentage of males dropped, the number of distinct female speakers went up. The number of distinct female speakers was also positively correlated with absolute number of females in the class. Mirroring this finding, the number of distinct male contributors rose as the percentage and absolute number of males in the class rose. Interestingly, the number of male speech turns rose as the percentage of males rose and the number of male speech turns dropped as the absolute number of females rose. However, these findings were not paralleled in the data for female speech turns.

The number of comments by the most frequently speaking female was positively correlated with the percentage of males, whereas this effect was not found for males. This

is to say that as the percentage of males in a class grew, the bulk of female speech turns came to fall increasingly on one female member of the class. However, when males found themselves in a class with a higher percentage of females, they did not rely on only one male member to contribute more. As the absolute number of females in a class rose, the number of speech turns taken per female dropped, whereas males kept their number of speech turns per member of the class more constant, regardless of their absolute numbers.

Occurrence of interruptions showed differences depending on the composition of the class. The number of male speech turns interrupted by the professor and the percentage of male speech turns interrupted by other males in the class both varied directly with the percentage of males in the class. As the absolute number of females in the class grew, so did the percentage of male speech turns interrupted by females, whereas the percentage of male speech turns interrupted by males dropped.

One factor negatively correlated with the number of females in the class is the rate at which females were interrupted by the professor. As absolute number of females in the class rose each female was less likely to be interrupted by the professor. Finally, as the percentage of females in a class rose, the percentage of speech turns that were categorized as opposing the turn before dropped.

## DISCUSSION

Overall, the findings of this study indicated that patterns of student participation at Rhodes were similar in some respects to those in the other institutions studied in the AAC report. Males were found to volunteer more frequently than females and the number of males making speech contributions was greater than the number of females making contributions.

Other findings have not been reported in previous research and were unanticipated. Among these, one of the more interesting is the finding that the number of speech turns taken by the most frequently speaking female in male professors' classes is almost twice that taken by the most frequently speaking female in classes taught by female professors. The mean of 6.78 females per class taught by male professors as compared to the 11.67 in classes taught by female professors may explain why there are on average 11.56 speech turns taken by the most frequently speaking female in classes taught by male professors as compared to the 6.00 speech turns in classes taught by females. If there are fewer females present, perhaps those that are present will each speak more, as representatives of the female sex. If more females were present, each female might feel less of an obligation to speak, thereby lowering the number of speech acts of the most frequently speaking female.

The findings that the number of distinct female speakers was positively correlated with the percentage and absolute number of females and that the number of distinct male speakers was positively correlated with the percentage of males indicate is that when there are more members of a particular gender in a class, more members of that gender speak, which is as to be expected. However the finding that the number of male speech turns is positively correlated with the percentage of males and negatively correlated with the absolute number of females indicates that as their percentage rises, only males increase their actual number of speaking turns as opposed to increasing only the number of participants. The number of female speaking turns does not rise as the percentage of females rises.

Another interesting finding was that the number of times females were interrupted in Humanities classes differed significantly from the number of times females were interrupted in Natural Science classes. The rate at which females were interrupted across divisions of the college, however, was not significantly different for any division of the college. This suggests that the marginally significant difference found in the number of females interrupted in the Humanities and the Natural Sciences may be due to a slight (but not significant) difference in the number of females in the Humanities and Natural Science classes.

The finding that the percentage of male speech turns interrupted by females was positively correlated with the number of females in the class but the percentage of male speech turns interrupted by males was negatively correlated might indicate that women students see a "safety in numbers" effect when it comes to interrupting males. Males seem to respond to increasing numbers of females by not interrupting their own gender. These results are not balanced however. Males do not take advantage of their higher numbers to interrupt females more, nor do females interrupt each other less in classes with higher numbers of males.

The finding that there was a negative correlation between the number of females in the class and the rate at which females were interrupted by the professor does not necessarily indicate malice on the part of a professor with small numbers of female students in his or her class. There was no significant correlation found between the number of females interrupted by the professor and the absolute number of females present. This finding suggests that the rate at which females were interrupted by the professor fell as the number of females rose simply because there were more females present, not because there were fewer interruptions by the professor.

In contrast to AAC findings, however, the rate at which males and females interrupted others was not significantly different nor was there a significant difference in the number of times women and men interrupted others. Also in contrast to AAC findings, no significant differences were found in either the number or rate at which females and males were interrupted by the professor, other males, or other females. These findings suggest that interruptions are not a contributing factor to a chilly classroom climate for female students at Rhodes.

Interestingly, no significant differences were found in the types of speech acts employed by women and men. No significant differences were found in the number of incidents or rate at which women and men were called upon. Furthermore, no significant differences in the number or rate of solicited or unsolicited comments made by women or men were found. Nor were there any significant differences in the number or rate of opposing comments made by men and women. Finally, no significant difference were found in the percentage of each type of comment made as a function of the total number of comments made by women and men. These findings suggest that men and women at Rhodes do not differ in the types of speech acts that make up their classroom communication. The results also suggest that in those speech acts that depend upon a professor's behavior (i.e. called upon and solicited comments), there was no significant gender bias. This indicates that Rhode's professors call upon female and male students at approximately the same rate. Other aspects of the act of calling upon someone (e.g. intonation, use of name, type of question asked), however, were not measured and could conceivably be different for women

and men.

Unfortunately, the results of this study indicated that not all is "balmy" for women in Rhodes classrooms. Why is it that women at Rhodes allow or accept male dominance of classroom discussion? Perhaps socialization causes such behavior. It could also be that both genders have internalized the idea that men are better students. Why is it that women tend to depend on one member of their gender to do the majority of the speaking? It is possible that speaking up in class is such a non-normative behaviour for women that it is implicitly understood that most women are not expected to speak and that those who do are the exceptions. What is it that produces a chilly climate for women? Research analyzing professors' behaviors such as types of examples used, types of questions asked of each gender, length of waiting time for a response, encouragement, and other behaviors needs to be conducted. The blame for an uncomfortable educational environment probably cannot be placed upon any one group- students or professors of either gender. Future research is needed to understand why such a climate exists and what can be done to improve the educational conditions that women may be experiencing.

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## THE VOCAL FIXED ACTION PATTERNS OF ROCK DOVES (*Columba livia*)

Mark West†

### ABSTRACT

*Rock doves (Columba livia) have three distinct vocal fixed action patterns. These calls are similar in pitch, but much like human speech, their duration, frequency of occurrence and rate of repetition varies. The rock dove's ability to discern its own call from that of a common outdoor pigeon also suggests that rock dove vocalizations may be used for communication.*

### INTRODUCTION

Many interesting examples of animal communication can be observed in the field of ornithology. Many birds, such as the peacock, present extravagant displays of communication through physical movement. Other birds communicate simply by their markings; certain species are branded with a certain badge that can serve as identification or territory protection. Perhaps the most intriguing example of communication in birds lies in birdsong.

Birdsongs serve many different functions, the most obvious of which is identification. Many species have a wide array of environmentally-determined dialects (Nottebohm 1955). Therefore, most birds are capable of recognizing not only birds of their species, but also birds who live in their immediate vicinity (Seebock 1960). Identification, however, is not the only purpose of birdsong, as birds may sound calls for mating, warning, territory staking, and other types of information transfer.

An easily observed type of birdsong is the coo of the rock dove. The dove has several vocal fixed action patterns (FAPs), each of which is simple and easily recorded. In this experiment, I have recorded (both manually and electronically) the bird's calls, have discovered its FAPs, and have constructed an ethogram from the collected data.

### MATERIALS AND METHODS

My specimen is a white rock dove, *Columba livia*. Most doves of this species have some coloration, and this particular solid white bird is a rarity commonly used in magic acts. The bird is somewhat domesticated as well. The dove is kept in a portable plastic cage, approximately two feet wide, one foot tall, and ten inches wide. The front is wire mesh with one-inch-square holes, and light may also enter through a series of holes near the top of the cage. The cage was lined with newspaper, and the bird was given birdseed and fresh water (placed in a cup at the rear of the cage) daily. At no time was anything placed over the bird's light source, so the bird had an unadulterated sense of night and day.

Because I did not have practical access to a sonograph or other such electronic recording device, I have recorded the bird's calls using standard musical notation. A note on

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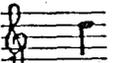
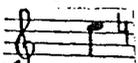
The third and least often heard FAP is FAP C:



FAP C never occurs in conjunction with any other FAP, and only occurs once or twice at a time.

There are also other elements of the dove's coo vocalizations that are difficult to show in diagrammatic form. The first is the relative length of time between vocal FAPs. FAPs are either contiguously connected or are separated by a silence of at least fifteen seconds. In figure 1, FAPs are shown as self-repetitive if they were either contiguous or were followed by silence.

Usually the length of separating silence was between thirty seconds and twenty minutes. On rare occasions, however, the dove began to coo after pauses varying from fifteen to thirty seconds. FAP A always followed one of these pauses.

Another element not fully explained by the diagram is the minor variation in both the pitch and duration of some FAPs, especially FAP A. (It is, of course, difficult to say whether or not these variations are just variations or are distinctly separate FAPs. Because these variations are very minute variations from the norm. However, it would be difficult to classify them as distinct FAPs). The second note in FAP A may vary in duration from the common  to an extreme length of . The first and third pitches of FAP A may also vary from a low pitch of  to a high pitch of .

The most fascinating observation of this experiment was the dove's response to outside stimuli. One observed response occurred if a loud noise, such as a human cough, was sounded. If the noise was sounded while the dove cooed, the dove immediately became silent. The same was true if there was a sudden physical movement in the dove's immediate area. This may suggest that the bird naturally lives in a predatory environment and becomes silent, perhaps an example of innate evolved behavior, if it perceives danger.

The dove's most intriguing response to outside stimuli was its reaction to hearing its own recorded voice or the recorded voice of another rock dove. The dove immediately begins to search for the source of the sound, jerking its head in the search for what it may perceive to be another dove. Often, the dove coos back at its recorded voice, in what appears to be an attempt at communication. The hypothesis that this display is communication is supported by the bird's lack of response when the coo of a common wild pigeon (which to the human ear sounds very similar to the dove's call) is played. The dove initially jerks its head in search for the source of the sound of its close relative, then simply ignores it, resuming normal behavior. The data gathered from these observations is presented in Table 1 on the next page:

**TABLE 1. Dove's Response to Recorded Vocalization**

	Coo*	Silence*
Conspecific (dove)	81	19
Non-conspecific (pigeon)	26	74

\* (each call was played 100 times)

### CONCLUSIONS

It is impossible from this limited data and beyond the scope of this paper to make real conclusions regarding the nature of the dove's FAPs, their evolution, or their separate purposes. However, some general observations can be suggested. The dove has three basic FAPs. One of these occurs much more frequently than the other two. Perhaps the two less frequently occurring FAPs convey information on some specific topic. It is equally possible that the minor variations in FAP A carry different information. Perhaps these seemingly random vocalizations have evolved into a sort of pseudo-language with phrase structure grammars similar to that of human speech (Seebok 564). A second and more easily substantiated conclusion states that the dove has the ability to discern the call of its species from a call of a similar species.

While each of these conclusions may be viable, one must be sure not to make any judgements based on this data set alone. Much more testing and study are needed before any conclusions can be formed.

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**REOXYGENATION INJURY IN THE ISOLATED  
PERFUSED WORKING HEART:  
Effects of Human Recombinant Manganese Superoxide  
Dismutase, Soybean Trypsin Inhibitor, and Ethanol**

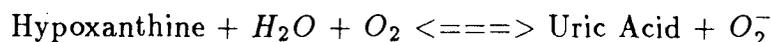
Jay Marecki†

**ABSTRACT**

*Much evidence has been presented implicating active oxygen species in myocardial tissue injury during the reoxygenation of the isolated perfused rat heart following a period of hypoxia. This study used a forty minute anoxic model with subsequent reoxygenation to investigate the role of human recombinant manganese superoxide dismutase, soybean trypsin inhibitor, and ethanol in cell damage as indicated by creatine kinase and lactate dehydrogenase release into the perfusate. The results indicate the possibility of free radical generation in the tissue and open new possibilities in the study of myocardial diseases.*

**INTRODUCTION**

Recent studies of myocardial cells subjected to periods of ischemia with subsequent reperfusion indicate that active oxygen species play an important role in the pathogenesis of cell injury [1,2]. Furthermore, the enzyme xanthine oxidase has also been shown to contribute as a major source of radicals as first proposed in 1981 by Granger *et al.*[3] and supported through further studies [4-6]. The basis of the proposal relies on a low energy state of a cell, induced by ischemia, that allows an influx of calcium-dependant cytosolic proteases. Once activated, the protease converts the NADH-producing enzyme xanthine dehydrogenase by limited proteolysis to the free radical generator xanthine oxidase. As the energy charge of the cell depleats, the stored glycogen reserves are used up resulting in a lose of cytosolic ATP. Concomitantly within the ischemic myocardium, the depletion of ATP results in the elevation of AMP, which is eventually catabolized to adenosine, iosine, and then hypoxanthine [7]. Hypoxanthine acts as an oxidizable purine substrate for xanthine oxidase. Thus, two very important schemes occur within the tissue: the new enzyme activity appears, and the addition of a suitable substrate. The dangers to the myocardium results from the univalent reduction of  $O_2$  by xanthine oxidase to generate superoxide,  $O_2^-$  [8], as shown by the following reaction.



The final substrate for the reaction, molecular oxygen, is supplied during reperfusion when oxygen enters the tissue and a strong burst of superoxide is produced. Furthermore, continued electron reduction through such reactions as the metal-catalyzed Haber Weiss reaction can give rise to other oxidants such as hydrogen peroxide ( $H_2O_2$ ) or the hydroxyl radical ( $OH^-$ ). These active oxygen species are capable of forming lipid peroxides and participating in free radical chain reactions resulting in cell damage and eventual cell death.

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Increased membrane permeability results in the uncoupling of the mitochondria and the escape of intracellular enzymes such as creatine kinase and lactate dehydrogenase [9].

The cells are equipped with detoxifying systems to deal with the harmful oxidants. These include superoxide dismutase (SOD) to scavenge superoxide radicals and catalase to convert hydrogen peroxide to harmless products. Within the ischemic myocardium, the large buildup of  $O_2^-$  can inactivate the catalase, and conversely the large amounts of  $H_2O_2$  can inactivate the SOD. With these crucial pathways blocked, the tissue is subjected to an oxidative stress and eventual cellular damage and death.

Most of the studies in the area of free radical production within the myocardium are performed with the models for global or regional ischemia rather than the models for hypoxia. Here, the oxygen tension of the buffer is lowered to point such that the mitochondria can no longer function, yet the cells remain exposed to constantly renewed extracellular fluid. The complete cessation of perfusion inevitably causes insult and injury beyond that caused by the xanthine oxidase pathway. This additional injury results in tissue lactic acid buildup and a drop in pH. In an effort to eliminate the other aspects of ischemic injury, we studied the buffer-perfused isolated working rat heart subjected to forty minutes of hypoxia followed by reoxygenation. We investigated the newly developed recombinant human manganese superoxide dismutase from human erythrocytes in our model to examine its ability to alleviate some of the free radical damage done in the myocardium. We assayed for the two marker enzymes creatine kinase and lactate dehydrogenase as an indicator for myocardial enzyme leakage via oxygen-induced damage. We also examined a polypeptide that was isolated from soybeans that has a trypsin-inhibiting characteristic. A past study indicates that it could inhibit the superoxide production in human neutrophils, and we investigated the possibilities of a scavenging characteristic within the hypoxia model [9]. Little is known in regards to the involvement of soybean trypsin inhibitor (STI) in the myocardium and thus was a novel approach to mediating active oxygen production. Finally, ethanol was used to see if the first metabolite, acetaldehyde, could also give rise to superoxide production within the myocardium since it is also a substrate for xanthine oxidase and could possibly add to the damage done during reoxygenation.

## MATERIALS AND METHODS

Male Wistar rats weighing 250 to 400 grams were obtained for use. Within one minute following decapitation, the hearts were quickly removed from the animal, rinsed in perfusion buffer, and cannulated on the apparatus. All hearts were perfused with a standard Krebs-Hanseleit buffer maintained at 37 degrees and gassed with either 95%  $O_2$  + 5%  $CO_2$  or 95%  $N_2$  + 5%  $CO_2$  resulting in a final pH of 7.4. The apparatus was modified from the description of Neely [10] for the working heart technique. Once the aorta was cannulated and perfusion begun from a filling reservoir 100 cm above the heart, one of the pulmonary veins leading to the left atrium was isolated and cannulated. A filling pressure of 13 cm  $H_2O$  was applied to the left atrium which resulted in cardiac work by the ejection of fluid against the aortic pressure head. Under the specified conditions, the average coronary flow was approximately 18 mL/min. While being paced at 300 beats/min, the hearts were maintained at 37 degrees in a jacketed chamber and allowed to stabilize for 15 minutes with the oxygenated buffer that contained 11 mM glucose. The hearts were

then switched to the anoxic, glucose-free buffer and perfused for 40 minutes, after which time they were returned to the original buffer. Coronary effluent was collected for one-minute fractions during hypoxia and reoxygenation. Myocardial damage was measured by assaying the perfusate for lactate dehydrogenase (LDH) and creatine kinase (CPK) [11].

To investigate the effects of recombinant human manganese superoxide dismutase (Biotechnology General), soybean trypsin inhibitor (Sigma), and ethanol on tissues with a hypoxic insult, the hearts were treated as above with the substances being added to both perfusion buffers prior to the removal of the hearts.

## RESULTS

The figures [1-5] illustrate the time course of the damage incurred by the myocardium following the reoxygenation of the hypoxic tissue as measured by the release of creatine kinase and lactate dehydrogenase. There was no significant release of the enzymes until oxygen was introduced following forty minutes of glucose-free anoxia. With the manganese SOD at  $10\mu\text{g/ml}$  there was a 51% decrease in the CPK activity as well as a 54% drop in the LDH activity, implicating superoxide production within the system.

Likewise, addition of the soybean trypsin inhibitor provided maximal protection at  $20\mu\text{g/ml}$  with a dramatic increase in CPK (52%) and LDH (44%). The lower concentration provided little protection within the system from the influx of the active species. The highest concentration, however, provided a two-fold increase in the release of both enzymes in response to the oxygenation insult.

Ethanol, however, provided no protection or exacerbation of enzyme release from hypoxic myocardium. These results are seen in Table 1 and Table 2. At the end of the paper is the data for this experiment plotted graphically.

Table 1. Treated Heart CPK Data.

Treatment	CPK IU/gbw	Significance p=	% of Control
Controls n=11	50.9±14.4		
HrMnSOD n=9	25.0±17.7	0.002	49
STI 5 $\mu\text{g/ml}$ n=4	47.6±11.1	0.7	94
STI 20 $\mu\text{g/ml}$ n=5	24.6±12.4	0.004	48
STI 40 $\mu\text{g/ml}$ n=4	103±53.1	0.008	202
Ethanol n=5	54.2±9.1	0.7	106

Table 2. Treated Heart LDH Data.

Treatment	LDH IU/gbw	Significance p=	% of Control
Controls n=11	9.9±2.8		
HrMnSOD n=9	4.5±2.7	0.007	45
STI 5µg/ml n=4	8.8±1.8	0.5	89
STI 20µg/ml n=5	5.6±2.4	0.01	57
STI 40µg/ml n=4	18.9±7.9	0.005	191
Ethanol n=5	9.8±1.6	0.9	99

## DISCUSSION

The present study attempted to eliminate any non-radical dependent mechanisms that would result in the build-up of toxic metabolites resulting in the failure of mechanical perfusion by providing the tissue with continuous buffer throughout the time course studied. With these aside, the oxygen mediated damage due to xanthine oxidase could be examined alone to evaluate the portion of injury to the heart resulting from reoxygenation. In this respect, the recombinant form of human manganese SOD was investigated as to its scavenging abilities within the myocardium. Since it provided considerable protection against the release of cellular enzymes into the perfusate, the results suggest that xanthine oxidase plays an important role in the injury sustained in this model. Because it was not nearly the level reported by other investigators using the copper/zinc form [12] it indicates that there are other mechanisms involved including a  $H_2O_2$ -specific injury, and an  $OH^-$  dependent injury requiring the presence of both  $O_2^-$  and  $H_2O_2$ . Further studies must be performed in order to establish the usefulness of the enzyme within the model, including dose-response and ischemic studies.

With only the indication that soybean trypsin inhibitor could possibly provide protection from superoxide, three concentrations were added to the perfusion buffers. The first, 5µg/ml, provided minimal, although some, protection from the release of enzymes into the perfusate. At 20µg/ml, there was nearly a 50% drop in both enzymes, indicating that the STI could possibly play a very important role within the radical generating system. If the STI can provide as much protection as the SOD, it may also be used to mediate  $O_2^-$  production by way of surface receptors or of other as yet unexplained mechanisms. The results establish its ability within the system to block damage. At high concentrations, however, the results reverse and actually induce a two-fold increase in damage. The results are inconclusive by the possibility of the contamination of the STI with endotoxins and thus providing inaccurate results. For the ethanol at high concentrations (0.5%), no effect

was seen in regards to the enzyme release. Presumably the ethanol was not metabolized within the myocardium and thus gave no rise to acetaldehyde and thus no added substrate for the xanthine oxidase system in the heart.

Through the work done, new possibilities are opened into the role of recombinant enzymes and soybean proteins in the protection of tissues from damage incurred from reoxygenation. The xanthine oxidase does play a role in the enzyme release in the hypoxic myocardium. With the recognition that free radicals are formed, their destructive role in the cell's metabolic activity may become more apparent in ischemia/reperfusion injury.

### ACKNOWLEDGEMENTS

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Figure 1

The effects of human recombinant manganese superoxide dismutase on the release of creatine kinase (CPK) and lactate dehydrogenase (LDH) from reoxygenated rat myocardium subjected to 40 min. of hypoxia. As indicated, all buffers contained superoxide at  $10\mu\text{g/ml}$ . The points represent fractions collected at 1 min. intervals following reoxygenation. For the control group,  $n = 11$ ; for the treated  $n = 9$ .

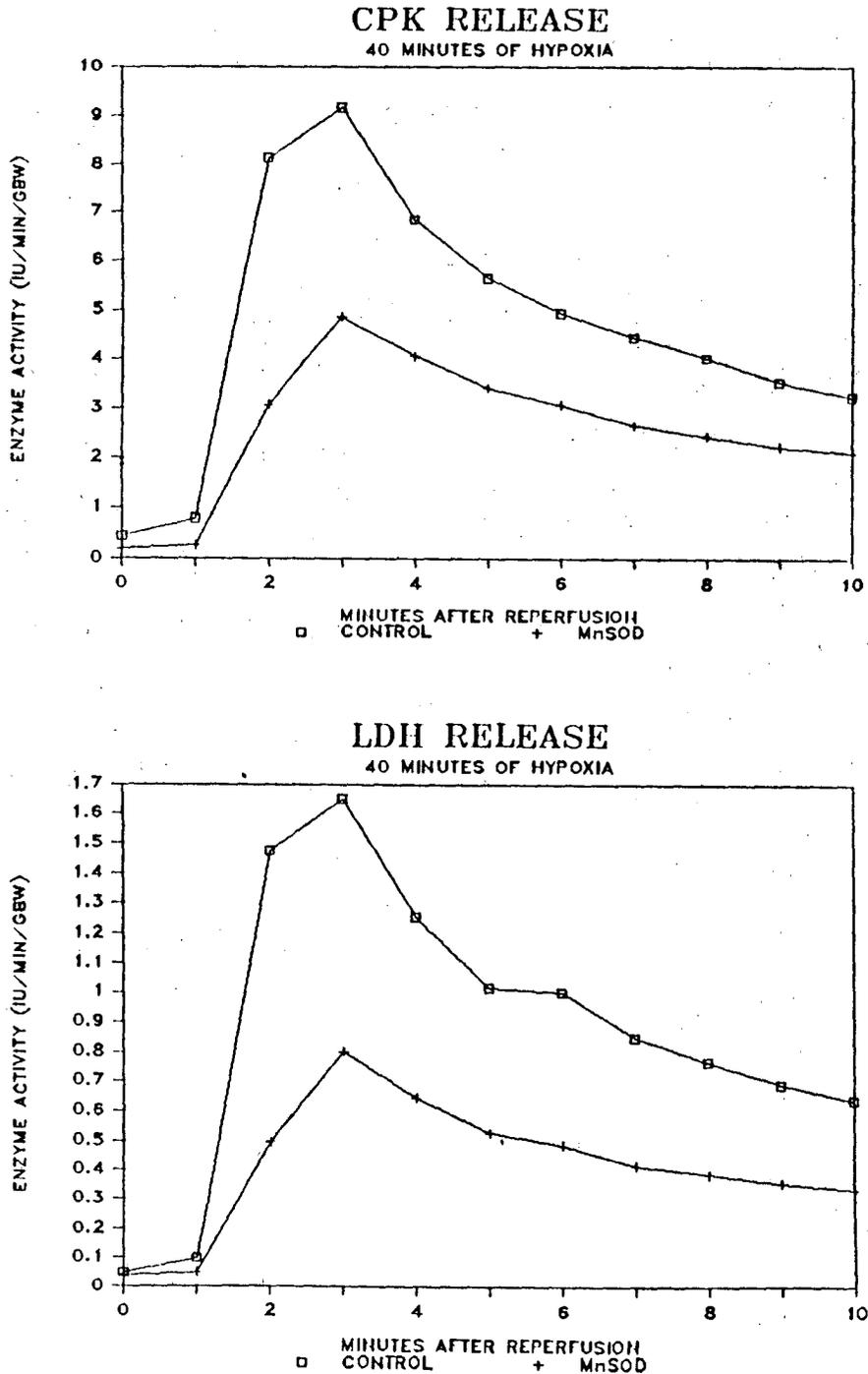


Figure 2

No effect of 0.5% ethanol in both buffers of rat myocardial enzyme release following 40 min. of hypoxia. Points represent the mean of 11 animals for the control and 5 animals for the treated group.

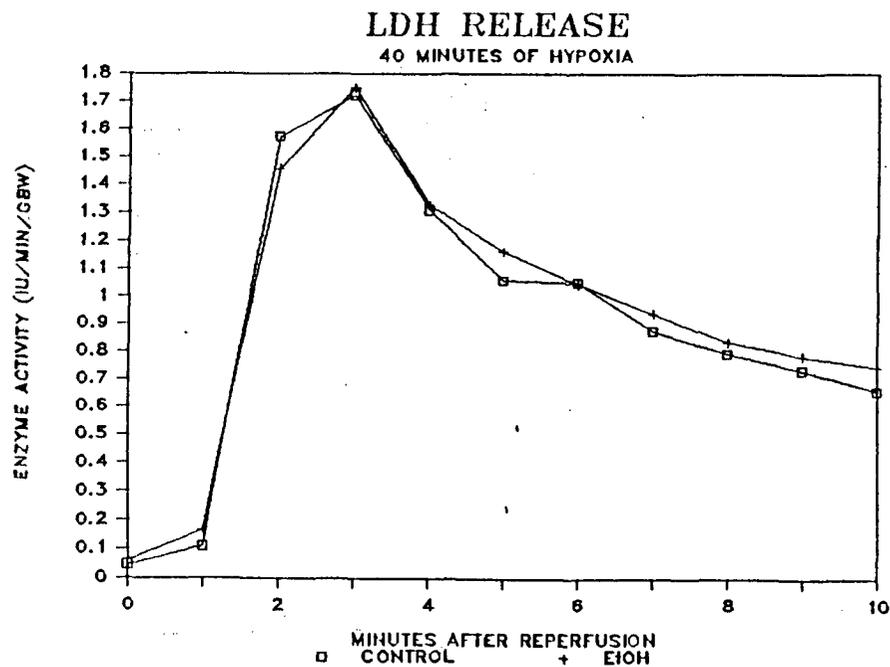
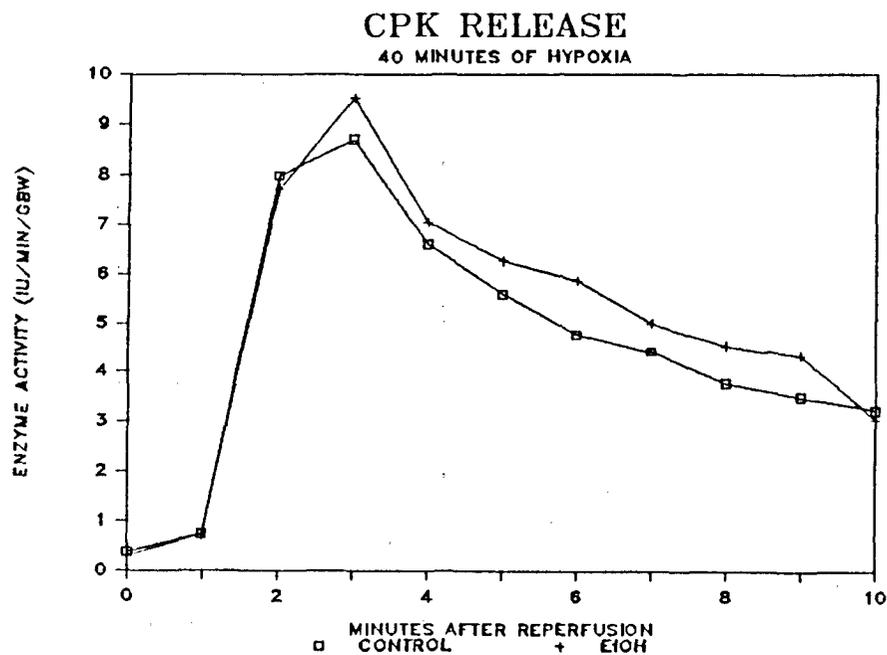


Figure 3

Little prevention of enzyme release upon reoxygenation of hypoxic tissue with a  $5\mu\text{g/ml}$  dose of soybean trypsin inhibitor added to both perfusion buffers. The points represent fractions collected at 1 min. intervals following the reintroduction of oxygen. For the control group  $n = 11$ ; for the treated group  $n = 4$ .

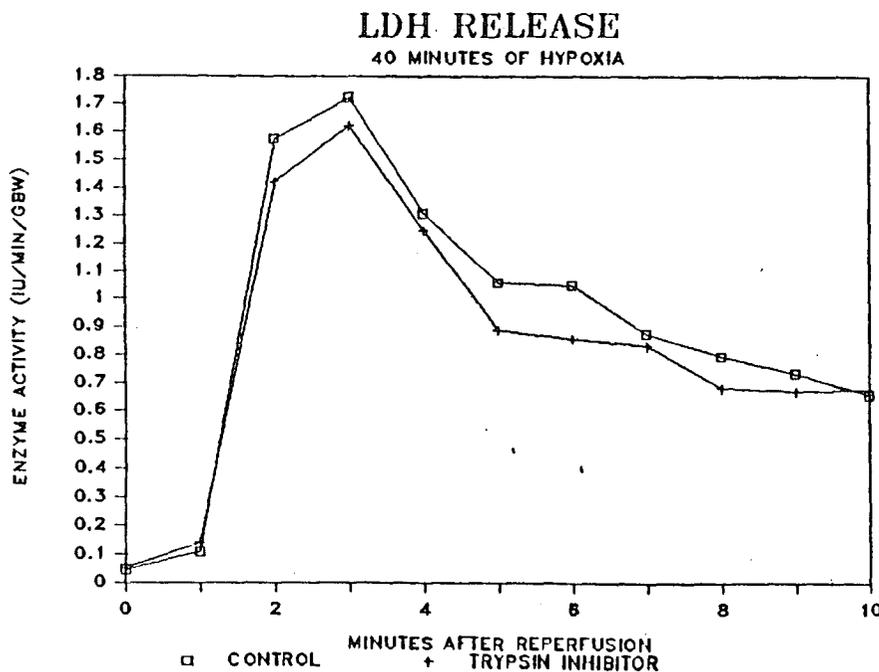
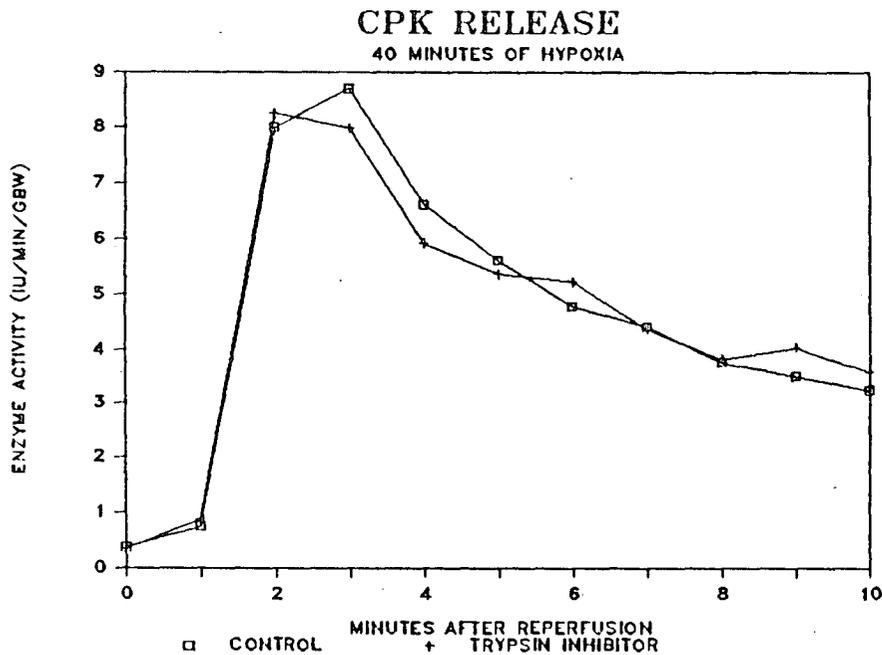


Figure 4

The dramatic prevention by soybean trypsin inhibitor of creatine kinase and lactate dehydrogenase release from myocardial tissue that was reoxygenated following a 40 min. period of anoxia. As indicated, soybean trypsin inhibitor was added to both perfusion buffers at  $20\mu\text{/ml}$ . Each point represents a fraction collected at 1 min. following the reintroduction of oxygen. For the control group,  $n = 11$ ; for the treated group  $n = 5$ .

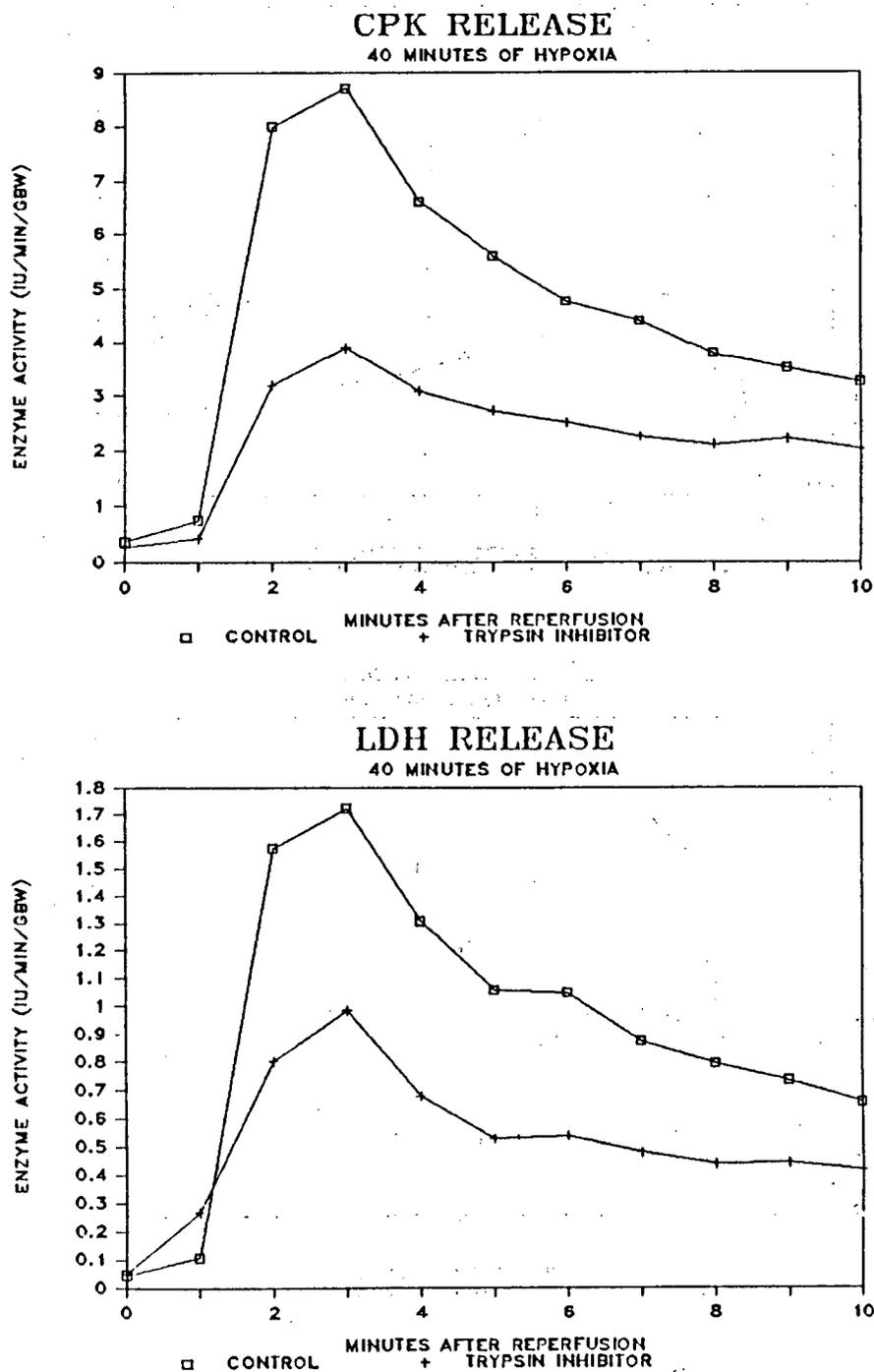


Figure 5

A two-fold increase in the enzyme release from reoxygenated myocardial rat tissue that was exposed to 40 min. of hypoxia with 40 $\mu$ g/ml of soybean trypsin inhibitor present in both perfusion buffers. For the control group, n = 11; for the treated group n = 5.

