BIOCHEMICALLY INDUCED AVOIDANCE

OF SACCHARIN: A PARAMETRIC STUDY

APPROVED:

[Signatures]

Major Professor

Minor Professor

Chairman of the Department of Psychology

Dean of the Graduate School

X-irradiation has been demonstrated to be an aversive stimulus. When paired with a discriminable solution it results in the subsequent avoidance of that solution. This has been demonstrated for a variety of solutions including saccharin, alcohol, chocolate milk, and morphine. Avoidance has also been observed following colloid injections when paired with such drinking solutions. The locus of this phenomenon has been discovered to lie in the reticulo-endothelial system of the body, which is affected by these events, thus generating the observed avoidance. The purpose of this study was to examine some of the parameters of saccharin avoidance relating to varying dose sizes of the colloidal suspension, Proferrin. Since studies reveal additive effects when irradiation and Proferrin are used together, it was hypothesized that different degrees of avoidance would be obtained by using various dose levels.

The subjects for this study were eight female albino rats placed into groups of two. Baseline data were collected prior to injection and each subject was given a choice between water and a saccharin solution to obtain percentages of saccharin intake. Following baseline, subjects in the experimental groups each received one of three different dose levels of Proferrin (.1cc, .2cc, and .3cc). The fourth group served as control subjects and received .4cc physiological
saline. Following injections, subjects were presented with the preferred saccharin solution for eight hours and the water-saccharin choice situation was again instituted subsequent to this period. Fluid consumption was measured and compared to baseline levels for three days following injections. Statistical significances were obtained between the middle dose group (.2cc Proferrin) and controls, showing a considerable decrement in saccharin intake. All experimental subjects revealed saccharin decreases from baseline while control animals showed no change; however, differences were not significant for the high and low dose groups due to the small N used and extinction of the phenomenon during the three days of data evaluated by the analysis of variance.

Following recovery from effects of the first injection, subjects in experimental groups received a second injection at a different dose level than the initial injection. Analysis was not computed on this data due to the omission of two subjects from this phase, but is was shown graphically and discussed. Implications for future research were discussed in terms of the present study, and recommendations for research attempting to replicate this were provided.
BIOCHEMICALLY INDUCED AVOIDANCE
OF SACCHARIN: A PARAMETRIC STUDY

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF ARTS

By

Judith E. Stowe, B. A.
Denton, Texas
January, 1971
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Summary of Analysis of Variance of Saccharin Index for Post-Injection Days and Dose Level</td>
</tr>
<tr>
<td>II</td>
<td>Newman-Keuls Test on Between Group Differences</td>
</tr>
<tr>
<td>III</td>
<td>Summary of Analysis of Covariance of Saccharin Volumes</td>
</tr>
<tr>
<td>IV</td>
<td>Analysis of Adjusted Mean Differences</td>
</tr>
<tr>
<td>V</td>
<td>Mean Differences from Baseline</td>
</tr>
<tr>
<td>VI</td>
<td>Dunnett Test Comparing Treatment and Control Means</td>
</tr>
</tbody>
</table>
## List of Illustrations

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saccharin Index for Injection One</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Saccharin Index for Injection Two</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Cumulative Deviations from Baseline for All Groups over Two Injections</td>
<td>20</td>
</tr>
</tbody>
</table>
Review of Research and Statement of the Problem

In contemporary research considerable interest has developed in seeking biological and biochemical influences on behavior, primarily due to recent concern over the behavioral effects of drugs. Joint efforts in the fields of biology, chemistry, and psychology have brought forth many new discoveries and reopened areas of study previously left by the wayside for other priorities. One area which has been neglected deals with various types of biochemically induced avoidance behavior. Review of early studies with x-irradiation reveal that exposure to this low dose stimulation serves as an unconditioned aversive stimulus (UCS). When a discriminable drinking solution (CS) is paired with exposure to low intensity irradiation (UCS), subjects will subsequently avoid (CR) this solution. Following the classical conditioning paradigm, researchers exposed subjects to a variety of drinking solutions during irradiation, and observed the same result. Avoidance of such solutions as saccharin (McLaurin and Scarborough, 1963; McLaurin, Scarborough and Farley, 1963; McLaurin, 1964; Scarborough and McLaurin, 1964; Garcia, Kimeldorf and Koelling, 1955), chocolate milk (Kimeldorf, Garcia and Rubadeau, 1960), NaCl (Perry, 1963), ethanol (Peacock and Watson, 1964), kool-aid (Harlow, 1962), chocolate on food (Hulse and Dempsey, 1964), and morphine (Mountjoy and Roberts, 1967) has been obtained reliably. In addition to exploring this phenomenon in relation to drinking solutions, researchers
investigated the avoidance in various organisms. Once again, there appeared to be no specificity, and avoidance occurred in such subjects as rats (McLaurin et al., 1963; Scarborough et al., 1964; Garcia et al., 1955), mice (Peacock et al., 1964), and monkeys (Harlow, 1962). Thus avoidance generated by pairing discriminable solutions to low doses of irradiation appears to be neither solution or species specific. In a further attempt to analyze the generality of this phenomenon, Scarborough and Addison (1962) succeeded in demonstrating suppression of instrumental responding in fish using high dosage irradiation.

These data thus seem to indicate broad implications for psychology, as well as raising the imposing question of why the avoidance behavior occurs. One of the more traditional explanations for such phenomena provided by the behavioral sciences, as briefly mentioned earlier, is based on the classical conditioning paradigm. Under these conditions, a conditioned stimulus (CS) is presented prior to or simultaneously with the unconditioned stimulus (UCS), resulting in the CS acquiring characteristics of the UCS. Thus the discriminable solution serves as the CS which is paired with the UCS, irradiation. Since the irradiation possesses aversive properties, these are acquired by the CS, and subsequent avoidance of the solution results. Until recently, this explanation was adequate since studies of pairing saccharin with irradiation revealed strongest avoidance behavior when CS and UCS were presented simultaneously, while a trace paradigm (introducing saccharin before irradiation) produced weaker avoidance. Likewise, when saccharin was presented 24 hours after irradiation, using a backward conditioning design, no avoidance was observed (Garcia,
Kimeldorf, and Hunt, 1961). Discrepant data, however, arose with the research of Scarborough, Whaley, and Rogers (1964) when these researchers investigated a backward conditioning paradigm but presented saccharin after shorter delays than 24 hours following irradiation. The results revealed that the greatest avoidance occurred using no delay, followed by delays of 3, 6, and 12 hours. No significant avoidance was observed when saccharin was given more than 12 hours following irradiation, thus accounting for the data of Garcia et al. (1961).

In light of these data, the backward conditioning explanation appears untenable since classical research using variables other than saccharin avoidance has failed to obtain results with delays of more than several minutes.

Consideration was also given the possibility that irradiation resulted in injury which was responsible for the avoidance. Examination of exposed tissue, however, revealed no damage and it was proposed that the decrement in saccharin consumption was dependent upon the experience of ingestion coupled with radiation exposure (Garcia et al., 1961).

To provide a more tenable alternative, Scarborough et al. (1964) hypothesized that since the avoidance phenomenon could be initiated long after termination of the irradiation, therefore, some other physiological mechanism remained active to account for this delayed effect. They further suggested that a physiological disturbance located in the humoral systems of the body might be responsible.

In accordance with this hypothesis, the earlier research of Garcia and Kimeldorf (1960) offered substantial support. In studying
the degree of avoidance obtained by irradiation of isolated areas of
the body, they found low dosage irradiation of the abdominal region
produced greater avoidance than exposure of other isolated areas,
with a cumulative effect of nearly 100 percent avoidance following
total body irradiation. These investigators made the point that the
abdominal region is the locus of a large vascular plexis and that
this phenomenon may therefore be a function of a humoral mechanism.

The work of Hunt and Kimeldorf (1967) gave considerable impetus
to the humoral system hypothesis. They linked together the circulatory
systems of two rats through a cutaneous vascular anastomosis. Following
this, the animals were shielded from one another and one rat was
irradiated while saccharin was available to both. The non-irradiated
rat was then tested and found to avoid the saccharin which had been
paired with the irradiation of his partner. To further explore this,
they performed the same circulatory linkage between pairs of rats to
serve as control subjects. In this case, one member of each pair
was irradiated with water available to both subjects. In a second
control group, animals were merely clamped together while saccharin
was available and one member of each pair was irradiated. The results
revealed no saccharin avoidance in any of the non-irradiated subjects
of both control groups. From this collection of data came the
proposition that the reticulo-endothelial system (RES), centered
in the abdominal region of the body, is the basis for the avoidance
phenomenon.

Anatomically, the RES consists of phagocytic cells located
primarily in the bone marrow, liver, lymph nodes, spleen, and
some subcutaneous tissues. The role of the RES has been specified by physiologists as important in clearing the blood of foreign particulate antigens and inert colloids (Pearsall and Weiser, 1970). The phagocytic cells of the RES attack and absorb foreign particles, thus serving the important function as protective agents against disease and infection. Research has demonstrated that immunity and disease resistance depends on the ability of the RES to remove bacterial toxins from the blood stream (Cohn and Parks, 1967). Until recently, such activity was considered the sole function of the RES; however, contemporary research has suggested that this system plays an integral role in other mechanisms of homeostasis. The RES has been shown to be involved in such situations as starvation (Gordon and Katsh, 1952) and shock, both traumatic (Zweifach and Thomas, 1957; McKenna and Zweifach, 1956) and hemorrhagic (Zweifach, Benacerraf, and Thomas, 1957). In particular, Talmage (1955) examined RES response to ionizing radiation revealing broad implications for survival under these conditions.

Earlier exploration of RES activity revealed evidence of its function in stress situations when animals were subjected to drum trauma in an apparatus designed by Nobie and Collip (1942). By exposing rats to tumbling and gradually increasing the number of revolutions every other day, subjects were capable of surviving degrees of trauma in excess of animals which had not undergone this procedure (Noble, 1942). He speculated that exposure to sublethal trauma results in the production of antitoxins which serve to immunize and heighten resistance to drum trauma. McKenna and Zweifach (1956)
were in agreement with this hypothesis and further proposed RES alteration was induced by many varieties of shock and infection. It was also suggested by these investigators that RES stimulation accounted for increased resistance to repeated exposure of drum trauma.

With this background reflecting the apparent role of the RES in drum trauma and stress situations, these researchers accompanied the data with exploration of biochemical influences on RES operation. They observed that when colloidal agents were injected intravenously, the system could be "overloaded" or "blockaded." This forces the protective phagocytic cells to take up these foreign particles until the cells have reached their capacity, rendering the RES non-functional until the particles are destroyed and the system can be cleared. These data have been supported by more recent studies involving RES stimulation and blocking with many different colloidal agents. Thorough examinations have been made of clearance rates of such suspensions including colloidal carbon (Normann and Benditt, 1965), saccharated iron oxide (Heller, 1958), ethyl palmitate (DiLuzio and Morrow, 1971), and heat denatured albumin (Mims, 1963). More relevant to this research was the behavioral observation that RES overloading by use of colloidal agents resulted in lowered resistance to drum trauma when subjects were exposed to sub-lethal doses of drum trauma (McKenna et al, 1956).

With these data which paralleled the evidence from irradiation studies, it was hypothesized that colloidal agents may affect the RES in the same manner, and thus such agents could induce the avoidance
phenomenon. To test this hypothesis, Feinberg (1966) experimented with both irradiation and the colloidal suspension, Proferrin (saccharated iron oxide). Saccharin avoidance was obtained with both Proferrin and irradiation individually, with an additive effect when used concomitantly.

At this stage of the research involving colloid induced avoidance, little is known of the parameters of this phenomenon. A variety of procedures have revealed comparable results. Gold (1967) used Proferrin injections of .2cc. A two hour delay was then followed by six hours of pairing with an alcohol solution before a choice situation was again instituted. In contrast to this, Feinberg (1966) used several dose levels, however, a saccharin-tap water choice situation was introduced immediately with no delay or isolated presentation of the discriminable solution. A variety of modifications of these procedures, particularly involving various dose sizes have also been used in pilot studies. The purpose of the present research is to parametrically investigate the possibility of differential effects due to the administration of several dose levels, and examine these in relation to avoidance obtained to a preferred saccharin solution.

Method

Subjects

The subjects for this study were eight female albino rats approximately 350 grams in weight. Animals were housed in home cages and maintained on free food, while liquid consumption was dictated by the experimental procedure.
Apparatus

Each cage was equipped with two standard Girton drinking bottles and tubes, one for tap water and a second for a saccharin and water solution. A 0.1% solution of saccharin in tap water was prepared for drinking as the preferred solution. Surgical supplies and anesthetics were used for the surgical implantation of prefabricated polyethylene cannulas. Installation of cannulas was necessary to facilitate intravenous injection. A 20 mg/cc solution of saccharated iron oxide (Proferrin) was prepared for injection of experimental subjects, and physiological saline was available for control subjects. Twenty-seven gage 1cc disposable tuberculin syringes were used for injection through the jugular cannulas. An Ohaus Triple Beam Balance was used for weighing subjects and solutions of water and saccharin.

Procedure

Prior to baseline, cannulas were constructed according to the design developed by Weeks (1967) and surgically implanted in all subjects in the right jugular vein as presented by Weeks and Davis (1964). Following surgical recovery, subjects were presented a choice between water and the 0.1% saccharin solution in the home cages. Drinking bottles were weighed at 24 hour intervals to obtain total volumes of water and saccharin consumed. An index of saccharin consumption (SI) was calculated daily from the gram-fluid intake for each subject.

\[
\text{Saccharin Index (SI)} = \frac{\text{total saccharin consumed}}{\text{total fluid consumed}} \times 100
\]

When four consecutive days of stable baseline were obtained for each animal, subjects were divided randomly into four groups of two. Three groups received different doses of Proferrin while the fourth
control group received saline. Three dosage levels of Proferrin were selected of .1cc, .2cc, and .3cc, and the control received .4cc physiological saline. This amount exceeded the largest Proferrin dose to maintain total volume consistency since saline was also given experimental subjects following colloid injections to rinse cannulas.

Preceding intravenous injections, subjects were exposed to a sixteen hour liquid deprivation to insure drinking during the period or pairing with saccharin. Immediately following injections, subjects were presented with the preferred saccharin solution for eight hours. At this point, the choice between water and saccharin was again instituted and bottles were weighed daily for calculation of the saccharin index. Experimental data were collected for a varying number of days, depending upon the rate of extinction of the avoidance response for individual subjects.

When subjects had returned to stable baseline SI values for four consecutive days, each experimental animal was then given a second dose of Proferrin. Subjects receiving a .3cc injection during the first phase, were given a subsequent dose of .2cc. Likewise .2cc animals were given .1cc injections as a second dose, while the low dose group which had received .1cc Proferrin was given .3cc. One subjects in the high dose group did not receive this second injection at .2cc due to an inoperable cannula, while another subject initially receiving .2cc was not injected with .1cc, because the animal's data failed to return to a satisfactory stable baseline level following the initial dosage. Control subjects were likewise not administered a second injection.
Throughout the experiment, data were recorded in grams of fluid intake and SI values calculated. Since these were percentage data, an arcsin transformation was computed. A 4x3 factorial design with repeated measures on one factor was used to analyze the percentage intake. The repeated measure was the first three days of post-injection and the levels of dosage were the non-repeated measures. Saccharin consumption in grams was also evaluated with an analysis of covariance for all subjects following the first injection with the baseline consumption being the covariate. The mean differences between baseline and the first day of post-injection data were also calculated in grams of saccharin consumed for each group. A Dunnett test was then used to compare these treatment and control means for the first injection. Statistical evaluation was not made of data collected following the second injection, since the omission of two animals resulted in an unequal number of subjects in two groups, significantly reducing degrees of freedom.

Results

An analysis of variance on the percentage data of the saccharin index was calculated as summarized in Table I. Significance between groups enabled further evaluation to determine where the differences occurred. A Newman-Keuls test located significant differences between the .2cc Proferrin group and all other groups at the .05 level (See Table II). The analysis of covariance of saccharin intake in grams likewise revealed a significant decrease in saccharin consumption as indicated in Table III, and the source of variance was found to lie between the .2cc group and control subjects only, shown in Table IV.
In spite of lack of significance, decreases in saccharin consumption followed the injections for all groups as can be seen in Figure 1. The greatest decrement for all subjects occurred after the first twenty-four hours with a gradual extinction effect taking place beginning within forty-eight hours in some subjects. The analysis of variance data evaluated three days of post-injection results and thus included this recovery phase, in particular, for subjects receiving .3cc and .1cc Proferrin. Although this interaction effect is observed graphically, it was not statistically significant, but was influential in reducing between group differences for the high and low dose groups.

To further evaluate this aspect, a comparison was made of treatment and control means to examine these differences. A mean of the saccharin baseline data in grams was calculated for each subject and this was compared to the gram-saccharin intake of only the first day of the post-injection phase (See Table V and Figure 3). This consideration of mean differences revealed a trend more in line with the experimental hypothesis that the high dose group should show a greater decrement in saccharin intake than the lower dose groups, with no appreciable change in control consumption. A Dunnett test was performed on this data showing significance at the .1 level, with differences occurring between the high dose group and saline controls as shown in Table VI.

Since subjects received a second injection at different dose levels, this data was plotted in Figure 2. Analysis was not done on these data due to the omission of two subjects, which reduced the N to the point of vitiating statistical analysis. Differences can be
observed, however, in all groups as compared to preceding baseline levels, established following extinction from the first injection. These data indicate in Table V and Figure 3 that subjects receiving a total of .5cc Proferrin (.3cc - injection one; .2cc - injection two) showed a greater overall decrement in saccharin intake when comparing the first day of post-injection data following each dosage than both lower dose groups. Likewise, the group that received the lowest total dosage of .3cc (.2cc - injection one; .1cc - injection two) revealed the least decrement in total saccharin intake, while the group receiving a total of .4cc Proferrin (.1cc - injection one; .3cc - injection two) was in between these.

Discussion

The significance of the results of this experiment was not as dramatic as the data in Figure 1 seem to indicate. All groups showed a definite decrement in saccharin intake; however, with only two subjects in each group, and considerable variability within groups, it is not surprising that statistical significance was not obtained. Further research with the avoidance phenomenon would necessitate using larger groups, particularly when evaluating a measure so sensitive as a dose response curve at these low dose levels. Feinberg (1966) used dose levels in his research of .2cc, .4cc, and .6cc which revealed greater differences between groups and probably would have indicated significances even with such small Ns as used here.
The statistical differences obtained between the middle dose group (.2cc Proferrin) and controls following the first injection was undoubtedly significant due to less variability between subjects in that group as well as the fact that extinction for these subjects did not begin to occur within the three days of post-injection data evaluated; whereas, extinction was nearly complete by the third day in both the high and low dose groups.

Another factor which may have influenced statistical outcomes is the baseline level of saccharin intakes prior to injection. Subjects in both the high and low dose groups had higher mean saccharin intakes before injection ($\bar{X} = 151$ grams) as opposed to subjects in the middle dose group which had a baseline mean of 88 grams. It is possible that subjects with a lower volume intake are more sensitive to drug effects than high volume subjects. Future research would indicate further consideration of this factor and an effort should be made to use subjects with more comparable levels of baseline saccharin volumes.

One factor which is influential, primarily when data is evaluated in terms of total fluid intake as in the saccharin index, is the extent of water consumption. Post-injection percentage data are greatly influenced when an increase in water intake occurs accompanied by the decrement in saccharin. When the total volume of both water and saccharin is divided into the saccharin intake (Saccharin index = total saccharin intake / total fluid intake x 100), the percentage would be greatly reduced by increased water consumption. Since all treatment groups revealed water volume increases from baseline
following Proferrin injections, a comparable water increase in all subjects would more greatly affect percentages in those subjects with saccharin volumes which were decreasing from a baseline level of 88 grams as opposed to subjects which were decreasing from a level of 151 grams saccharin. Here again the influences of baseline saccharin volumes may alter statistical outcomes and should be carefully considered in future research.

The only data which do not seem tarnished by these factors are seen in Table V. This shows that in spite of volume differences during baselines and significances differentiating the middle dose group, results were obtained more in line with expectation of the varying dose sizes. These data represent the differences from baseline of the first day of post-injection saccharin intake.

Although statistical significances were not impressive, the graphs indicate the presence of a phenomenon meriting further exploration. Implications of such avoidance behavior are far reaching. Gold (1968) revealed alcohol avoidance and Mountjoy et al. (1967) obtained avoidance of morphine. At this point, these studies have worked only with animal populations, but seem promising for use with human in such areas as addiction and alcoholism. Most common treatments of such behavioral problems involve aversive conditioning such as drug-induced nausea, shock, etc. However, making use of the physiological system of the RES and artificially inducing an aversive situation without its common uncomfortable or painful accompaniments, a new and exciting method of treatment may be available. In none of the studies previously discussed where consideration was made of factors such as
tissue damage, pain, etc., was there any evidence of discomfort or pain in the subjects. Interestingly enough, the use of Proferrin for this area of research stemmed from the fact that it was commercially manufactured and used for humans with iron anemia (Nissum, 1947; Lucas and Hagadorn, 1952). No report of discomfort or behavioral sideeffects of avoidance to stimuli paired with the injections were indicated; however, the latter could have occurred without the patient's awareness.

Biochemically induced avoidance is a phenomenon necessitating much further research before its expansion to dealing with human problems is justified. Research at present is very impressive, but little is known of the exact physiological correlates with the RES. Since this system is known to be necessary for behavioral activity under stress situations, if the RES were destroyed or totally blockaded through the use of colloids or irradiation, it is conceivable that an organism could not respond or learn to escape or avoid a truely aversive situation. This facet of the research is most exciting, and if the hypothesis holds, it would point out a most important aspect of behavior; that is, learning of such escape and avoidance behavior is at the cellular level, under the control of the reticulo-endothelial system.

Other areas of equal interest in this research are examination of the types of stimuli which may be paired with colloidal injections to obtain avoidance. At present, most research has dealt with drinking solutions, although Scarborough et al. (1962) obtained suppression of instrumental responding with irradiation. It is conceivable,
however, that avoidance of visual, auditory, and other sensory stimulation may be observed when research in this area is expanded and refined.

In conclusion, it is not overly presumptuous to say that research in this area may provide a contribution to the understanding of the biochemical bases of behavior. Independent of the less than conclusive results obtained here, the area of biochemical avoidance should not be underestimated or overlooked. This research will undoubtedly become more sophisticated, necessitating the interdisciplinary cooperation of the biochemist and his refined instrumentation, along with the behavioral techniques of the psychologist.
APPENDIX A
Figure 1--Saccharin Index for Injection One
Figure 2--Saccharin Index for Injection Two
Figure 3--Cumulative Deviations from Baseline for All Groups over Two Injections
### Table I

**SUMMARY OF ANALYSIS OF VARIANCE OF SACCHARIN INDEX FOR POST-INJECTION DAYS AND DOSE LEVEL**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose level (A)</td>
<td>6.9061</td>
<td>3</td>
<td>2.3020</td>
<td>16.1204*</td>
</tr>
<tr>
<td>Error (A)</td>
<td>.5713</td>
<td>4</td>
<td>.1428</td>
<td></td>
</tr>
<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days (B)</td>
<td>.4559</td>
<td>2</td>
<td>.2280</td>
<td>2.2975</td>
</tr>
<tr>
<td>A x B</td>
<td>.6818</td>
<td>6</td>
<td>.1136</td>
<td>1.145</td>
</tr>
<tr>
<td>Error (B)</td>
<td>.7939</td>
<td>8</td>
<td>.0992</td>
<td></td>
</tr>
</tbody>
</table>

*p < .05

### Table II

**NEWMAN-KEULS TEST ON BETWEEN GROUP DIFFERENCES**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 .2cc Proferrin</th>
<th>Group 2 .3cc Proferrin</th>
<th>Group 3 .1cc Proferrin</th>
<th>Group 4 Saline Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>.2cc</td>
<td>-----</td>
<td>.8574*</td>
<td>1.1579*</td>
<td>1.4286*</td>
</tr>
<tr>
<td>.3cc</td>
<td>-----</td>
<td></td>
<td>.3005</td>
<td>.5712</td>
</tr>
<tr>
<td>.1cc</td>
<td></td>
<td></td>
<td></td>
<td>.2707</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
<td>-----</td>
</tr>
</tbody>
</table>

*p < .05
### Table III
SUMMARY OF ANALYSIS OF COVARIANCE
OF SACCHARIN VOLUMES

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose level (A)</td>
<td>32824.987</td>
<td>1</td>
<td>32824.987</td>
<td>10.239*</td>
</tr>
<tr>
<td>Error (A)</td>
<td>3205.859</td>
<td>3</td>
<td>1068.619</td>
<td></td>
</tr>
<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days (B)</td>
<td>3631.75</td>
<td>2</td>
<td>1815.875</td>
<td>4.504</td>
</tr>
<tr>
<td>A x B</td>
<td>4892.917</td>
<td>6</td>
<td>815.486</td>
<td>2.022</td>
</tr>
<tr>
<td>Error (B)</td>
<td>2822.00</td>
<td>7</td>
<td>403.143</td>
<td></td>
</tr>
</tbody>
</table>

*p < .05

### Table IV
ANALYSIS OF ADJUSTED MEAN DIFFERENCES

<table>
<thead>
<tr>
<th></th>
<th>Group 1 .3cc Proferrin</th>
<th>Group 2 .2cc Proferrin</th>
<th>Group 3 .1cc Proferrin</th>
<th>Group 4 Saline Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>.3cc</td>
<td>.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.2cc</td>
<td></td>
<td>11.72*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>.1cc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05
Table V
MEAN DIFFERENCES FROM BASELINE (IN GRAMS)

<table>
<thead>
<tr>
<th>Group</th>
<th>Injection 1 Proferrin Dose</th>
<th>Difference</th>
<th>Injection 2 Proferrin Dose</th>
<th>Difference</th>
<th>Totals Dose/Grains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>.3cc</td>
<td>-108</td>
<td>.2cc</td>
<td>-198</td>
<td>.5cc/-306</td>
</tr>
<tr>
<td>Group 2</td>
<td>.2cc</td>
<td>-73</td>
<td>.1cc</td>
<td>-53</td>
<td>.3cc/-126</td>
</tr>
<tr>
<td>Group 3</td>
<td>.1cc</td>
<td>78</td>
<td>.5cc</td>
<td>-118</td>
<td>.4cc/-196</td>
</tr>
<tr>
<td>Group 4</td>
<td>saline</td>
<td>+13</td>
<td></td>
<td></td>
<td>.4cc/saline/+13</td>
</tr>
</tbody>
</table>

Table DVT
DUNNETT TEST COMPARING TREATMENT AND CONTROL MEANS

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>12625</td>
<td>3</td>
<td>4208</td>
<td>5.42*</td>
</tr>
<tr>
<td>Experimental error</td>
<td>3106</td>
<td>4</td>
<td>776</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15731</td>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .1
REFERENCES


