AN ABSTRACT OF THE THESIS OF

Gary Lee Owings (Name) for the MASTER OF SCIENCE (Degree)

in Pharmaceutical Science (Major) presented on November 16, 1973 (Date)

Title: THE INFLUENCE OF CALCIUM CARBONATE AND CALCIUM GLUCONATE ON TETRACYCLINE ABSORPTION IN MAN

Abstract approved: Redacted for Privacy

Dr. Robert W. Sager

This experiment was designed to investigate the in vivo effects of calcium ions on tetracycline absorption when oral doses of tetracycline and calcium-containing medications were administered concurrently. Twenty-one human volunteers were selected and divided into three equal-sized groups: Group A, Group B, and Group C. Each volunteer in Group A received a 250 mg. tetracycline hydrochloride capsule, administered one day with and one day without, a concurrent dose of calcium carbonate. Each volunteer in Group B received a 250 mg. dose of tetracycline syrup, administered one day with and one day without, a concurrent dose of calcium carbonate. Each volunteer in Group C received a 250 mg. tetracycline hydrochloride capsule, administered one day with and one day without, a concurrent dose of calcium gluconate.

On each of the two days of medication administration, a series of five blood samples was drawn at selected times from each

volunteer. These blood samples were used for subsequent serum tetracycline determinations. Data from individual serum tetracycline determinations was used to compute mean serum tetracycline levels for each of the medication groups.

The mean serum tetracycline level data obtained from the medications groups was evaluated statistically by conducting independent-sample mean comparisons and paired-sample mean comparisons. The independent-sample mean comparisons revealed that both calcium carbonate and calcium gluconate significantly decreased absorption of tetracycline. In addition, independent-sample mean comparisons revealed that the depressant effect of calcium carbonate on tetracycline absorption was significantly greater than the depressant effect of calcium gluconate on tetracycline absorption. Paired-sample mean comparisons performed at a 95% confidence level revealed no significant difference between effects of calcium carbonate and calcium gluconate on tetracycline absorption. However, when a 90% confidence level was used, calcium carbonate was again found to have a significantly greater depressant effect on tetracycline absorption than calcium gluconate.

An explanation for the decreased absorption of tetracycline in the presence of calcium carbonate and calcium gluconate has been offered in terms of calcium ion chelation with tetracycline. An additional explanation, one involving gastric pH influences of calcium carbonate, has been offered as a possible explanation for the

significantly greater depressant effect of calcium carbonate on tetracycline absorption.

In addition to the statistical evaluation of the effects of calcium-containing medications on tetracycline absorption, a clinical evaluation of the experimental data was also conducted. The clinical evaluation involved an examination of the median minimum inhibitory concentrations of tetracycline needed for various microorganisms, and a discussion of the effects which calcium-containing medications may have on obtaining these median minimum inhibitory concentrations.

The Influence of Calcium Carbonate and Calcium Gluconate on Tetracycline Absorption in Man

by

Gary Lee Owings

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1974

APPROVED:

Redacted for Privacy

Professor of Pharmaceutical Science
in charge of major

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Date thesis is presented November 16, 1973

Typed by Susie Kozlik for Gary Lee Owings

ACKNOWLEDGEMENT

I would like to express sincere gratitude to all the members of my Graduate Committee, with a special note of thanks to Dr. R. W. Sager and Dr. J. W. Ayres who offered valuable advice and assistance throughout the entire research project.

I would also like to express a deep appreciation to Marvin D. Prince, M. S., and R. A. MacHaffie, M. D., for their consultation and assistance in this research.

A special thanks is offered to all of the volunteers who took part in this research, and to the many other people who gave considerable time and effort in helping to complete this research. Their cooperation and assistance enabled this research to be completed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE INFLUENCE OF CALCIUM CARBONATE AND CALCIUM GLUCONATE ON TETRACYCLINE ABSORPTION IN MAN

I. INTRODUCTION

Literature Review

Tetracycline is an antibiotic which is used in the treatment of certain gram-negative and gram-positive bacterial infections. It is also effective against some microorganisms insensitive to other antimicrobial agents, and against some bacteria that have become resistant to other antibiotics (Goodman and Gilman, 1965). According to a recent national survey, tetracycline is currently the fourth most widely prescribed medication in the United States (National Prescription Audit, 1972).

Like all other antibiotics, tetracycline produces a variety of untoward reactions, perhaps the most distressing of which are the abdominal discomfort, nausea, and vomiting that sometimes occur after oral administration (Kunin and Finland, 1961; Goodman and Gilman, 1965; Osol et al., 1967; Garrod and O'Grady, 1968; Reilly, 1972; Kucers, 1972). It has been estimated that 5% to 25% of the patients who take oral tetracycline may experience some unpleasant gastrointestinal effects (Osol et al., 1967). A direct gastric mucosal irritation by tetracycline appears to be the cause of these gastrointestinal disturbances (DiPalma, 1965; Osol et al., 1967).

Physicians have found that if oral tetracycline is administered with food or milk, many of its undesirable gastrointestinal side effects can be prevented. In fact, when tetracycline was first available for clinical use, it was recommended that troublesome gastric distress be controlled by administration of tetracycline with milk or food, and by sedatives or antacids (Goodman and Gilman, 1965).

Since that time, several studies have been conducted which indicated that certain foods or antacids containing Al^{+3}, Mg^{+2}, Fe^{+2}, or Ca^{+2} impair absorption of tetracycline analogues (Michel et al., 1950; Waisbren and Hueckel, 1950; Albert and Rees, 1956; Price et al., 1957; Weinberg, 1957; Schiener and Altemeier, 1962; Rosenblatt et al., 1966).

In addition, a few experiments have been conducted investigating the effects of certain cations on absorption of tetracycline. These experiments indicated that Mg^{+2}, Fe^{+3}, and the commonly used capsule filler dicalcium phosphate decrease absorption of orally administered tetracycline (Dearborn et al., 1957; Harcourt and Hamburger, 1957; Sweeney et al., 1957; Neuvonen et al., 1970). From the results of the research involving tetracycline and tetracycline analogues, investigators have concluded that certain metallic cations impaired absorption of orally administered tetracycline and tetracycline analogues. These investigators encouraged physicians not to give these drugs with materials which may contain aluminum, magnesium, iron,

or calcium cations. However, physicians still use magnesium, calcium, or aluminum-containing antacids or milk to decrease the incidence of gastrointestinal upset from tetracycline, and their patients do get better (Francke and Whitney, 1972).

Some of the early experiments involving metallic cations and the tetracycline series were poorly designed and may have yielded inaccurate results. These results, in turn, may have led the researchers into making false conclusions. A portion of these early experiments were uncontrolled, while others utilized tetracycline capsules which contained dicalcium phosphate and magnesium salts. Some early experiments did not state the active content of antibiotic in the preparations used, and still other experiments involved the use of two unknowns (Kunin and Finland, 1961). Thus, it appears that these experiments involving tetracycline and metallic cations may have resulted in inaccurate evaluations of the effects of some of the metallic cations on tetracycline absorption.

The basis for impaired absorption of tetracycline in the presence of certain cations appears to be dependent upon the chemical structure of the tetracycline molecule (Weinberg, 1957; Mitscher et al., 1969). The tetracycline molecular structure contains numerous sites with which metallic cations can bind to form insoluble tetracycline:cation chelates. These insoluble chelates may prevent absorption of the tetracycline (DiPalma, 1965).

Recent evidence has accumulated, however, indicating that chelate formation may be dependent upon the pH of the medium surrounding the tetracycline and cation. Mitscher et al., (1969) reported that at pH 4.9, Al^{+3} clearly chelates with tetracycline, but Ca^{+2} and Mg^{+2} gave little evidence for binding at this pH. They also discovered that as the pH was increased, chelation of Ca^{+2} and Mg^{+2} with tetracycline increased.

Another group of investigators has proposed that pH alone influences the absorption of tetracycline (Barr et al., 1971). These investigators used sodium bicarbonate (2 grams) to show that increased pH resulting from the antacid properties of sodium bicarbonate decreased absorption of tetracycline by an average of 50% when concurrent doses of both tetracycline and sodium bicarbonate were given orally.

Statement of the Problem

It appears that if decreased absorption of tetracycline occurs when it is administered with an antacid or food containing aluminum, magnesium, iron, or calcium ions, three possible mechanisms exist which may explain the decreased absorption. First, insoluble chelate formation may be preventing absorption; second, the antacid or food may be increasing the gastric pH sufficiently to prevent absorption;

or third, both pH increases and chelation may be occurring simultaneously to prevent tetracycline absorption.

To date, it seems that no researchers have attempted to determine which one of the above three mechanisms is ultimately responsible for decreased tetracycline absorption in the presence of these cations. In addition, after extensive literature review, it appears that no researchers have investigated the influence of calcium carbonate, a widely used antacid containing Ca^{+2}, on tetracycline absorption.

Purpose of the Study

The purpose of this experiment was to investigate the *in vivo* effects of calcium ions on tetracycline absorption when oral doses of tetracycline and calcium-containing medications were administered concurrently. In addition, should any effects on absorption be observed, the experiment was designed to investigate whether these effects were the result of pH influences of the calcium-containing medication, chelation of the calcium ion, or both pH and chelation.

To aid in the investigation of the possible cause of any effects of calcium ions on tetracycline absorption, calcium carbonate and calcium gluconate were used in this experiment. Both calcium carbonate and calcium gluconate supply calcium ions for possible chelation with tetracycline. However, a difference exists between calcium

carbonate and calcium gluconate in that calcium carbonate is used to increase gastric pH, while no such effect has been reported for calcium gluconate. As a result, calcium carbonate was used in this experiment to determine the extent to which calcium ions influence tetracycline absorption when concurrent pH influences may have been a contributing factor. Calcium gluconate was used to determine the extent to which calcium ions influence tetracycline absorption when concurrent pH influences were not a contributing factor. By using doses of calcium carbonate and calcium gluconate which contained the same amount of calcium ion, and were therefore subject to the same amount of chelation, the investigator also attempted to determine if pH changes produced by calcium carbonate influenced tetracycline absorption.

II. EXPERIMENTAL METHODS AND MATERIALS[1]

Experimental Subjects

A total of 21 healthy adult volunteers between the ages of 21 and 35 served as the subjects for this investigation. Volunteers were screened and it was found that none had a history of acute or chronic renal or hepatic disease, nor a history of gastrointestinal disease or gastrointestinal surgery. None of the volunteers were currently taking other medications, had a known hypersensitivity to medications being used in the study, or had any other medical condition which in the opinion of a physician or pharmacist would endanger the health of the volunteer or adversely influence the results of the study.

All volunteers were informed by a physician of possible adverse reactions to medications that they would be consuming as a participant in the experiment. They were informed by the investigator of the controls they were to follow while participating in this experiment.

The volunteers were required to sign an informed consent statement indicating that they were aware of the nature of the experiment, and that they were freely volunteering to engage in the experiment as a subject. (A copy of the informed consent statement is provided in

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[1] A table listing materials used in this experiment is provided in the Appendix, page 40.

the Appendix, page 41). The 21 volunteers were then randomly divided into three equal-sized groups, Group A, Group B, and Group C.

Experimental Controls

All volunteers were required to fast overnight prior to each of the two days of medication administration. The evening meal of the night before dosing was to be consumed no later than 8:00 P. M. Liquids including tea, water, and black coffee were permitted throughout the experiment. No food or other liquid was permitted until two hours after administration of medication, after which time these items were permitted ad libitum.

All medications were consumed immediately after the control blood sample had been drawn. Volunteers were required to be present for additional blood sampling at one, two, four, and six hours after medication consumption. Normal activities, other than rigorous physical activities, were permitted after medication consumption.

Medication Administration

The three equal-sized groups of volunteers (Group A, Group B, and Group C) participated in the investigation of serum tetracycline levels produced by three different medication regimens. All three

medication regimens consisted of two separate days of medication therapy.[2]

The individuals in Group A each followed a medication regimen consisting of a 250 mg. tetracycline hydrochloride capsule[3] and 180 ml. of distilled water. This medication was administered one day with, and one day without, a 1.181 gm. dose of calcium carbonate. (A dose of 1.181 gm. calcium carbonate is equivalent in acid neutralizing capacity to 2 gm. sodium bicarbonate.) The calcium carbonate was administered suspended in 180 ml. of distilled water.[4]

The individuals in Group B each followed a medication regimen consisting of 10 ml. of a non-buffered tetracycline syrup (equivalent to 250 mg. tetracycline hydrochloride) and 180 ml. of distilled water. This medication was administered one day with, and one day without, a 1.181 gm. dose of calcium carbonate suspended in 180 ml. of distilled water.

The individuals in Group C each followed a medication regimen consisting of a 250 mg. tetracycline hydrochloride capsule and 180 ml. of distilled water. Their medication was administered one day with,

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[2] A table showing the experimental design after randomization is provided in the Appendix, page 42.

[3] A copy of the manufacturer's quality control assay report on the tetracycline capsules used in this study is provided in the Appendix, page 43.

[4] A table showing the medication administration schedule for all groups is provided in the Appendix, page 42.

and one day without, a 5.098 gm. dose of calcium gluconate. (A dose of 5.098 gm. calcium gluconate is stoichiometrically equivalent in calcium to 1.181 gm. calcium carbonate.) The calcium gluconate was administered dissolved in 180 ml. of distilled water.

The two separate days of medication administration were each preceded by an overnight fast and separated by a six day interval. The overnight fast was used to eliminate any influence which foods may exert on the absorption of the administered medications. The purpose of the six day interval was to eliminate possible detectable serum carry-over of medications administered on the first day of the study. The half-life ($t_{\frac{1}{2}}$) of oral tetracycline in patients with normal liver and kidney function is estimated to be from six to eight hours (Greenborg et al., 1967; Bennett et al., 1970). Thus, the six day interval was believed to be sufficient time to eliminate any serum carry-over. (No serum carry-over appeared experimentally, as evidenced by the fact that results from paired-sample mean comparisons at Time 0 hr. were not significantly different from zero.)

Medication administration was randomized so that all 21 volunteers did not receive their assigned calcium-containing medication on the first day of the experiment. However, upon completion of the second day of medication administration, each volunteer had received his assigned calcium-containing medication.

On the days of medication administration, the volunteers were dosed immediately after their control blood sample had been drawn (Time 0 hr.). Dosing was conducted at three minute intervals, starting with Group A at 8:00 A.M., and ending with Group C at 8:59 A.M.

Sample Collection

On the two days of medication administration, each volunteer had blood samples drawn at the following times:

Time 0 hr. - Immediately prior to medication consumption.
Time 1 hr. - One hour after medication consumption.
Time 2 hr. - Two hours after medication consumption.
Time 4 hr. - Four hours after medication consumption.
Time 6 hr. - Six hours after medication consumption.

Thus, each volunteer provided a series of five blood samples on each day of the experiment.[5]

Blood samples were drawn into evacuated 7 ml. glass tubes using 20 gauge, one-inch needles. The blood samples consisted of 5 ml. to 7 ml. of venous blood drawn aseptically from the medial aspect of the forearm of each volunteer by a registered medical technician. The samples were coded and later used for serum tetracycline concentration determinations.

The serum was separated from the whole-blood samples by allowing the blood samples to remain at room temperature for approximately

[5] A table showing the times at which blood samples were drawn is provided in the Appendix, page 44.

30 minutes after drawing. By the end of this period of time, each blood sample had clotted. The blood samples were then centrifuged at 1000G for 20 minutes. This was a sufficient length of time to separate the clot from the pale yellow supernatant serum. The serum samples were then immediately frozen and retained for tetracycline analysis.

Several researchers have shown that freezing of serum samples does not interfere with later serum tetracycline analyses (Dearborn et al., 1957; Harcourt et al., 1957; Foltz, 1958; Holvey et al., 1969; Barr et al., 1971).

Preparation of Reagents

The method of serum tetracycline determination used in this experiment was described by D. M. Wilson (1972). This method is based upon evidence that tetracycline forms fluorescent chelates in the presence of calcium and barbital (Kohn, 1961).

The calcium barbital reagent needed for the analytical procedure was prepared by making two batches of 0.1M sodium barbital and 0.05M calcium acetate solution. After preparation, each batch of solution was adjusted to pH 9 using acetic acid. Each batch of solution was prepared within 24 hours of its use and stored at room temperature in tightly closed amber glass containers. One batch of

this solution was used in the analysis of samples from each of the two days of sample collection.

In addition to the calcium barbital reagent, a Triton X-100: chloroform reagent was needed. This was prepared by making two identical batches of a 1:4 (v/v) mixture of Triton X-100:chloroform. The batches of this solution were also prepared within 24 hours of use and stored at room temperature in tightly closed amber glass containers. One batch of this solution was also used in the analysis of samples from each of the two days of sample collection.

Preparation of Samples for Analysis

Immediately prior to analysis of the serum samples, each sample was removed from the freezer and allowed to thaw at room temperature for one hour. This was found to be sufficient time to permit the samples to thaw completely. A 0.5 ml. portion of each thawed serum sample was removed and placed into an appropriately coded 15 ml. test tube. Next, 10 ml. of the calcium barbital reagent was added to each test tube, followed by 4 ml. of the Triton X-100: chloroform reagent. The tubes were then sealed using rubber stoppers and taped to prevent any leakage. After the tubes had been prepared in this manner, they were shaken manually for 15 minutes. To insure complete mixing, all tubes were inverted several times during the shaking process. After shaking had been completed, the

tubes were centrifuged at 1000G for 10 minutes. The tubes were then removed from the centrifuge and placed in a test tube rack while awaiting fluorometric analysis.

Care was taken to insure that the solution in the centrifuged tubes was not disturbed because centrifugation resulted in the formation of two distinct layers of solution in the test tubes. The upper layer was aqueous, while the lower layer consisted of chloroform containing the fluorescent tetracycline chelate.

Analysis of Samples[6]

The samples were analyzed fluorometrically for tetracycline content by first extracting a sufficient quantity of the lower layer from each of the centrifuged samples. A 1.5 ml. to 2.0 ml. portion of the clear lower layer was drawn from each sample using disposable glass capillary pipets. The solution was then carefully pipetted into a quartz fluorometric cuvette. A single pair of matched cuvettes was used for the entire analytical procedure. Care was taken to insure that the solution in the cuvette was clear and not contaminated by any particles or aqueous droplets which could influence the fluorescence reading of the sample. The cuvette was then placed into an Aminco-Bowman Spectrophotofluorometer which had been allowed to warm up for 30

[6] A copy of the experimental data tables is provided in the Appendix, pages 45-48.

minutes prior to sample analysis. After the sample had been placed into the fluorometer, the lamp shutter was depressed and the sample's fluorescence value was read on an Aminco Photomultiplier Microphotometer. A meter multiplier setting of 0.03 was used on the microphotometer to permit sample fluorescence values to fall on the face of the meter. An activating wavelength of 395 nm and emission wavelength of 525 nm was used on the Spectrophotofluorometer for all sample analyses (Wilson, 1972).

Preparation of a Standard Curve

A plot of the fluorescence of several "known" tetracycline concentrations in serum was prepared to aid in the conversion of sample fluorescence values into actual tetracycline concentrations. The "known" tetracycline concentrations in serum were prepared by first pooling 1.0 ml. portions of the control serum (obtained from the Time 0 hr. blood sample) from each of the 21 volunteers. The pooled serum was then thoroughly mixed to insure uniformity. Next, six "known" tetracycline hydrochloride concentrations in distilled water were prepared. The "known" tetracycline hydrochloride solutions were of such concentrations that when a 0.125 ml. portion of one of the tetracycline solutions was diluted with 0.375 ml. of the pooled serum (quantities measured using a 1.0 ml. glass tuberculin syringe), it would yield a 0.5 ml. "known sample" containing one of the following

six tetracycline concentrations: 0.5 μg/ml., 0.625 μg/ml., 1.0 μg/ml., 1.25 μg/ml., 2.0 μg/ml., or 2.5 μg/ml. The 0.5 ml. "known samples" prepared in this manner were then treated exactly like the 0.5 ml. unknown serum samples and analyzed by the fluorometric procedure previously described. The fluorescence values for these various "known samples" were recorded and a plot of fluorescence vs. "known" tetracycline concentrations was prepared (Figure 1. page 17).[7]

Four 0.5 ml. "known samples" were prepared and analyzed for each of the six tetracycline concentrations listed above. These repeated observations allowed the investigator to obtain a more precise plot of fluorescence vs. known serum tetracycline concentration.

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[7] A table showing the fluorescence values of the six tetracycline concentrations is provided in the Appendix, page 49.

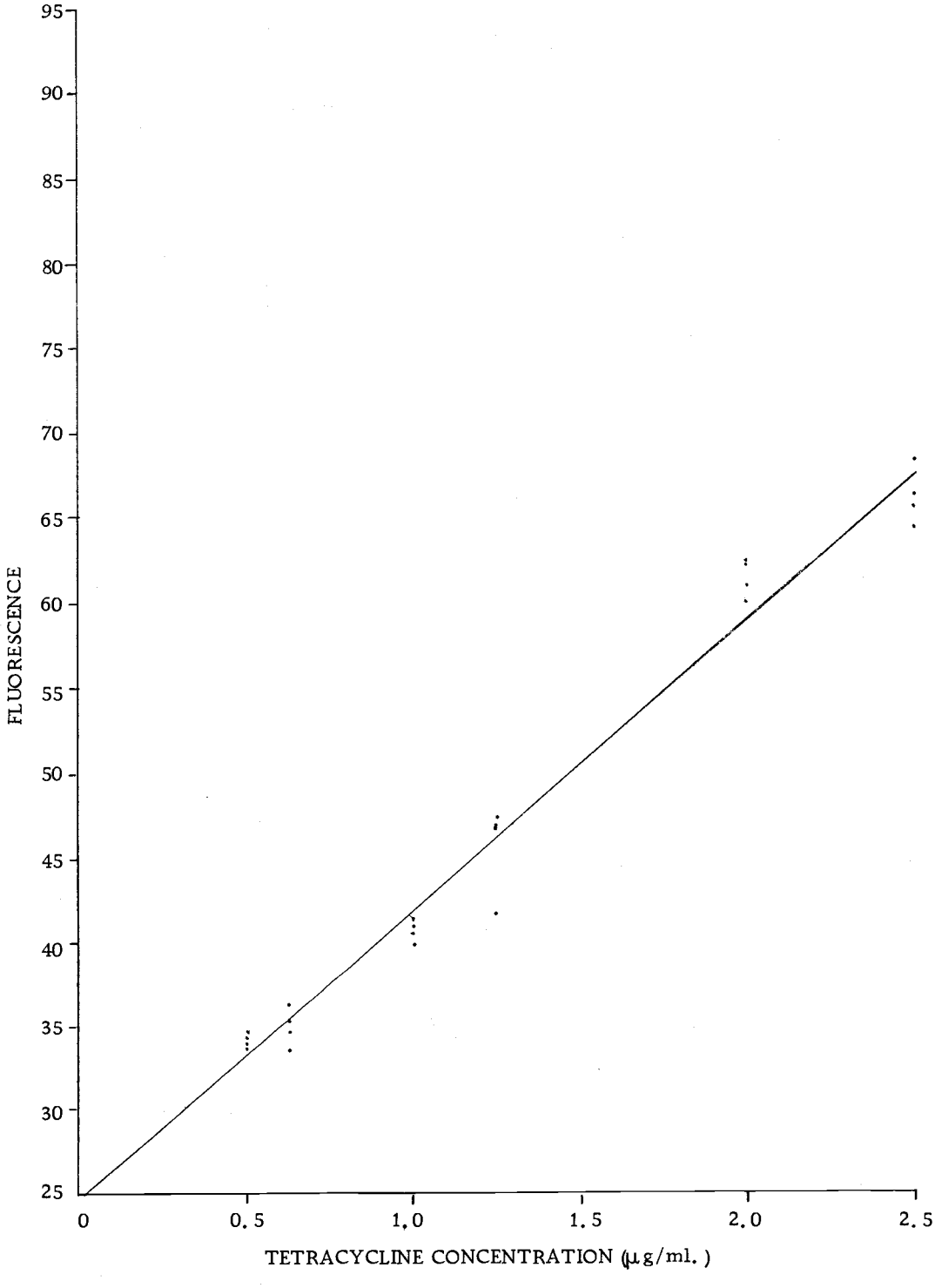

Figure 1. Graph of Fluorescence of Six Known Tetracycline Concentrations

III. RESULTS AND DISCUSSION

The effects of calcium carbonate and calcium gluconate on tetracycline absorption were evaluated by two methods:

1. Independent-Sample Mean Comparisons
2. Paired-Sample Mean Comparisons

Independent-Sample Mean Comparisons

The independent samples were prepared by calculating the mean serum tetracycline concentrations achieved at the five sampling times for the following medication sub-groups:

1. Tetracycline capsule sub-group (a sub-group of Group A), referred to as "TCcc."
2. Tetracycline capsule plus calcium carbonate sub-group (a sub-group of Group A), referred to as "TC+CC."
3. Tetracycline syrup sub-group (a sub-group of Group B), referred to as "TCS."
4. Tetracycline syrup plus calcium carbonate sub-group (a sub-group of Group B), referred to as "TCS+CC."
5. Tetracycline capsule sub-group (a sub-group of Group C), referred to as "TCcg."
6. Tetracycline capsule plus calcium gluconate sub-group (a sub-group of Group C), referred to as "TC+CG."

Independent-sample mean comparisons were then made between the sub-groups listed above. The independent-sample mean comparisons were accomplished by comparing the mean serum tetracycline concentrations achieved with one sub-group to the mean serum

tetracycline concentrations achieved with another sub-group.[8] In all cases, comparison of two sub-groups resulted in a series of five comparisons, one corresponding to each of the five sampling times.[9]

Comparison of the Tetracycline Capsule (TCcc and TCcg) Sub-Groups

A comparison was made between the two tetracycline capsule sub-groups (i.e., TCcc and TCcg). It was found that no significant difference ($\alpha = .05$) existed at sampling times between mean serum levels achieved with the two tetracycline capsule sub-groups. Examination of the graph of "Mean Serum Tetracycline Concentration vs. Time" reveals that each tetracycline capsule sub-group reached peak serum tetracycline levels approximately three hours after medication consumption (Figure 2, page 26).

Comparison of the Tetracycline Capsule (TCcc and TCcg) and the Tetracycline Syrup (TCS) Sub-Groups

When the mean serum tetracycline levels achieved with the two tetracycline capsule sub-groups were compared individually, and collectively, to those achieved with the tetracycline syrup sub-group,

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[8] A table showing all statistical formulas used during independent-sample and paired-sample mean comparisons is provided in the Appendix, page 50.

[9] A table summarizing the results of the independent-sample mean comparisons is provided in the Appendix, page 53.

no significant difference ($\alpha = .05$) was found between mean serum tetracycline levels achieved with these sub-groups at any of the five sampling times. Examination of the graph (Figure 2, page 26), however, reveals that the tetracycline syrup sub-group appeared to reach peak serum concentration more rapidly than either of the tetracycline capsule sub-groups. This observation could be expected and may be explained on the basis of disintegration and dissolution times. No disintegration or dissolution time was needed for the tetracycline syrup, whereas both are required for tetracycline capsules. Thus, more rapid absorption of the tetracycline syrup could be expected.

Comparison of the Tetracycline Capsule (TCcg) and the Tetracycline Capsule Plus Calcium Gluconate (TC+CG) Sub-Groups

The results from comparison of the tetracycline capsule sub-group (TCcg) and the tetracycline capsule plus calcium gluconate sub-group (TC+CG) reveals that at Times 1 hr., 2 hr., 4 hr., and 6 hr., the TC+CG sub-group had significantly lower ($\alpha = .05$) mean serum tetracycline levels than the TCcg sub-group. The TC+CG sub-group achieved serum tetracycline levels which were approximately 50% lower than those of the TCcg sub-groups (Figure 2, page 26). Thus, it appears that the peak serum level obtained from tetracycline administered with calcium gluconate is about one-half of that obtained from tetracycline administered alone.

A possible explanation for this depressed absorption of tetracycline in the presence of calcium gluconate may be made in terms of the formation of insoluble tetracycline:calcium chelates. As proposed by other investigators, calcium ions are believed to form insoluble complexes (called chelates) with the tetracycline molecules. These insoluble chelates prevent absorption of tetracycline, resulting in decreased serum tetracycline levels (Weinberg 1957; Mitscher et al., 1969; DiPalma, 1965).

Comparison of the Tetracycline Capsule (TCcc) and the Tetracycline Capsule Plus Calcium Carbonate (TC+CC) Sub-Groups

The tetracycline capsule plus calcium carbonate sub-group (TC+CC) achieved at Times 1 hr., 2 hr., 4 hr., and 6 hr., mean serum tetracycline levels which were significantly lower ($\alpha = .05$) than those achieved at the same times by the tetracycline capsule sub-group (TCcc). The levels of the TC+CC sub-group were only 25% to 30% of the serum tetracycline levels of the TCcc sub-group. Thus, it appears that calcium carbonate administered concurrently with tetracycline decreased peak serum tetracycline levels to about one-fourth of those of tetracycline administered alone.

A portion of the depressed absorption of tetracycline in the presence of calcium carbonate (a portion equivalent to the depression caused by calcium gluconate) may be explained in terms of

the insoluble, non-absorbed tetracycline:calcium chelates discussed above. However, it appears that further explanation is needed to explain why calcium carbonate caused more depression of tetracycline absorption than calcium gluconate.

A possible explanation for this difference in effects on tetracycline absorption may be offered in terms of the abilities of calcium gluconate and calcium carbonate to influence gastric pH. Calcium carbonate is a well-known antacid which is often used clinically to increase the pH of gastric contents. Calcium gluconate, on the other hand, forms a solution of neutral pH and has not been reported to have antacid capabilities. The influence of pH on tetracycline absorption was pointed out by Barr, et al. (1971) when they demonstrated that increased gastric pH resulted in decreased absorption of tetracycline administered in a solid dosage form. The mechanism of this decreased absorption, as proposed by these investigators, was that increased pH resulted in inhibition of dissolution of solid tetracycline. Since the tetracycline was not dissolved, absorption was reduced. It seems likely that the lower serum tetracycline levels of the TC+CC sub-group, as compared to the TC+CG sub-group, may be a function of the ability of calcium carbonate to increase gastric pH. This ability to increase gastric pH and inhibit dissolution may explain why calcium carbonate produced greater suppression of tetracycline absorption than calcium gluconate.

Comparison of the Tetracycline Syrup (TCS) and the Tetracycline Syrup Plus Calcium Carbonate (TCS+CC) Sub-Groups

Comparison between the tetracycline syrup sub-group (TCS) and the tetracycline syrup plus calcium carbonate sub-group (TCS+CC) revealed that the TCS+CC sub-group had significantly lower ($\alpha = .05$) mean serum tetracycline levels at all sampling times than the TCS sub-group. Examination of the graph (Figure 2, page 26) reveals that calcium carbonate suppressed absorption of tetracycline from syrup to approximately the same levels as it suppressed absorption of tetracycline from capsules.

An explanation for the decreased absorption of tetracycline from syrup in the presence of calcium carbonate can be made only in terms of tetracycline:calcium chelation. The pH effects of calcium carbonate cannot be used to explain decreased absorption of tetracycline from syrup because the tetracycline in tetracycline syrup is present in solution, and Barr et al. (1971) reported that increasing gastric pH does not affect the absorption of tetracycline when administered in solution.

An explanation then, as to why calcium carbonate decreased tetracycline absorption from syrup to approximately the same levels that it decreased tetracycline absorption from capsules, may be offered in terms of extensive tetracycline:calcium chelation. Since the tetracycline in tetracycline syrup was present in solution, it

would be more readily available for chelation with calcium ion than tetracycline administered in the capsule form. As a result, more extensive tetracycline:calcium chelation may have occurred with tetracycline syrup than with tetracycline capsules. This more extensive chelation may explain why serum tetracycline levels obtained with tetracycline syrup in the presence of calcium carbonate were approximately the same as those obtained with tetracycline capsules in the presence of calcium carbonate.

Comparison of the Tetracycline Capsule Plus Calcium Carbonate (TC+CC) and the Tetracycline Capsule Plus Calcium Gluconate (TC+CG) Sub-Groups

Comparisons were made between mean serum tetracycline levels achieved with tetracycline capsules plus calcium carbonate (TC+CC) and those achieved with tetracycline capsules plus calcium gluconate (TC+CG). Results here reveal that the serum tetracycline levels produced by TC+CC were consistently lower at all five sampling times than those produced by TC+CG. This difference, however, is statistically significant ($\alpha = .05$) only at Time 2 hr. It can be seen from the graph (Figure 2, page 26) that at Time 2 hr., serum tetracycline levels achieved by TC+CC are only 57% of those achieved by TC+CG. Thus, it appears that oral doses of calcium carbonate and calcium gluconate, which contain stoichiometrically equivalent

amounts of calcium, suppress tetracycline absorption to different degrees.

A possible explanation for this difference in effects on tetracycline absorption has been offered in a previous section (see page 22). The explanation deals with differences in pH influencing capabilities of calcium carbonate and calcium gluconate.

Comparison of the Tetracycline Capsule Plus Calcium Carbonate (TC+CC) and the Tetracycline Syrup Plus Calcium Carbonate (TCS+CC) Sub-Groups

Comparison of the TC+CC sub-group with the TCS+CC sub-group revealed no significant difference ($\alpha = .05$) in mean serum tetracycline levels achieved at sampling times by either group. However, it appears from the graph (Figure 2, page 26) that again, the sub-group receiving the tetracycline syrup reached peak serum tetracycline levels earlier than the sub-group receiving the tetracycline capsule.

Paired-Sample Mean Comparisons

Paired-samples were formed for Group A, Group B, and Group C. This was accomplished by first pairing each individual's serum tetracycline levels produced by tetracycline alone with their serum tetracycline levels produced by tetracycline plus calcium-containing medication. After the pairing process was completed, the difference

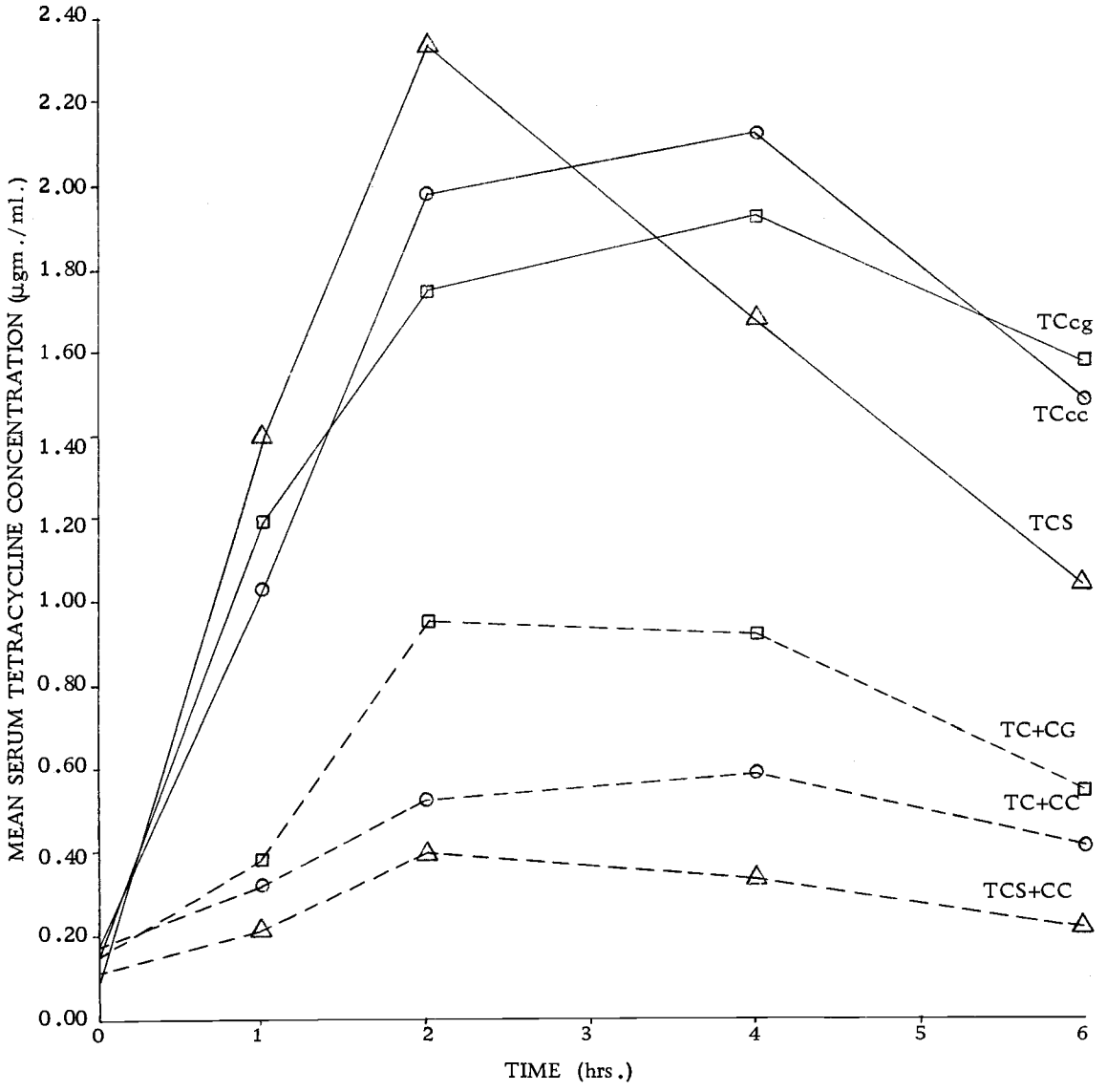

Figure 2. Graph of Mean Serum Tetracycline Concentrations*

TCcg = Mean concentrations achieved with tetracycline capsule (Group C).
TCcc = Mean concentrations achieved with tetracycline capsule (Group A).
TCS = Mean concentrations achieved with tetracycline syrup (Group B).
TC+CG = Mean concentrations achieved with tetracycline capsule + calcium gluconate (Group C).
TC+CC = Mean concentrations achieved with tetracycline capsule + calcium carbonate (Group A).
TCS+CC = Mean concentrations achieved with tetracycline syrup + calcium carbonate (Group B).

*A table showing mean serum tetracycline concentrations along with standard deviations and variances is provided in the Appendix, page 52.

in each pair was computed. This resulted in a series of five "differences" for each individual, one difference corresponding to each of the five sample times (Time 0 hr., 1 hr., 2 hr., 4 hr., and 6 hr.). From these data, group means were calculated for Groups A, B, and C for all five sample times. These group means represent the effects of calcium carbonate on tetracycline capsule absorption for Group A, the effects of calcium carbonate on tetracycline syrup absorption for Group B, and the effects of calcium gluconate on tetracycline capsule absorption for Group C. (In this study, the effects of calcium carbonate and calcium gluconate on tetracycline absorption were found to be effects of depression of tetracycline absorption. A graph of these mean depressant effects of tetracycline absorption is shown in Figure 3, page 31.) The means of the paired-samples, or the mean depressant effects, from Groups A, B, and C were then compared to determine if significant differences existed among the three groups. By performing the pairing process before comparison, the investigator was able to eliminate biological variation among individuals in the study and obtain a more precise estimate of the depressant effects of calcium carbonate and calcium gluconate on tetracycline absorption.

Comparison of Paired-Sample Means from Groups A and B

When paired-sample means of Group A were compared to those of Group B, no statistically significant difference ($\alpha = .05$) was found between the paired-sample means at any of the sampling times. Thus, it appears that in this study, the magnitude by which calcium carbonate depressed tetracycline absorption from capsules was not significantly different from the magnitude by which calcium carbonate depressed tetracycline absorption from syrup.

This result was unexpected. It was anticipated that increased gastric pH resulting from calcium carbonate would inhibit solid tetracycline dissolution, resulting in greater depression of tetracycline absorption from capsules than from syrup. This expectation was based upon work by Barr et al. (1971), in which it was demonstrated that increased gastric pH decreased absorption of tetracycline from capsules but had no effect on absorption from tetracycline solution.

A possible explanation as to why calcium carbonate depression of tetracycline absorption from capsules was not greater than calcium carbonate depression of tetracycline absorption from syrup has been offered in a previous section (see page 23). The explanation suggested that perhaps tetracycline syrup, which contains dissolved

tetracycline, underwent more extensive tetracycline:calcium chelation than solid tetracycline capsules whose dissolution was impaired by increased pH resulting from calcium carbonate. This difference in chelation may explain the disparity between the expected results and those observed experimentally.

Comparison of Paired-Sample Means from Groups A and C

Comparison of the paired-sample means from Groups A and C indicated that there was no significant difference ($\alpha = .05$) at sampling times between the effect of calcium carbonate and calcium gluconate on tetracycline absorption from capsules. However, as can be seen from Figure 3, page 31, calcium carbonate depression of tetracycline absorption was consistently greater (after approximately one hour) than that of calcium gluconate. If a 90% confidence level is used (i. e., $\alpha = 0.1$) as the significance level for the comparison of Groups A and C, the depressant effects of calcium carbonate on tetracycline absorption are shown to be significantly greater at Times 2 hr. and 4 hr. than those of calcium gluconate. Therefore, it appears that the depressant effects of calcium carbonate on tetracycline absorption are somewhat greater than the depressant effects of calcium gluconate on tetracycline absorption.

An explanation for the difference observed between calcium carbonate and calcium gluconate effects on tetracycline absorption has been presented in an earlier section (see page 22), and deals with the difference in pH effects of the two calcium-containing medications.

Comparison of Paired-Sample Means from Groups B and C

The last comparison conducted was between the paired-sample means of Groups B and C. The comparison indicated that at Time 2 hr. the depressant effect of calcium gluconate on tetracycline absorption from capsules was significantly lower ($\alpha = .05$) than the depressant effect of calcium carbonate on tetracycline syrup absorption. No efforts were made to describe the cause of the difference observed between these two groups because not only were two different calcium-containing preparations compared, but also two different tetracycline preparations were compared.

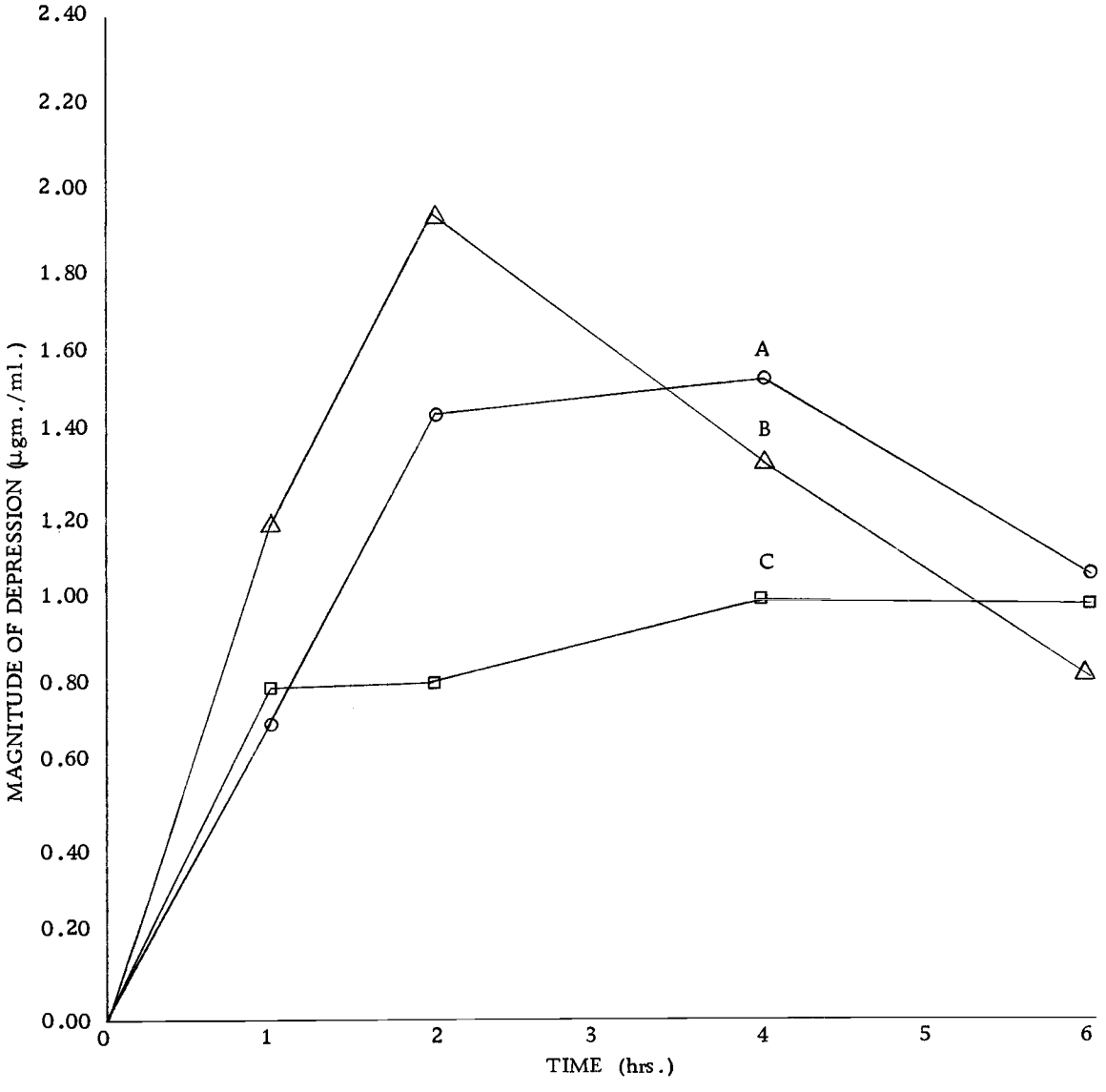

Figure 3. Graph of Mean Depressant Effects of Calcium Carbonate and Calcium Gluconate on Tetracycline Absorption.*

A = Depressant effect of calcium carbonate on tetracycline absorption from capsule (Group A).
B = Depressant effect of calcium carbonate on tetracycline absorption from syrup (Group B).
C = Depressant effect of calcium gluconate on tetracycline absorption from capsule (Group C).

*A table showing the mean depressant effects of calcium carbonate and calcium gluconate on tetracycline absorption, along with standard deviations and variances is provided in the Appendix, page 54.

IV. CONCLUSION

Oral tetracycline, when administered with concurrent doses of calcium carbonate, produced serum tetracycline levels which were only 25% to 30% of those normally achieved with tetracycline administered alone. Thus, it appears from this experiment, that calcium carbonate significantly decreased absorption of orally administered tetracycline.

Oral tetracycline, when administered with concurrent doses of calcium gluconate, produced serum tetracycline levels which were 50% to 55% of those normally achieved with tetracycline administered alone. Thus, it appears from this experiment, that calcium gluconate also significantly decreased absorption of orally administered tetracycline.

The mechanism by which calcium gluconate, and to some extent calcium carbonate, depressed tetracycline absorption may have been through calcium ion chelation of tetracycline. Calcium ions are believed to form insoluble, non-absorbable chelates with tetracycline, which prevent absorption and result in decreased serum tetracycline levels.

Since doses of calcium carbonate which contained the same amount of calcium ions as calcium gluconate resulted in greater depression of tetracycline absorption, calcium ion chelation alone

cannot fully explain calcium carbonate depression of tetracycline absorption. Therefore, it appears that calcium carbonate decreased tetracycline absorption by a second mechanism. This second mechanism is in addition to calcium ion chelation and may be a result of pH influences of calcium carbonate. Calcium carbonate increases gastric pH and this increased gastric pH may have inhibited tetracycline dissolution, resulting in greater depression of tetracycline absorption than was produced by calcium ion chelation alone.

Thus, both of the calcium-containing medications used in this experiment resulted in decreased tetracycline absorption. The decreased tetracycline absorption caused by calcium gluconate has been explained by calcium ion chelation, while the decreased tetracycline absorption caused by calcium carbonate has been explained by calcium ion chelation and pH changes produced by calcium carbonate.

V. CLINICAL EVALUATION

In previous sections of this paper, it was pointed out that both calcium carbonate and calcium gluconate decreased absorption of orally administered tetracycline. Although the decrease in absorption was shown to be statistically significant, no conclusions were drawn as to the clinical significance of this decreased tetracycline absorption.

Before clinical conclusions may be drawn, it is necessary to determine the minimum inhibitory concentrations of tetracycline needed for the effective treatment of susceptible organisms. According to Kucers (1972), organisms inhibited by 1 μg/ml. or less can be regarded as highly sensitive to tetracycline, those inhibited by 1 to 5 μg/ml. as intermediately sensitive, and those not inhibited by at least 5 μg/ml. as tetracycline resistant.

The minimum inhibitory concentrations of tetracycline needed for some selected bacterial species are provided in Table X, page 55. This table is condensed from work by Steigbigel, et al. (1968), and shows the in vitro susceptibility of 223 strains of various bacterial species to tetracycline. (Organisms included in this table are organisms requiring a median minimum inhibitory concentration of less than 5 μg/ml. tetracycline.) The majority of organisms tested by Steigbigel, et al. were isolated from patients who were believed to

have an infection of the isolated organism. Using the median minimum inhibitory concentration as the concentration required for effective treatment of the organisms listed in Table X, it is possible to draw some conclusions regarding the clinical significance of the decreased tetracycline absorption caused by calcium carbonate and calcium gluconate.

As shown in Figure 2, page 26, the mean peak serum tetracycline concentration of the various groups after ingestion of a 250 mg. dose of tetracycline ranged from approximately 2.2 μg/ml. to 2.4 μg/ml. According to the work by Steigbigel, et al., a concentration of 2.2 μg/ml. to 2.4 μg/ml. exceeds the median minimum inhibitory concentration for 135 strains of organisms. Therefore, according to data obtained in this study, a 250 mg. dose of tetracycline administered alone provides serum tetracycline levels in excess of the median minimum inhibitory concentration for 135 strains of organisms listed in Table X.

When tetracycline was administered with concurrent doses of calcium carbonate, the mean peak serum tetracycline concentration achieved was approximately 0.7 μg/ml. According to Table X, a tetracycline concentration of 0.7 μg/ml. exceeds the median minimum inhibitory concentration for only 35 strains of microorganisms (all diplococcus pneumoniae). Thus, when calcium carbonate was administered concurrently with tetracycline, absorption of the

tetracycline was impaired to the extent that the minimum inhibitory concentration needed for many sensitive organisms was not obtained.

Concurrent administration of calcium gluconate and tetracycline resulted in mean peak serum tetracycline levels of approximately 1.2 μg/ml. According to Table X, a concentration of 1.2 μg/ml. exceeds the minimum inhibitory concentration for 126 strains of microorganisms. This is more strains than would be inhibited by tetracycline administered concurrently with calcium carbonate, but less than tetracycline administered alone. Therefore, even though calcium gluconate decreased tetracycline absorption to a lesser extent than calcium carbonate, the decrease in absorption was still sufficient to prevent the attainment of serum tetracycline concentrations needed to inhibit all 135 strains of sensitive microorganisms.

Thus, it appears that the decreased absorption of tetracycline administered concurrently with either calcium carbonate or calcium gluconate may be sufficient to prevent attainment of the minimum inhibitory concentrations of drug needed to inhibit the organisms normally inhibited by doses of tetracycline administered alone. Therefore, in order to prevent the possibility of decreasing tetracycline absorption below that needed for successful therapy, tetracycline should not be administered concurrently with calcium carbonate or calcium gluconate.

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APPENDIX

Table I. Materials Used in Experiment

Tetracycline hydrochloride capsules, 250 mg. Achromycin V, Lederle Laboratories. Control No. 347-554.

Tetracycline syrup, cherry flavored, equivalent to 125 mg./5 ml., Achromycin V, Lederle Laboratories. Control No. 350-415.

Calcium carbonate, reagent grade, Mallinckrodt Chemical Works.

Calcium gluconate, reagent grade, Mallinckrodt Chemical Works.

Calcium acetate, reagent grade, Mallinckrodt Chemical Works.

Acetic acid, reagent grade, Mallinckrodt Chemical Works.

Sodium barbital, reagent grade, Merck Chemical Company.

Chloroform, reagent grade, Mallinckrodt Chemical Works.

Triton X-100, J. T. Baker Chemical Company.

Silicone-coated glass Vacutainer tubes, 7 ml. and 15 ml., Becton, Dickinson & Company.

Vacutainer needles, 20 gauge, one inch, Becton, Dickinson & Company.

Tuberculin syringe, glass, 1.0 ml., Becton, Dickinson & Company.

Disposable syringe, glass, 10 ml., Becton, Dickinson & Company.

Disposable capillary pipets, glass, 9" x 7.0 - 7.4 mm. O. D., Van Waters & Rogers.

Aminco-Bowman Spectrophotofluorometer, Catalog No. 4-8100, American Instrument Company, Inc., 1954.

Aminco Photomultiplier Microphotometer, Catalog No. 4-8100, American Instrument Company, Inc., 1954.

Fused quartz fluorometric cuvettes, Catalog No. 4-8110, American Instrument Company, Inc., 1954.

INFORMED CONSENT STATEMENT

I, _____, state that I am over twenty-
(Name of Participant)

one (21) years of age and have been informed of the nature of the experiment I am about to engage in as a subject when I will ingest tetracycline with and without calcium carbonate or tetracycline with and without calcium gluconate. I have been told by R. MacHaffie, M. D., of the possible adverse pharmacological and allergic reactions I may encounter with these drugs, and the precautions to be taken to prevent them. I hereby freely and voluntarily consent to engage in the experiment as a subject.

_____
(Signature of Volunteer)

_____
(Signature of Physician)

_____
Witness to Explanation
(not to signature)

_____
(Date)

Table II. Table of Experimental Design after Randomization

Patient	Medication Administered on Day 1	Medication Administered on Day 2
	Group A	
1	TC+CC	TC
2	TC	TC+CC
3	TC	TC+CC
4	TC+CC	TC
5	TC	TC+CC
6	TC+CC	TC
7	TC+CC	TC
	Group B	
8	TCS	TCS+CC
9	TCS+CC	TCS
10	TCS	TCS+CC
11	TCS+CC	TCS
12	TCS+CC	TCS
13	TCS	TCS+CC
14	TCS	TCS+CC
	Group C	
15	TC+CG	TC
16	TC+CG	TC
17	TC	TC+CG
18	TC+CG	TC
19	TC	TC+CG
20	TC	TC+CG
21	TC+CG	TC

TC = Tetracycline hydrochloride capsule, 250 mg.*

TCS = Tetracycline syrup, 10 ml. (equivalent to 250 mg. tetracycline hydrochloride)*

TC+CC = Tetracycline hydrochloride capsule, 250 mg., taken with 1.181 gm. calcium carbonate*

TC+CG = Tetracycline hydrochloride capsule, 250 mg., taken with 5.098 gm. calcium gluconate*

TCS+CC = Tetracycline syrup, 10 ml. (equivalent to 250 mg. tetracycline hydrochloride) taken with 1.181 gm. calcium carbonate*

*Administered with 180 ml. distilled water

QUALITY CONTROL ASSAY REPORT

LEDERLE LABORATORIES

	ANALYTICAL NO.	CODE NO.	BATCH NO.	MATERIAL	DEPT. CHARGE	COUNTRY	VENDOR	MONOGRAPH	DATE RECEIVED	REVIEWED
1	21094	4880	292	ACHROMYCIN V CAPSULES 250 mg.	631			10444	11/13/72	
	ACCT. CODE	DAYS TO TEST	DATE DUE	SAMPLE SIZE / RETENTION SIZE	BATCH SIZE/FILL WGT	PRODUCT TYPE CODE	DISPOSITION	APPROVED BY		DISPOSED
	1041654	3	11/16	I 1x30 B 1x150 / REMAINDER		b	1	[signature] 11/14/72		12/06/
	TEST DEPT.	SPECIAL INSTRUCTIONS								
2	822	F.D.A. 1/5000 (min.30)								

	NO.	ASSAY CODE	TEST FOR	RESULTS				LINE CODE	# DET	HOURS	BADGE
2	1	0001-	DESCRIPTION	PASSES							
3	2	9444	COLOR (COLOR HARMONY MANUAL)	12 1/2	pc	1 1/2	ne				
3	3	3411-	WEIGHT VARIATION	AVE g.	0.4798	3.0%hi	4.6%lo				
3	4	9576	DISINTEGRATION	5	min.						
3	5	3497	MOISTURE OF CONTENTS (VACUUM OVEN 3 hrs., 60°C)	0.3%							
2	821		F.D.A. 1/5000 (min.30)								
2	1	0001-	DESCRIPTION	PASSES							
3	6	8539	TETRACYCLINE HCl (CHROM.)	255	mg/cap	102.0%	L.S.				
3	7	1114	TETRACYCLINE HCl (MICROBIAL)	261	mg/cap	104%	L.S.				
			EXPIRATION DATE - JANUARY 1978								
			J-3								
			1) CERT. NO.- 100,136 2) CERT. DATE- 12.04.72 3) ANTI. No.- TC 7955Y 4) EXP. DATE- JAN. 1978								

4880-292
347-554
6-4-73

Table III. Times at Which Blood Samples Were Drawn.

Patient	Time				
	0 hr.	1 hr.	2 hr.	4 hr.	6 hr.
1	8:00 am	9:00 am	10:00 am	12:00 am	2:00 pm
2	8:03	9:03	10:03	12:03	2:03
3	8:06	9:06	10:06	12:06	2:06
4	8:09	9:09	10:09	12:09	2:09
5	8:12	9:12	10:12	12:12	2:12
6	8:15	9:15	10:15	12:15	2:15
7	8:18	9:18	10:18	12:18	2:18
8	8:21	9:21	10:21	12:21	2:21
9	8:24	9:24	10:24	12:24	2:24
10	8:27	9:27	10:27	12:27	2:27
11	8:30	9:30	10:30	12:30	2:30
12	8:33	9:33	10:33	12:33	2:33
13	8:36	9:36	10:36	12:36	2:36
14	8:39	9:39	10:39	12:39	2:39
15	8:42	9:42	10:42	12:42	2:42
16	8:45	9:45	10:45	12:45	2:45
17	8:48	9:48	10:48	12:48	2:48
18	8:51	9:51	10:51	12:51	2:51
19	8:54	9:54	10:54	12:54	2:54
20	8:57	9:57	10:57	12:57	2:57
21	8:59	9:59	10:59	12:59	2:59

Table IV. Experimental Data Tables

Sample Time	Medication Administered on Day 1		Medication Administered on Day 2	
	Fluorescence Value	Serum Tetracycline Concentration	Fluorescence Value	Serum Tetracycline Concentration
		Patient 1		
0 hr.	28.0	0.19	28.0	0.19
1 hr.	29.1	0.25	50.2	1.48
2 hr.	34.0	0.54	54.3	1.71
4 hr.	33.4	0.50	65.8	2.38
6 hr.	29.5	0.27	39.8	0.87
		Patient 2		
0 hr.	25.5	0.04	23.8	0.00
1 hr.	49.6	1.44	23.9	0.00
2 hr.	64.8	2.32	27.0	0.13
4 hr.	59.5	2.01	26.1	0.08
6 hr.	52.1	1.59	23.3	0.00
		Patient 3		
0 hr.	28.0	0.19	27.0	0.13
1 hr.	43.5	1.09	32.1	0.43
2 hr.	45.2	1.19	34.1	0.54
4 hr.	58.0	1.93	30.5	0.33
6 hr.	56.4	1.83	30.4	0.33
		Patient 4		
0 hr.	28.5	0.22	28.0	0.19
1 hr.	28.8	0.23	31.5	0.39
2 hr.	36.1	0.66	57.0	1.87
4 hr.	33.8	0.52	69.9	2.62
6 hr.	33.3	0.49	54.9	1.75
		Patient 5		
0 hr.	26.2	0.08	26.0	0.07
1 hr.	47.2	1.30	30.4	0.33
2 hr.	64.5	2.30	39.0	0.83
4 hr.	51.0	1.52	46.1	1.24
6 hr.	48.9	1.40	41.3	0.96
		Patient 6		
0 hr.	27.1	0.14	25.9	0.07
1 hr.	30.3	0.32	40.2	0.90
2 hr.	31.2	0.37	62.0	2.16
4 hr.	34.4	0.56	55.5	1.78
6 hr.	30.3	0.32	53.2	1.65

(continued)

Table IV. (Continued)

Sample Time	Medication Administered on Day 1		Medication Administered on Day 2	
	Fluorescence Value	Serum Tetracycline Concentration	Fluorescence Value	Serum Tetracycline Concentration
		Patient 7		
0 hr.	32.0	0.42	32.0	0.42
1 hr.	36.2	0.66	35.0	0.59
2 hr.	36.4	0.67	65.0	2.33
4 hr.	40.0	0.88	70.6	2.66
6 hr.	35.0	0.59	47.9	1.34
		Patient 8		
0 hr.	21.9	0.00	22.0	0.00
1 hr.	43.0	1.06	23.0	0.00
2 hr.	61.0	2.10	26.5	0.10
4 hr.	50.2	1.46	25.2	0.03
6 hr.	39.2	0.84	24.7	0.00
		Patient 9		
0 hr.	28.7	0.23	26.8	0.12
1 hr.	29.8	0.29	64.4	2.29
2 hr.	32.2	0.43	100.9	4.42
4 hr.	33.0	0.48	53.5	1.67
6 hr.	30.0	0.30	40.7	0.92
		Patient 10		
0 hr.	22.5	0.00	23.0	0.00
1 hr.	34.0	0.54	23.6	0.00
2 hr.	49.0	1.41	26.0	0.07
4 hr.	43.2	1.08	27.5	0.16
6 hr.	38.6	0.80	25.5	0.04
		Patient 11		
0 hr.	27.6	0.16	27.8	0.18
1 hr.	31.1	0.38	57.1	1.88
2 hr.	35.2	0.61	66.0	2.39
4 hr.	34.0	0.54	80.0	3.20
6 hr.	32.0	0.42	63.9	2.27
		Patient 12		
0 hr.	28.4	0.21	27.1	0.14
1 hr.	33.3	0.50	60.4	2.07
2 hr.	34.0	0.54	64.0	2.28
4 hr.	31.1	0.37	46.6	1.27
6 hr.	29.5	0.27	38.0	0.77

(continued)

Table IV. (Continued)

Sample Time	Medication Administered on Day 1		Medication Administered on Day 2	
	Fluorescence Value	Serum Tetracycline Concentration	Fluorescence Value	Serum Tetracycline Concentration
		Patient 13		
0 hr.	24.6	0.00	22.7	0.00
1 hr.	47.2	1.30	25.0	0.01
2 hr.	59.7	2.03	32.0	0.42
4 hr.	52.6	1.61	27.0	0.13
6 hr.	42.9	1.05	26.0	0.07
		Patient 14		
0 hr.	28.8	0.23	28.0	0.19
1 hr.	36.7	0.69	30.5	0.33
2 hr.	55.0	1.75	36.0	0.65
4 hr.	49.5	1.43	36.0	0.65
6 hr.	35.0	0.59	31.8	0.41
		Patient 15		
0 hr.	32.0	0.42	32.1	0.43
1 hr.	33.0	0.48	33.5	0.51
2 hr.	37.0	0.71	41.2	0.95
4 hr.	43.0	1.06	65.3	2.35
6 hr.	30.5	0.33	48.0	1.35
		Patient 16		
0 hr.	25.3	0.03	25.4	0.04
1 hr.	30.9	0.36	49.0	1.41
2 hr.	39.0	0.83	53.5	1.67
4 hr.	38.5	0.80	65.0	2.33
6 hr.	31.0	0.36	48.4	1.37
		Patient 17		
0 hr.	28.9	0.24	28.7	0.23
1 hr.	40.2	0.89	31.0	0.36
2 hr.	58.1	1.93	47.2	1.30
4 hr.	61.0	2.10	49.9	1.46
6 hr.	60.3	2.06	39.0	0.83
		Patient 18		
0 hr.	25.0	0.01	23.2	0.00
1 hr.	26.1	0.08	51.5	1.55
2 hr.	34.6	0.57	66.0	2.39
4 hr.	32.5	0.45	57.0	1.87
6 hr.	29.6	0.28	54.0	1.70

(continued)

Table IV. (Continued)

Sample Time	Medication Administered on Day 1		Medication Administered on Day 2	
	Fluorescence Value	Serum Tetracycline Concentration	Fluorescence Value	Serum Tetracycline Concentration
		Patient 19		
0 hr.	28.0	0.19	27.1	0.14
1 hr.	38.9	0.82	30.2	0.32
2 hr.	46.0	1.23	42.0	1.00
4 hr.	49.0	1.41	45.7	1.21
6 hr.	47.1	1.30	40.0	0.88
		Patient 20		
0 hr.	26.2	0.08	26.7	0.11
1 hr.	63.0	2.22	35.3	0.61
2 hr.	66.0	2.39	38.0	0.77
4 hr.	59.4	2.01	35.0	0.59
6 hr.	58.0	1.93	34.5	0.56
		Patient 21		
0 hr.	27.0	0.13	26.5	0.10
1 hr.	33.0	0.48	40.5	0.91
2 hr.	49.7	1.45	54.0	1.70
4 hr.	40.3	0.90	50.0	1.46
6 hr.	34.5	0.56	49.2	1.42

Table V. Fluorescence of Six Known Tetracycline Concentrations

Known Tetracycline Concentrations (μg/ml.)	Fluorescence Values			
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 4
2.5	68.3	65.6	64.4	66.2
2.0	62.3	62.2	60.0	61.0
1.25	47.3	47.0	47.0	41.6
1.0	41.4	40.5	40.0	41.0
0.625	35.5	36.2	34.7	33.6
0.5	34.0	34.2	33.9	34.8
0.0 (control)	24.1	26.3	29.0	23.6

Table VI. Table of Statistical Formulas*

I. Statistical Formulas used for Independent-Sample Mean Comparisons

A. $\bar{x} = \sum_{i=1}^{n} (x_i)/n$

B. $s^2 = \sum_{i=1}^{n} (x_i - \bar{x})^2/n-1$

C. $s = \sqrt{s^2}$

D. $s_{\bar{d}}^2 = \frac{s_1^2 + s_2^2}{n}$

E. $L(u_{\bar{d}}) = (\bar{x}_1 - \bar{x}_2) \pm t_{\alpha/2} \sqrt{s_{\bar{d}}^2}$

F. Assume: $n_1 = n_2 = n = 7$; $\sigma_1^2 \neq \sigma_1^2$

Use (n-1) degrees of freedom and 95% confidence level

x_i = the individual observations

$\bar{x}$ = the mean of individual observations

n = the number of individual observations

Σ = mathematical symbol indicating "the sum of"

s^2 = the sample variance

s = the sample standard deviation

$s_{\bar{d}}^2$ = sample variance of mean difference

s_1^2 and s_2^2 = the variances from two samples being compared

$L(u_{\bar{d}})$ = confidence interval estimate of mean difference

$\bar{x}_1$ and $\bar{x}_2$ = the means from two samples being compared

t = students "t" $\alpha/2$ = error rate; = 0.05

II. Statistical Formulas used for Paired-Sample Mean Comparisons

A. $d_i = x_{i1} - x_{i2}$

B. $\bar{d} = \sum_{i=1}^{n} (d_i)/k$

C. $s_d^2 = \sum_{i=1}^{n} (d_i - \bar{d})^2/k-1$

D. $s_{\bar{d}}^2 = s_{d_1}^2 + s_{d_2}^2 / k$

E. $L(u_{\bar{d}}) = (\bar{d}_1 - \bar{d}_2) \pm t_{\alpha/2} \sqrt{s_{\bar{d}}^2}$

F. Asume $k_1 = k_2 = k = 7$; $\sigma_1^2 = \sigma_2^2$

Use $2(k-1)$ degrees of freedom and 95% confidence level

x_{i1} and x_{i2} = a pair of observations made on one individual

d_i = the difference between a pair of observations made on one individual. A series of these "differences" constitute a new sample.

$\bar{d}$ = the mean "difference" of several pairs of observations

k = the number of pairs of observations

s_d^2 = the sample variance

$s_{d_1}^2$ and $s_{d_2}^2$ = the sample variances from two samples being compared

$\bar{d}_1$ and $\bar{d}_2$ = the sample means from two samples being compared.

* Formulas extracted from Exercises in Statistical Inference by Roger G. Peterson, 1972, O. S. U. Book Stores, Inc., Corvallis, Ore.

Table VII. Serum Tetracycline Concentrations

Time		Group A		Group B		Group C	
		TCcc	TC+CC	TCS	TCS+CC	TCcg	TC+CG
0 hr.	mean	0.17	0.17	0.09	0.11	0.15	0.15
	std. dev.*	0.13	0.13	0.10	0.11	0.15	0.14
	variance	0.02	0.02	0.01	0.01	0.02	0.02
1 hr.	mean	1.03	0.32	1.40	0.21	1.19	0.38
	std. dev.	0.42	0.20	0.69	0.21	0.58	0.17
	variance	0.18	0.04	0.47	0.04	0.33	0.03
2 hr.	mean	1.98	0.53	2.34	0.40	1.75	0.95
	std. dev.	0.43	0.23	0.97	0.23	0.54	0.32
	variance	0.18	0.05	0.95	0.05	0.30	0.10
4 hr.	mean	2.13	0.59	1.68	0.34	1.93	0.92
	std. dev.	0.43	0.38	0.70	0.24	0.38	0.35
	variance	0.18	0.14	0.50	0.06	0.15	0.12
6 hr.	mean	1.49	0.42	1.04	0.22	1.59	0.54
	std. dev.	0.32	0.30	0.56	0.18	0.31	0.24
	variance	0.11	0.09	0.32	0.03	0.10	0.06

TCcc = Mean concentrations achieved with tetracycline capsule from Group A

TC+CC = Mean concentrations achieved with tetracycline capsule + calcium carbonate from Group A

TCS = Mean concentrations achieved with tetracycline syrup from Group B

TCS+CC = Mean concentrations achieved with tetracycline syrup + calcium carbonate from Group B

TCcg = Mean concentrations achieved with tetracycline capsule from Group C

TC+CG = Mean concentrations achieved with tetracycline capsule + calcium gluconate from Group C

*Abbreviation for standard deviation.

Table VIII. Table Summarizing Independent-Sample Mean Comparisons

Sub-group	Group A		Group B		Group C	
	TCcc	TC+CC	TCS	TCS+CC	TCcg	TC+CG
			Group A			
TCcc	-	+	0	-	0	-
TC+CC	+	-	-	0	-	x
			Group B			
TCS	0	-	-	+	0	-
TCS+CC	-	0	+	-	-	-
			Group C			
TCcg	0	-	0	-	-	+
TC+CG	-	x	-	-	+	-

+ Indicates that a significant difference ($\alpha = .05$) was found at Sampling Times 1, 2, 4, and 6 hr. when the mean serum tetracycline concentrations of the two sub-groups were compared. (Comparisons were conducted for Sampling Times 0, 1, 2, 4, and 6 hrs.)

x Indicates that a significant difference ($\alpha = .05$) was found only at Sampling Time 2 hr. when the mean serum tetracycline concentrations of the two sub-groups were compared. (Comparisons were conducted for Sampling Times 0, 1, 2, 4, and 6 hrs.)

0 Indicates that no significant difference ($\alpha = .05$) was found when the mean serum tetracycline concentrations of the two sub-groups were compared. (Comparisons were conducted for Sampling Times 0, 1, 2, 4, and 6 hrs.)

- Indicates that statistical comparisons were not conducted.

Table IX. Table of Depressant Effects of Calcium Carbonate and Calcium Gluconate on Tetracycline Absorption (μg/ml.)

Time		Group A[1]	Group B[2]	Group C[3]
0 hr.	mean	0.00	-0.01	0.00
	std. dev.*	0.04	0.05	0.03
	variance	0.00	0.00	0.00
1 hr.	mean	0.71	1.19	0.80
	std. dev.	0.55	0.59	0.58
	variance	0.30	0.34	0.34
2 hr.	mean	1.45	1.94	0.81
	std. dev.	0.50	0.95	0.67
	variance	0.25	0.90	0.45
4 hr.	mean	1.54	1.34	1.01
	std. dev.	0.62	0.65	0.53
	variance	0.39	0.42	0.28
6 hr.	mean	1.07	0.82	1.00
	std. dev.	0.46	0.52	0.35
	variance	0.21	0.27	0.12

[1]Calcium carbonate depression of tetracycline absorption from capsule.

[2]Calcium carbonate depression of tetracycline absorption from syrup.

[3]Calcium gluconate depression of tetracycline absorption from capsule.

*Abbreviation for standard deviation.

Table X. Susceptibility of 223 Strains of Various Bacterial Species to Tetracycline *in vitro*

Organism	No. of Strains	Minimum inhibitory Concentration*	
		Range	Median
Staphylococcus aureus	56	1.6 - 100	3.1
Diplococcus pneumoniae	35	0.19 - 3.1	0.39
Streptococcus, group A	63	0.19 - 50	0.78
Streptococcus, group B	6	0.8 - 1.6	1.1
Streptococcus, group G	3	1.6	1.6
Streptococcus viridans	31	0.2 - 100	3.1
Neisseria gonorrhoeae	25	0.39 - 6.3	0.78
Mima polymorpha	1	3.1	
Bacillus cereus	3	0.78	0.78

*Concentrations (range and median) expressed as μg/ml.