TABLE I.—COEFFICIENTS OF VARIATION CALCULATED FROM SERUM SALICYLATE CONCENTRATION AND PER CENT ABSORBED DATA[a]

Time, Min.	First Test S.S.	First Test P.A.	Second Test S.S.	Second Test P.A.	Combined Tests S.S.	Combined Tests P.A.
15	69	66	94	105	83	90
30	53	51	44	52	48	51
60	24	22	28	28	25	25
90	19	13	18	15	18	14

[a] S.S., Coefficients based on serum salicylate concentration; P.A., coefficients based on per cent absorbed.

tion, where plasma albumin concentration is greatly reduced (15). It is also to be expected that correlation of blood level and per cent absorbed data would be poor when drug absorption rate is not considerably higher than the rate of drug elimination (assuming that elimination rate shows the usual intersubject variability).

From a similar point of view, it is of interest to compare the homogeneity of per cent absorbed values to that of blood level data. Considering the possible intersubject variations in volume of distribution and elimination rate, one would expect that actual absorption data would be more homogenous than blood level data (which would reflect also the variations in V and K values). As shown in Table I, coefficients of variation of blood level data and of absorption data obtained in the present study were generally the same, except for the greater homogeneity of absorption data when absorption was near completion (90 minutes). The latter tendency reflects the intersubject differences in elimination rates, which become most apparent in the post-absorption period. The reasons for the lack of greater homogeneity of absorption data (compared to blood level data) in the present study can be attributed to the similarity of volumes of distribution among the test subjects and also the rapid gastrointestinal absorption (compared to elimination) of aspirin. The chance exists also that in this relatively small group of subjects a fortuitous occurrence of opposite effects in any given subject may have dampened the blood level variations. In general, however, and particularly in situations where a greater diversity of V and K values is encountered and where k_a is not much greater than K, the use of actual absorption values should yield more homogenous data than are obtained by use of blood level values. This would permit a more sensitive statistical comparison of drug absorption from different dosage forms and would provide a more direct indication of absorption rates.

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Metal-Acid Complexes with Members of the Tetracycline Family I

Introduction

By EDWARD G. REMMERS, WILLIAM C. BARRINGER, GEORGE M. SIEGER, and ALBERT P. DOERSCHUK

Metal-acid complexes with a number of members of the tetracycline family have been prepared for chemical and biological evaluation. The studies reported here indicate that these preparations exhibited properties not displayed by the uncomplexed antibiotic. The most interesting properties characteristic of selected complexes in this series include enhanced solubility at pH 4.0–7.0, enhanced alkaline stability, reduced acute toxicity, reduced tissue irritation and rapid tissue diffusion, and enhanced blood levels.

THIS PUBLICATION is the first in a series describing the pharmaceutical properties of metal-acid complexes with members of the tetracycline family. The experimental data reported in this and subsequent publications have been obtained over the last 5 years through the cooperative efforts of a large number of investigators.

Many metal-acid complexes have been prepared having the following general formula: (tetracycline - group antibiotic) - (aluminum)a-(calcium)b-(gluconic acid)c, where a, b, and c vary widely. As the molar ratios of the constituents are varied, the pharmaceutical proper-

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6-Demethylchlortetracycline, chlortetracycline, and tetracycline are marketed as Declomycin, Aureomycin, and Achromycin, respectively, by the American Cyanamid Co., Pearl River, N. Y. 5-Hydroxytetracycline is marketed as Terramycin by the Chas. Pfizer Co., New York, N. Y.

ties, which are not displayed by salts of these antibiotics, also vary. Much of the experimental effort with these complexes has been devoted to investigation of the effects of different molar ratios on pharmaceutical properties and to determination of the minimum molar ratios of constituents in the complex which still give the desired properties.

EXPERIMENTAL

Preparation of Metal-Acid Complexes.[1]—The aluminum-acid complex was first prepared by adding aluminum isopropoxide to a solution of the acid in water or other suitable solvent to give the desired molar ratios. The resulting mixture was stirred overnight until an essentially clear solution was obtained. The antibiotic was then added as the base or hydrochloride, and stirring was continued until a clear solution was obtained. If a second metal, such as calcium or magnesium, was to be incorporated into the complex, the appropriate amount of the metal oxide was added, and the mixture was stirred until a clear solution was obtained. The pH of the mixture was elevated to 5.0–7.0 with concentrated NaOH if necessary, and the solution was filtered to remove traces of unreacted solids. The complex was recovered by lyophilization or by solvent precipitation in 10–20 vol. of a 2:1 isopropanol–petroleum ether (b.p. 60–68°) mixture. The resulting precipitate was aged for 30 minutes or longer, collected by filtration, washed with isopropanol, and dried *in vacuo* over phosphorus pentoxide at 30–40°.

Solubility Determinations.—The complexes were added to distilled water at a level of about 50 mg./ml. of antibiotic with stirring at room temperature. The pH of the solution was adjusted with either 5 *N* HCl or saturated NaOH. The solution was stirred for approximately 0.5 hour, during which the pH was readjusted to the desired level if necessary. It was then filtered; the filtrate was diluted in water and read in a Cary U.V. spectrophotometer to determine the solubility of the antibiotic.

Blood Level Studies.—Mongrel dogs of each sex, weighing 40 to 60 lb., were divided into groups of three, assigned so that the average body weight of each group was approximately the same. The antibiotic preparations were dissolved in distilled water to give a concentration of 25 to 50 mg. antibiotic/ml. and were injected intramuscularly into the biceps femoris. Blood samples were withdrawn from the cephalic vein at 1, 4, 7, and 24 hours following administration of the antibiotic. The samples were allowed to clot, ringed, and centrifuged. The clear serum was pipeted from the clot and assayed in duplicate microbiologically as tetracycline·HCl, using *B. cereus* as the test organism. The details of this assay procedure have been reported from these laboratories (1). The area under the blood level–time curve from 0 to 24 hours was computed and reported as area under the curve (AUC).

In all blood level experiments, a tetracycline·HCl standard was used in all serum assays. This procedure was followed to reduce all blood level experiments to a common standard and to conform to previous work, as reported by Kunin and Finland (2) and Sweeney *et al.* (3). Thus, all blood levels are reported in terms of tetracycline·HCl equivalents.

LD_{50} Determinations.—The method used for estimating the LD_{50} values has been described by Reed and Muench (4). Swiss albino mice (Taconic Farms) were caged in groups of 10 each; dosage was graduated so that more than 50% of the mice receiving the highest dosage succumbed, and more than 50% survived the lowest dose. The preparation to be tested was dissolved in distilled water at levels such that 0.5-ml. and 0.2-ml. quantities could be administered by intraperitoneal and intravenous routes, respectively. The intravenous dose was injected over an interval of 5–7 seconds. The mice were observed daily for a 6-day test period.

Rabbit Intradermal Irritation Test.—Rabbits weighing 4–6 Kg. were used, usually with three rabbits per group. On the day before the test, the abdomen of each rabbit was clipped with an electric clipper. Immediately before injection, the rabbits were anesthetized by administration of sodium pentobarbital into the marginal ear vein at a dose of 0.2 ml. of a 6% solution per pound of body weight.

Solutions of two chlortetracycline standards (class I "good" and class IV "bad") and two experimental preparations were made at a concentration of 50 mg. antibiotic in 10 ml. of pyrogen-free distilled water. One-milliliter tuberculin syringes were fitted with No. 27 gauge needles with intradermal bevels. One-tenth of a milliliter of each of the four solutions was injected as superficially as possible, just lateral to the mammary glands and away from the movable skin near the front and hind legs. A separate needle and syringe were used for each preparation. The injection sites were examined 16 to 20 hours after injection. The reactions of the test solutions were then compared to the good and bad standards. An average rating was computed from the results obtained with three rabbits. Preparations must have a class I (good irritation) rating before they may be used in parenteral and topical products.

RESULTS AND DISCUSSION

Table I lists some of the antibiotics, metals, and acids used in these complexes. To date, every tetracycline antibiotic tried in these preparations has undergone complex formation. Of the various

TABLE I.—ANTIBIOTICS, METALS, AND ACIDS USED IN METAL-ACID COMPLEXES

Antibiotics	Metals	Acids
Chlortetracycline	Aluminum	Gluconic
6-Demethylchlortetracycline	Calcium	Metaphosphoric
Tetracycline	Magnesium	Pyrophosphoric
Oxytetracycline	Iron	Polyphosphoric
6-Demethyltetracycline	Tungsten	Orthophosphoric
6-Deoxy-6-demethyltetracycline	Uranium	Lactic
6-Deoxytetracycline		Boric
9-Amino-6-demethyl-6-deoxytetracycline		

[1] The procedures employed in preparing these complexes are described more fully by Ritter, I., U. S. pat. 2,736,725 and by Sieger, G. M., and Weidenheimer, J. F., U. S. pat. 3,053,892.

TABLE II.—PHARMACEUTICAL PROPERTIES OF METAL-ACID COMPLEXES

(*a*) Increased solubility in the pH range of 4.0–7.0.
(*b*) Enhanced alkaline stability.
(*c*) Reduced acute toxcity.
(*d*) Reduced tissue irritation and rapid tissue diffusion.
(*e*) Enhanced blood levels.

metals used, aluminum, calcium, magnesium, and iron have produced the most interesting pharmaceutical properties. Unfortunately, complexes containing iron have a relatively dark color due to reaction of iron with a phenolic hydroxyl group of the antibiotic. As a result, a major portion of effort was devoted to complexes with the other metals. Of the various acids employed to date, polyhydroxy acids and phosphorus-containing acids have given the most interesting results. In preparing metal-acid complexes, the two following criteria have been employed in determining if complex formation has occurred: (*a*) changes in the ultraviolet absorption spectrum and (*b*) solubility behavior in the region of pH 4.0–7.0.

If complex formation has occurred, the ultraviolet absorption peak in the 355–370 mμ region, due to the C-7 to C-12 chromophore (5), is shifted to longer wavelengths. The complex will also remain in solution in the region of pH 4.0–7.0, where simple salts of the tetracycline family are hydrolyzed, and the neutral form of the antibiotic precipitates from solution.

The important pharmaceutical properties that have been observed with selected preparations are shown in Table II. Each property will be discussed in detail.

Increased Solubility.—Compared to the uncomplexed antibiotic, metal-acid complexes generally are more soluble in the pH range of 4.0 to 7.0, in which many pharmaceutical preparations are formulated. Figure 1 compares the pH solubility curve for 6-demethylchlortetracycline (DMCTC) to the corresponding curves for two DMCTC-metal-acid complexes. Over the pH range of 3.0 to 7.0, the uncomplexed antibiotic is soluble to the extent of 3 to 7 mg./ml. In contrast, the DMCTC complexes exhibit comparatively high solubility over this pH range. The solubility of DMCTC-aluminum-gluconate (molar ratio 1:4:6.6) exceeds 50 mg./ml. of antibiotic over the pH range of 2.0 to 10.0. The pH solubility curve for DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:2:12) indicates the effect on solubility of incorporation of

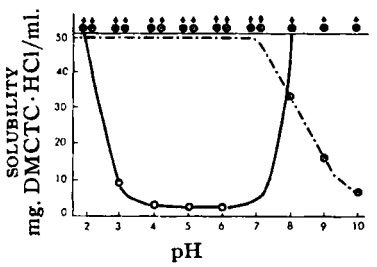

Fig. 1.—pH solubility curves for DMCTC and two DMCTC-metal-acid complexes. Key: O, DMCTC; ●, DMCTC-Al-gluconate (1:4:6.6); ⊙, DMCTC-Al-Ca-gluconate (1:4:2:12).

calcium into the complex. When DMCTC-aluminum-gluconate complexes are prepared with graduated amounts of calcium in the complex, the solubility on the alkaline side of neutrality is depressed.

Enhanced Alkaline Stability.—The enhanced alkaline stability of aluminum-gluconate complexes with graduated amounts of calcium has been reported by Remmers *et al.* (6), where the incorporation of 1.0 or more molar parts of calcium into the complex markedly increased the alkaline stability of most, but not all, of the tetracycline antibiotics. Of those tested to date, only chlortetracycline is unusual since studies with this antibiotic have repeatedly failed to demonstrate greatly enhanced alkaline stability.

Acute Toxicity.[2]—Prior to extensive biological evaluation of these complexes, representative preparations were submitted for acute toxicity studies in mice. Table III presents the LD_{50} determinations for DMCTC·HCl and three selected DMCTC-metal-acid complexes in mice following intraperi-

TABLE III.—LD_{50} VALUES FOR SELECTED METAL-ACID COMPLEXES IN MICE[a]

Derivative	Administration Rt. i.p.	i.v.
DMCTC·HCl	273	89
DMCTC-aluminum-gluconate (molar ratio 1:4:6.6)	495	698
DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12)	420	306
DMCTC-aluminum-pyrophosphate (molar ratio 1:1:1)	1,810	260

[a] Milligrams DMCTC·HCl per kilogram body weight.

TABLE IV.—RESULTS FROM RABBIT INTRADERMAL IRRITATION TEST

Prepn.	Rating
Bad chlortetracycline standard	Class IV
DMCTC neutral (starting material for complex)	Class II
DMCTC-aluminum-calcium-gluconate (1:4:5:12)	<Class I
Good chlortetracycline standard	Class I

toneal and intravenous administration. The LD_{50} values for the complexes are approximately 1.5 to 8.0 times higher than the values obtained with the uncomplexed antibiotic when compared on an antibiotic-content basis.

The DMCTC-aluminum-pyrophosphate complex (molar ratio 1:1:1) resulted in a comparatively high LD_{50} value after intraperitoneal administration and a relatively low LD_{50} value after intravenous administration. This phenomenon is probably the result of serum binding of calcium; complexes whose calcium binding has not been satisfied produce blood pressure depression when administered intravenously By incorporating calcium into the complex or by administering the complex in a solution containing available calcium ions, the blood pressure depression can be essentially eliminated.

Changes in Tissue Irritation and Diffusion.—An important characteristic of these complexes is their ability to minimize or to eliminate the irritating

[2] Acute toxicity studies and blood level experiments were performed under the direction of Dr. J. J. Corbett and Mr. N. Anagnostakos, Biological Assay Development Laboratory.

TABLE V.—MOLECULAR WEIGHT DETERMINATIONS FOR SELECTED METAL-ACID COMPLEXES

Derivative	Mol. Wt.	Degree of Homogeneity
DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12)	990	Homogeneous
DMCTC-aluminum-pyrophosphate (molar ratio 1:1:1)	1,350	Homogeneous
DMCTC-aluminum-metaphosphate (molar ratio 1:4:12)	2,000 [Higher molecular wt. fraction]	Two fractions

properties of selected batches of antibiotic in the rabbit intradermal irritation test.[3] Table IV presents the results obtained with a metal-acid complex; approximately 20 hours following intradermal administration, the complex has diffused away from the injection site and has produced no signs of erythema. Metal-acid complexes consistently diffuse so rapidly from the injection site that in many cases it is difficult to identify the site 16 to 20 hours later. When DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12) is prepared from DMCTC having a class II rating, the resulting complex has an irritation rating less than class I.

Molecular weight determinations were made on selected metal-acid complexes in the ultracentrifuge.[4] Table V indicates that molecular weights ranged from 990 to 2,000. In most cases the complexes behaved as a homogeneous entity in the ultracentrifuge. The DMCTC - aluminum - metaphosphate complex (molar ratio 1:4:12) was the only preparation displaying heterogeneity. The molecular weight of the lighter fraction was too low to be estimated accurately. Disk diffusion studies performed on several microbiological media indicated that DMCTC-metal-acid complexes diffused as rapidly as uncomplexed DMCTC·HCl.[5]

Blood Levels.—A third important property observed with selected metal-acid complexes is the enhanced blood levels obtained compared to levels resulting from salts of the corresponding uncomplexed antibiotic. Figure 2 shows blood levels obtained with DMCTC-aluminum-magnesium-gluconate (molar ratio 1:4:5:12) and DMCTC·HCl when administered intramuscularly to dogs at a level of 1.5 mg. antibiotic per pound of body weight. Blood levels of the group of three dogs receiving the DMCTC·HCl resulted in an AUC of 20.3 mcg./hours/ml. When the dogs received the antibiotic as the DMCTC-aluminum-magnesium-gluconate complex, an AUC of 99.6 mcg./hours/ml. was obtained or nearly a fivefold enhancement. After administration at a level of 1.5 mg. per pound of body weight, irritation evidenced by limping was noted in both groups of dogs in this experiment.

Figure 3 presents the results of a blood level study in which the DMCTC complex was administered at a reduced dosage. The control group of three dogs received DMCTC·HCl at a level of 2.5 mg. per pound of body weight. A second group received DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12) at a level of 0.8 mg. DMCTC·HCl per pound of body weight. After a dosage reduction, in terms of antibiotic content that was approximately threefold, the DMCTC complex resulted in blood levels nearly twice as high in terms of AUC as those obtained with DMCTC·HCl. The level of 2.5 mg. DMCTC·HCl per pound of body weight produced considerable irritation in the dogs, while little or no irritation occurred in dogs receiving the DMCTC complex. Thus, it is possible to reduce the dosage of the antibiotic when administered as the complex to a level where little or no irritation is produced, but where acceptable blood levels are maintained.

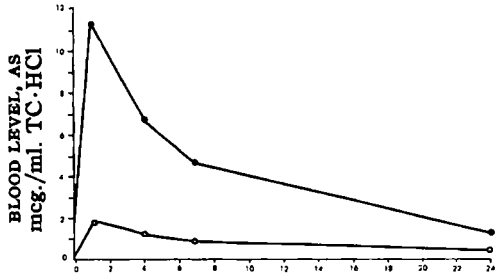

Fig. 2.—Average blood levels of three dogs per group following intramuscular administration of DMCTC·HCl and DMCTC-metal-acid-complex at a dose of 1.5 mg. DMCTC·HCl/lb. body weight. Key: O, DMCTC·HCl, AUC = 20.3 mcg./hours/ml. (formulation pH 4.0); ●, DMCTC-Al-Mg-gluconate (1:4:5:12), AUC = 99.6 mcg./hours/ml. (formulation pH 4.5).

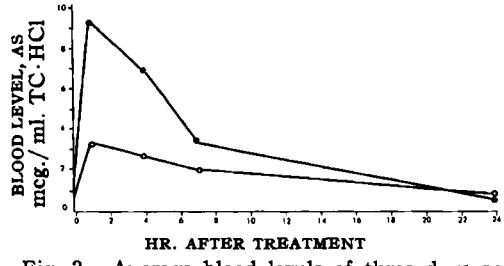

Fig. 3.—Average blood levels of three dogs per group following intramuscular administration of DMCTC·HCl and DMCTC-metal-acid complex. Key: O, DMCTC·HCl at 2.5 mg./lb., AUC = 45.5 mcg./hours/ml. (formulation pH 4.0); ●, DMCTC-Al-Ca-gluconate (1:4:5:12) at 0.8 mg. DMCTC·HCl/lb., AUC = 80.1 mcg./hours/ml. (formulation pH 4.5).

[3] Rabbit intradermal irritation studies were performed under the direction of Mr. G. R. Personeus and Mr. A. Tonelli, Quality Control Section.

[4] Molecular weight determinations were made under the direction of Mr. R. L. Davies, Biochemical Research Section.

[5] Disk diffusion studies were performed under the direction of Mr. A. C. Dornbush, Biochemical Research Section.

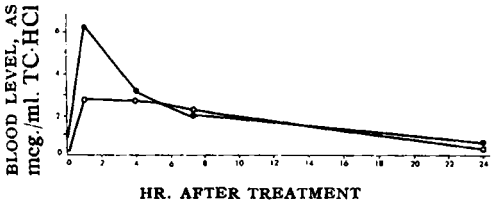

Fig. 4.—Average blood levels of three dogs per group following intramuscular administration of OTC and DMCTC-Al-Ca-gluconate. Key: O, OTC at 2.5 mg. OTC neutral/lb., AUC = 38.6 mcg./hours/ml. (formulation pH 8.5). ●, DMCTC-Al-Ca-gluconate (1:4:5:12) at 0.3 mg. DMCTC·HCl/lb., AUC = 45.3 mcg./hours/ml. (formulation pH 4.5).

Figure 4 shows the blood levels obtained with DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12) and a commercially available preconstituted oxytetracycline intramuscular formulation in 75% propylene glycol (Pfizer) for human administration. The DMCTC complex was administered intramuscularly to dogs at 0.3 mg. DMCTC·HCl per pound of body weight and the oxytetracycline (OTC) at a level of 2.5 mg. OTC base per pound of body weight. On an antibiotic-weight basis, approximately one-eighth as much antibiotic was administered as the complex. However, the blood levels produced nearly equal AUC's in terms of tetracycline·HCl equivalents (Fig. 4). Some of the blood level enhancement observed in this experiment results from the fact that DMCTC is more active microbiologically on a weight basis than OTC when assayed against a tetracycline·HCl standard. To date, many blood-level studies have been made in dogs, rabbits, and rats with complexes containing various tetracycline antibiotics orally, intramuscularly, and intravenously. The degree of blood level enhancement, which has been demonstrated repeatedly, is dependent on the route of administration and the constituents in the complex.

The mechanism by which blood-level enhancement occurs is not completely understood. One hypothesis is that blood-level enhancement results from limited tissue penetration of the antibiotic complex from the blood stream.

Blood Level Experiments in Humans.—Katz (7) and Katz and Fedorko (8) reported the results of a clinical trial in humans using DMCTC-aluminum-calcium-gluconate (molar ratio 1:4:5:12). They found that intramuscular administration of this complex in single doses equivalent to 25 and 50 mg. of DMCTC·HCl produced significantly higher serum levels than would have been expected from considerably larger doses of tetracycline. These doses were in a range normally expected to be therapeutic. However, several patients with tetracycline-antibiotic-susceptible infections did not respond satisfactorily at a dose of 50 mg. per day. The response was improved by increasing the dose to 100 mg. per day.

SUMMARY

Chemical and biological evaluation of metal-acid complexes with members of the tetracycline family indicate that these preparations exhibited properties that were not displayed by the uncomplexed antibiotic. The most interesting properties which are characteristic of selected complexes include enhanced solubility and alkaline stability, reduced acute toxicity and tissue irritation, and enhanced blood levels.

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ERRATUM

In the paper titled "Preparation of a Phase Diagram for Coacervation" (1), Eq. 6 on page 519 should read:

$$n = \frac{(\Delta \text{ sp. gr.})(D) - (\Delta \text{ R. I.})(\Delta \text{ sp. gr. } + B)}{(AD - BC) - (\Delta \text{ sp. gr.})(C - D)} \qquad \text{(Eq. 6)}$$

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