Metal-Acid Complexes with Members of the Tetracycline Family III

Summary of Blood Level Studies

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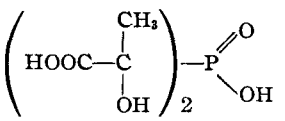
Metal-acid complexes with tetracycline (TC) and demethylchlortetracycline (DMCTC) have been evaluated for their ability to increase the levels of antibiotic in the blood of animals, where the acid constituents of the complexes were gluconic acid, various phosphoric acids, or bis-(1-carboxy-1-hydroxyethyl) phosphinic acid. Selected members of these three major groups each have generally resulted in higher blood levels by two of the following three routes of administration: oral, intramuscular, and intravenous. No single group of complexes has produced blood-level enhancement by all three routes.

PREVIOUS PAPERS in this series (1, 2) described the chemical and biological evaluation of selected aluminum - (calcium) - gluconate complexes with members of the tetracycline family. The purpose of this paper is to extend the evaluation of metal-acid complexes containing gluconic acid to aluminum complexes with acids containing phosphorus.

Such complexes may be classified into two broad groups: (*a*) those containing modifications of phosphoric acid (*ortho-*, *meta-*, poly-, and pyrophosphoric acids) and (*b*) those containing substituted phosphinic acids. Of the latter groups, bis - (1 - carboxy - 1 - hydroxyethyl) phosphinic acid, or phosphinicodilactic acid (PDLA), has been studied most extensively. The structure of PDLA is



The pH solubility curves for complexes containing modifications of phosphoric acid are shown in Fig. 1. Solubility characteristics of this group differed from the complexes containing gluconic acid described previously (1). Attempts to incorporate calcium into aluminum complexes with various phosphoric acids have been uniformly unsuccessful. As a result, this group did not possess the increased alkaline stability reported earlier (3) with aluminum-calcium complexes containing gluconic acid.

Metal-acid complexes containing PDLA were under alkaline conditions almost as soluble and stable as complexes with gluconic acid. Those containing PDLA differ from complexes with various phosphoric acids because sufficient calcium can be incorporated in the former to impart stability under alkaline conditions similar to that reported earlier (3) with aluminum-calcium complexes with gluconic acid.

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Tetracycline and 6-demethylchlortetracycline are marketed as Achromycin and Declomycin, respectively, by the American Cyanamid Co., Pearl River, N. Y.

EXPERIMENTAL

Aluminum-Phosphate Complexes.—The method of preparing these complexes was described in a recent U. S. patent (4).

Aluminum-PDLA Complexes.—A solution was prepared by suspending aluminum isopropoxide and PDLA in the desired molar ratio in distilled water. The suspension was shaken for 24 hours, then filtered. The antibiotic and calcium oxide (if desired) were then added. The reaction mixture was stirred for 2 hours until a clear solution was obtained. The pH was raised to 5.0 with 10% NaOH, and the complex was recovered by freeze drying.

Aluminum-Gluconate Complexes.—The method of preparation has been described (1).

Blood Level Studies in Rats.—Adult rats of the Sprague-Dawley strain, weighing approximately 300 Gm., were divided into groups of four animals. For oral administration, the antibiotic preparations were dissolved in distilled water to give a concentration of

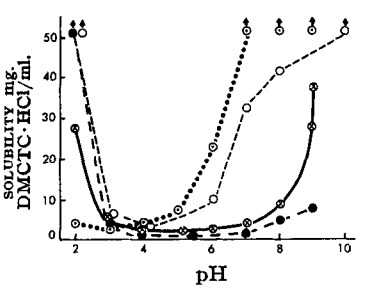

Fig. 1.—pH solubility curves for four DMCTC-metal-acid complexes. Key: ⊙, DMCTC-Al-pyrophosphate; O, DMCTC-Al-metaphosphate; ⊗, DMCTC-Al-polyphosphate; ●, DMCTC-Al-orthophosphate.

TABLE I.—BLOOD LEVELS[a] IN RATS FOLLOWING ORAL ADMINISTRATION OF TC AND DMCTC-METAL-GLUCONATE COMPLEXES

	pH[b]	Hr. After Injection[c] 1	4	7	AUC (0–7 Hr.) mcg./Hr./ml.	Ratio
TC·HCl	2.0	5.10	4.15	2.79	26.8	1.00
TC-aluminum-calcium-gluconate complex (1:4:5:12)	5.0	0.71	0.47	0.31	3.3	0.12
DMCTC·HCl	2.0	10.5	7.75	5.55	52.6	1.00
DMCTC-aluminum-calcium-gluconate complex (1:4:5:12)	5.0	1.00	1.12	0.45	6.0	0.11

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Averages of four rats per group. [b] pH of the aqueous solution at time of administration. [c] Dose = 25 mg. antibiotic HCl/300 Gm. body weight.

TABLE II.—BLOOD LEVELS[a] IN RATS FOLLOWING ORAL ADMINISTRATION OF DMCTC-METAL-PHOSPHATE COMPLEXES

	pH[b]	Hr. After Injection[c] 1	4	7	AUC (0–7 Hr.) mcg./Hr./ml.	Ratio
DMCTC-aluminum-pyrophosphate complex (1:1:1)	7.0	48.0	18.6	10.1	167.1	3.17
DMCTC-aluminum-metaphosphate complex (1:4:12)	7.0	24.5	31.2	33.2	192.4	3.66
DMCTC-aluminum-polyphosphate complex (1:4:12)	7.0	6.22	1.92	1.39	20.3	0.39
DMCTC-aluminum-orthophosphate complex (1:4:12)	2.0	1.53	0.79	0.74	6.5	0.12
DMCTC·HCl	2.0	10.5	7.75	5.55	52.6	1.00

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Averages of four rats per group. [b] pH of the aqueous solution at time of administration. [c] Dose = 25 mg. DMCTC·HCl/300 Gm. body weight.

TABLE III.—BLOOD LEVELS[a] IN DOGS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF DMCTC-METAL-PHOSPHATE COMPLEX

	pH[b]	Hr. After Injection[d] 1	4	7	24	AUC, mcg./Hr./ml.	Ratio
DMCTC-aluminum-pyrophosphate complex (1:1:1)	7.0	5.10	2.79	1.99	0.57	43.3	2.13
DMCTC-aluminum-metaphosphate complex (1:4:12)	2.0	0.41	0.39	0.47	0.51	11.0	0.54
DMCTC·HCl in commercial intramuscular formulation[e]	4.0	1.81	1.17	0.92	0.47	20.3	1.00

[a] Based on microbiological assays expressed as micrograms per milligram tetracycline·HCl. Averages of three dogs per group. [b] pH of the aqueous solution at time of administration. [c] Formulation contained DMCTC·HCl, 100 mg.; magnesium gluconate, 330 mg.; citric acid, 50 mg.; lidocaine base, 33 mg.; and niacinamide, 100 mg. [d] Dose = 1.5 mg. DMCTC·HCl/lb. body weight.

10 mg. antibiotic/ml. The resulting solution was administered by inserting a rubber catheter attached to a syringe in the throat of the animal. Blood samples were withdrawn from the caudal vein and assayed by the method described previously (1).

For intramuscular administration to rats, the antibiotic preparations were dissolved in distilled water to give a concentration of 25 to 50 mg. antibiotic/ml. and were injected into the biceps femoris. Blood samples were obtained as described above.

Blood Pressure Measurements.—Dogs were anesthetized by intravenous administration of veterinary pentobarbital sodium (Abbott) with a dose of 1.0 ml./5 lb. body weight. The femoral vein in the hind leg was cannulated for direct administration of test compounds. The carotid artery in the throat was cannulated for direct recording of blood pressure by a manometer which recorded continuously on a kymograph.

Each of the complexes was injected in three doses at 10, 25, and 50 mg. antibiotic/Kg. body weight, allowing 1 to 2 hours between injections. The injections were made at a rate of 5 mg. antibiotic/Kg./minute.

RESULTS

Complexes Containing Gluconic Acid.—Previous papers (1, 2) reported elevation of antibiotic blood levels in dogs following intramuscular and intravenous administration of metal-acid complexes containing gluconic acid. After oral administration to rats, complexes containing gluconic acid resulted in comparatively low blood levels, as indicated in Table I. Aluminum-calcium-gluconate complexes with tetracycline (TC) and 6-demethylchlortetracycline (DMCTC) produced blood levels only 11 to 12% as high as the uncomplexed antibiotic.

Complexes Containing Phosphoric Acids.—Complexes containing various phosphoric acids were evaluated by oral, intramuscular, and intravenous administration to rats, rabbits, and dogs. The results are presented in Tables II–IV. The DMCTC-aluminum-orthophosphate and DMCTC-aluminum-polyphosphate complexes gave unusually low blood levels after oral administration to rats. In contrast, the corresponding aluminum-pyrophosphate and aluminum-metaphosphate complexes produced

blood levels more than threefold higher than those obtained with DMCTC·HCl. After intramuscular administration to dogs and rabbits, the DMCTC-aluminum-pyrophosphate complex resulted in blood levels higher than those obtained with other aluminum-phosphate complexes tested.

Studies in dogs following intravenous administration of DMCTC-aluminum-pyrophosphate and DMCTC-aluminum-metaphosphate complexes indicated that these preparations can produce serious and even fatal depression in blood pressure at certain dosages. The aluminum-pyrophosphate complex produced no blood pressure depression or adverse side effects when administered at a dose of 10.0 or 25.0 mg. DMCTC·HCl/Kg. However, at a dose of 50.0 mg./Kg., there was serious depression of blood pressure, increased heart beat, and labored breathing and panting which lasted for 2 hours before the dog recovered. At a dose of 25.0 mg. DMCTC·HCl/Kg., the DMCTC-aluminum-metaphosphate complex produced severe blood pressure depression, but the animal recovered. The dose of 50 mg./Kg. was fatal within 5 minutes.

The mechanism by which blood pressure depression occurs following intravenous administration of aluminum-phosphate complexes is believed to be a binding or precipitation of the serum calcium.

Complexes Containing PDLA.—Complexes containing PDLA were evaluated in rats following oral and intramuscular administration. The blood levels obtained are presented in Tables V and VI, respectively. After oral administration, considerable enhancement in blood levels was observed with the TC-aluminum-calcium-PDLA complex. When administered intramuscularly, the aluminum-calcium-PDLA complex with TC produced blood levels comparable to TC·HCl in a commercial intramuscular formulation. Although complexes containing PDLA were not evaluated intravenously, this type of preparation would be expected to increase blood levels of antibiotic by this route without severe blood pressure depression if sufficient calcium were incorporated in the preparation.

The mechanism by which blood-level enhancement occurs is not understood completely. One hypothesis is that it results from limited diffusion of the antibiotic from the blood stream. Preliminary evidence supporting this has been obtained in an experiment in dogs following intravenous administration of DMCTC-aluminum-calcium-gluconate complex (molar ratio 1:4:5:12); a threefold increase of antibiotic level occurred in the serum which was not accompanied by an increase in the cerebrospinal fluid.

SUMMARY

After oral administration to rats, TC and DMCTC-aluminum-calcium-gluconate complexes (1:4:5:12) produced blood levels only 11 to 12% as high as those obtained with TC·HCl and DMCTC·HCl. Previous work with this type of preparation following intramuscular or intravenous administration to dogs resulted in enhanced blood levels.

TABLE IV.—BLOOD LEVELS[a] IN RABBITS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF DMCTC-ALUMINUM-PHOSPHATE COMPLEXES

	pH[b]	Hr. After Injection[c] 1	4	7	24	AUC, mcg./Hr./ml.	Ratio
DMCTC-aluminum-pyrophosphate complex (1:1:1)	7.0	7.20	2.97	1.55	0.38	42.1	2.86
DMCTC-aluminum-metaphosphate complex (1:4:12)	2.0	2.61	1.52	1.17	0.37	23.4	1.59
DMCTC-aluminum-orthophosphate complex (1:4:12)	2.0	0.89	1.03	0.75	0.29	14.7	1.00
DMCTC-aluminum-polyphosphate complex (1:4:12)	2.0	3.74	1.94	1.43	0.43	30.8	2.10

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Averages of three rabbits per group. [b] pH of the aqueous solution at time of administration. [c] Dose = 1.5 mg. DMCTC·HCl/lb. body weight.

TABLE V.—BLOOD LEVELS[a] IN RATS FOLLOWING ORAL ADMINISTRATION OF TC-METAL-PDLA COMPLEX

	pH[b]	Hr. After Injection[c] 1	4	7	24	AUC, mcg./Hr./ml.	Ratio
TC-aluminum-calcium-PDLA complex (1:4:3:12)	2.5	16.5	9.6	6.6	0.73	134.0	2.75
TC·HCl	1.9	5.3	4.4	2.5	0.0	48.8	1.00

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Averages of four rats per group. [b] pH of the aqueous solution at time of administration. [c] Dose = 25 mg. TC·HCl/300 Gm. body weight.

TABLE VI.—BLOOD LEVELS[a] IN RATS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF TC-METAL-PDLA COMPLEX

	pH[b]	Hr. After Injection[d] 1	4	7	24	AUC, mcg./Hr./ml.	Ratio
TC-aluminum-calcium-PDLA complex (1:4:3:12)	2.5	19.1	18.8	14.1	2.0	252.6	0.96
TC·HCl in commercial intramuscular formulation[c]	1.9	34.8	20.0	11.8	1.9	263.8	1.00

[a] Based on microbiological assays expressed as micrograms per milliliter tetracycline·HCl. Averages of four rats per group. [b] pH of the aqueous solution at time of administration. [c] Formulation contained TC·HCl, 100 mg.; procaine HCl, 40 mg.; $MgCl_2 \cdot 6H_2O$, 46.8 mg.; and ascorbic acid, 250 mg. [d] Dose = 25 mg. TC·HCl/300 Gm. body weight.

The DMCTC-aluminum-orthophosphate and DMCTC-aluminum-polyphosphate complexes produced unusually low blood levels after oral administration, while the corresponding aluminum-pyrophosphate and aluminum-metaphosphate complexes gave blood levels more than threefold higher than those obtained with DMCTC·HCl. Following intramuscular administration, the DMCTC-aluminum-pyrophosphate complex gave blood levels higher than those obtained with other aluminum-phosphate complexes tested. When administered intravenously, the aluminum-phosphate complexes caused severe blood pressure depression.

In rats, the TC-aluminum-calcium-PDLA complex (1:4:3:12) yielded blood-level enhancement following oral administration. No blood-level enhancement followed intramuscular administration. Although complexes containing PDLA were not evaluated intravenously, these would be expected to give good results by this route if sufficient calcium were present in the preparation to minimize blood pressure depression.

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Absorption of Sodium Bisulfite from Peritoneal Dialysis Solutions

By SAMI F. HALABY* and ALBERT M. MATTOCKS

Toxic symptoms have been observed in using peritoneal dialysis fluids containing 7 per cent dextrose. The one ingredient suspected as a possible cause of toxicity is sodium bisulfite, present in concentrations of 500 mg./L. On reviewing the literature it was obvious that in the quantities this fluid may be used—as much as 10–12 L. per day—the amount of bisulfite would be dangerous if appreciable absorption occurs from the dialysis fluid. Five rabbits were injected intraperitoneally with the dialysis fluid containing a portion of the bisulfite labeled with S^{35}. Blood levels and urinary excretions were measured, and tissue distribution was evaluated. Rapid absorption occurs from the peritoneum, and the ability of the organism to remove the bisulfite *via* oxidation or urinary excretion is lost as the blood levels are increased. Thus, the conclusion is that the use of 500 mg./L. of sodium bisulfite in peritoneal dialysis fluids is dangerous and should be discontinued.

RECENTLY, THE AUTHORS found unexpected toxic symptoms in using certain peritoneal dialysis fluids; the symptoms observed were typical of central nervous system stimulation. Examination of control data on the fluids used revealed no basis for the difficulty, and the authors were prompted to examine the formula for inherent toxic properties. The formula for the suspected dialysis solution is a common one:

INGREDIENT	%
Dextrose	7.0
Sodium chloride	0.62
Sodium lactate	0.39
Calcium chloride	0.026
Magnesium chloride	0.015
Sodium bisulfite	0.05

Dextrose and all the salts of this formula are commonly used in injections without difficulty; the only substance present in abnormally high amounts, considering the quantities of fluid used, is the sodium bisulfite. Sodium bisulfite is used extensively in concentrations up to 0.31% (equivalent to 0.2% SO_2) as a preservative to prevent discoloration of dextrose, but rarely are these fluids injected in volumes comparable to that used in peritoneal dialysis. So the literature on the toxicity of sodium bisulfite was investigated.

Most studies of sodium bisulfite have dealt with the oral ingestion of small quantities over extended periods to prove its safety as a food preservative. Until 1951, little attention had been given to the toxicity of this substance by intraperitoneal injection or injection by other routes. Reiss and Gerstl (1) found that 0.18 mmoles or more given intraperitoneally to mice caused death within a few minutes. Two of five mice died after injection with 0.12 mmoles, and none died from 0.06 mmoles. These workers reported that 1.02 mmoles/Kg. was toxic to rabbits; they failed to find morphological changes in the animals so injected. The bisulfite in toxic doses was said to have an immediate effect on the

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