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

Received September 4, 1964, from the College of Pharmacy, University of Michigan, Ann Arbor.
Accepted for publication October 6, 1964.
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central nervous system indicated by clonic convulsions—particularly on the cardiovascular center—with subsequent rapid fall of blood pressure. Reiss and Gerstl thought the effect of bisulfite was a blocking of the oxidation mechanism of the animal.

Bhaghat and Lockett (2) injected rats intraperitoneally and reported that an isotonic solution of bisulfite at a level of 3% of body weight killed half of those injected. These workers reported that toxic doses caused restlessness, cyanosis, prostration, and finally cardiovascular collapse. Also, Bhaghat and Lockett found that 80–90% of the injected bisulfite (sublethal doses) was converted to sulfate prior to excretion in the urine.

Hoppe and Goble (3) reviewed the literature on this subject and determined the toxicity by intravenous injection in several species. The following values were found: mouse, LD_{50} 130 mg./Kg.; rat, LD_{50} 115 mg./Kg.; hamster, LD_{50} 95 mg./Kg.; rabbit, LD_{50} 65 mg./Kg.

From the above figures, the toxicity appears to increase progressively in larger animals. If this trend is applied to man, the lethal dose might be expected to be quite small. It is noteworthy that animals receiving a sublethal dose have shown no apparent toxic effects, even after repeated doses. One might state that if a dangerous dose is administered, the patient will either die or show no ill effects. This explanation may account for the failure to note toxic effects prior to this time.

With peritoneal dialysis, as much as 50 L. of dialysis fluid may be injected within a 24-hour period. The solution containing the high amount of dextrose and a correspondingly high amount of bisulfite is seldom used exclusively, but it may be used to the extent of 10–12 L. in a 24-hour period. This represents a total quantity injected of as much as 60 mmoles of bisulfite or 1 mmole/Kg. If the bisulfite is absorbed completely and accumulated in the body, this amounts to a lethal dose if man has sensitivity the same as the rabbit. Considering the poor physical condition of the patient who is being treated by this procedure, the conclusion must be made that if there is significant absorption of bisulfite from the peritoneum, the use of such amounts in dialysis fluids is dangerous and should be discontinued.

Thus, a set of experiments was planned to determine the extent of absorption of bisulfite from intraperitoneal injection of dialysis fluid containing 500 mg. of sodium bisulfite per liter and to assess the accumulation following repeated injections. Rabbits were used as the test animals.

EXPERIMENTAL

Methods and Materials

Peritoneal Dialysis Fluids.—Peritoneal dialysis fluid was prepared and sterilized using the formula presented above except that the bisulfite was omitted. Weighed quantities of S^{35}-sodium sulfite (about 10 mg. with an activity of 1–2 mc./mmole) and a sufficient quantity of inactive sodium bisulfite to make 500 mg. were dissolved in 1 L. of the dialysis fluid using the aseptic technique. As noted under *Discussion*, the amount of bisulfite normally present in peritoneal dialysis fluids may vary, depending on conditions of preparation and handling. By preparing these solutions by aseptic addition, the full amount of bisulfite was present, representative of the maximum quantity one might expect to encounter.

Injection and Sampling.—Animals were tied down on an animal board, blood samples were taken from the ear vein [as described by Nace and Spradlin (4)] and—with all but the first experiment—a catheter was inserted into the bladder so that urine samples could be taken at will.

Injection into the peritoneum was made *via* an indwelling catheter using a Cornwall syringe and intravenous infusion set. Each dose was 100 ml. of the dialysis fluid.

Measurement of Samples.—Blood or plasma samples were treated with an equal volume of 15% trichloroacetic acid mixed vigorously, and centrifuged. A 1-ml. aliquot was added to dioxane phosphor {7 Gm. of PPO (2,5-diphenyloxazole), 0.05 Gm. of POPOP[1,4-bis-2-(5-phenyloxazolyl)-benzene], and 50 Gm. of naphthalene, and dioxane to make 1 L.}. Samples, were counted on a Tri-Carb liquid scintillation counter (Packard Instrument Co., LaGrange, Ill.) in duplicate and again counted after addition of an internal standard of S^{35}-sodium sulfite.

Urine samples were diluted if necessary, then treated in a manner the same as the blood.

Dissected tissues were minced with water in a high-speed blender, centrifuged, and an aliquot treated in the same manner.

To differentiate between bisulfite and sulfate excreted in urine, 1-ml. aliquots of urine samples were treated with 0.25 ml. of 1% sulfuric acid, 0.25 ml. of normal hydrochloric acid, and 0.5 ml. of 10% barium chloride. Sulfate was added to prevent loss

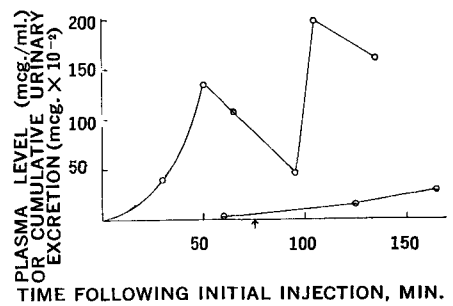

Fig. 1.—Rabbit *A*. Plasma levels and urinary excretions of bisulfite following injection of peritoneal dialysis fluid. Key: O, plasma level; ⊖, cumulative amounts in urine. Injections made at 0 and 75 minutes.

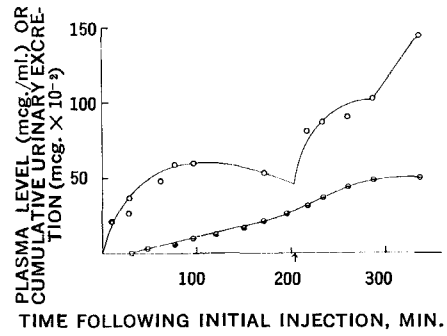

Fig. 2. —Rabbit *B*. Plasma levels and urinary excretions of bisulfite following injection of peritoneal dialysis fluid. Key: O, plasma level; ⊖, cumulative amounts in urine. Injections made at 0 and 204 minutes.

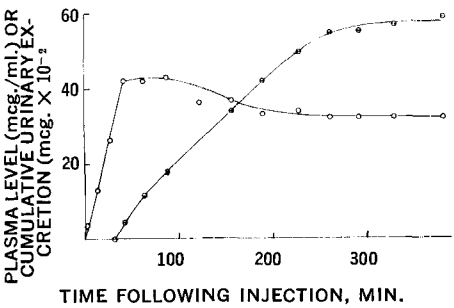

Fig. 3. —Rabbit *C*. Plasma levels and urinary excretions of bisulfite following injection of peritoneal dialysis fluid. Key: O, plasma level; ⊖, cumulative amounts in urine. Single injection given at 0 time.

of S^{35}-barium sulfate as colloidal particles. The mixture was then centrifuged and the supernatant treated as previously.

The usual blood and urine samples taken before injection were run as controls, and a sample of the dialysis fluid used was counted with each set of samples.

Toxicity in Mice.—To determine whether the other contents of the dialysis fluid may modify the toxicity of sodium bisulfite, solutions of sodium bisulfite in water and in dialysis fluid were injected intraperitoneally. Solutions of various concentrations were prepared so that 0.5 ml. was injected in each instance. Albino mice weighing 20–25 Gm. were used.

RESULTS

Rabbit A.—A female, weighing 3.14 Kg., was given two injections; the animal died approximately 9 hours after the first injection. The blood and urine data are shown in Fig. 1. The return fluid obtained at 140 minutes contained 0.04% of the total dose in 72 ml. of fluid.

Rabbit B.—A male, weighing 4.08 Kg., was given two injections, and the animal died in convulsions 337 minutes after the first injection. The blood and urine data are shown in Fig. 2. Only 1.7 ml. of dialysis fluid could be recovered at 183 minutes, and this contained 0.4% of the amount injected (one dose). At the time of death (337 minutes), 34 ml. of fluid containing 5.9% of the amount injected (two doses) was recovered.

Rabbit C.—A male, weighing 3.14 Kg., was given only one injection; the animal died about 30 hours after injection. The blood and urine data are shown in Fig. 3. Twenty milliliters of dialysis fluid was recovered at 382 minutes, and this contained 2% of the dose.

Rabbit D.—A male, weighing 3.31 Kg., was given three injections. The animal survived, and blood and urine data are shown in Fig. 4.

Rabbit E.—A male, weighing 2.57 Kg., was given three injections. The animal was sacrificed 382 minutes after the first injection to determine tissue distribution. Immediately following sacrifice, the abdomen was drained of fluid to obtain 259 ml. This volume plus rinsings of the cavity contained 14% of the radioactivity administered (three doses). The blood and urine data are shown in Fig. 5. The tissue distribution data are shown in Table I, and sulfate-bisulfite content of urine is presented in Table II.

Toxicity in Mice

The mortality data are given in Table III. These results indicate that the peritoneal dialysis fluid does not increase the toxicity of bisulfite, although it may decrease it slightly.

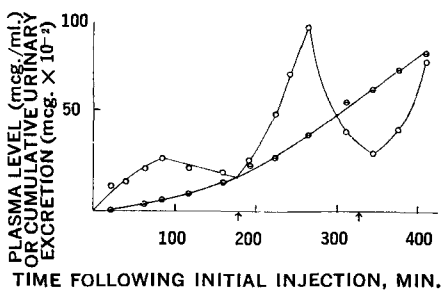

Fig. 4. —Rabbit *D*. Plasma levels and urinary excretions of bisulfite following injection of peritoneal dialysis fluid. Key: O, plasma level; ⊖, cumulative amounts in urine. Injections made at 0, 179, and 329 minutes.

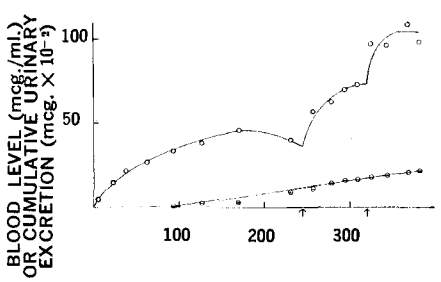

Fig. 5.—Rabbit *E*. Blood levels and urinary excretions of bisulfite following injection of peritoneal dialysis fluid. Key: O, blood level; ⊖, cumulative amounts in urine. Injections made at 0, 244, and 319 minutes.

TABLE I.—TISSUE DISTRIBUTION OF S^{35}-BISULFITE IN RABBIT *E*

Tissue	% of Radioactivity Recovered from Body
Blood	35.95
Urine	5.36
Liver	8.43
Stomach	2.49
Colon	2.64
Small intestine	6.51
Heart	2.14
Lungs	1.25
Kidneys	1.99
Spleen	0.13
Gall bladder	0.13
Brain	0.15
Muscle (est. as 40% body wt.)	32.86

TABLE II.—AMOUNT OF URINARY SULFUR APPEARING AS SULFATE IN RABBIT *E*

Time of Sample, Min. after First Injection	% of S as SO_4
22	0
93	68.4
170	74.9
231	38.2
278	16.1
294	5.4
325	2.7
369	0.8

TABLE III.—COMPARISON OF TOXICITY TO MICE OF BISULFITE IN WATER AND IN PERITONEAL DIALYSIS FLUID

Amt. Sodium Bisulfite Injected i.p., mg.	Mice Injected with Each Soln., No.	Mice Killed, No.	
		Water Soln.	Dialysis Fluid Soln.
25	5	5	5
15	5	5	3
12.5	5	2	1
10	5	0	0
2.5	5	0	0

DISCUSSION

The blood curves show that in every case the bisulfite was rapidly absorbed from the peritoneal dialysis fluid. The blood levels obtained were variable, probably a reflection of varying rates of both absorption and distribution in the tissues. The extent of absorption, as indicated by the amounts of bisulfite in the return fluid, was quite high in each case. The most dependable recovery of return fluid was from rabbit *E*, where the animal was sacrificed and the fluid drained from the abdomen which was rinsed to insure complete recovery. In this case only 14% of the injection amount was found in the peritoneal cavity, even though three injections had been given, the third 63 minutes before sacrifice.

An unexpected finding was the low rate of urinary excretion of radioactive sulfur. The rates of excretion varied but not in relation to the blood concentration. Also, the urinary excretion rate decreased greatly after 300–400 minutes and became almost zero in some instances.

The urinary sulfate values (Table II) indicate that the animal oxidizes bisulfite to sulfate rather slowly and that there is a finite limit to the amount of such oxidation the organism can accomplish. This, coupled with the decreased excretion rate and continued absorption, causes an ever-increasing concentration of bisulfite in the body.

The examination of isolated tissues (Table I) shows no concentration of bisulfite in a given tissue other than the blood but demonstrates a broad distribution throughout the body. Unfortunately, only 44% of the injected radioactivity was accounted for; this was probably the result of incomplete extraction. Bisulfite may be expected to react chemically with numerous chemical groups and thus may attach itself quite firmly to tissues.

Collectively, these findings show that the distribution and action of bisulfite from peritoneal dialysis fluid may be described as follows:

The bisulfite is rapidly absorbed into the blood from the dialysis fluid; the rate of absorption becomes less as the blood level increases. Bisulfite is rapidly distributed from the blood to the body tissues in a nonspecific manner. At the same time, bisulfite is oxidized to sulfate (probably in the liver), the rate of oxidation decreasing gradually to zero as the source of oxygen for this purpose is depleted or the enzyme system poisoned. The excretion of bisulfite is low enough to play no significant part in protecting the animal and becomes lower as the kidney is damaged by the high bisulfite levels. Finally, tissue concentration of bisulfite reaches the point where the central nervous system is affected and cardiovascular collapse results.

Although these limited results indicate the above action, more study is required to demonstrate these concepts unequivocably. Further work is in progress to clarify this mechanism.

At the beginning of this work, it was believed that if bisulfite is absorbed to an appreciable extent from peritoneal dialysis fluid the inclusion of as much as 500 mg. per liter would be hazardous to the life of the patient. These results indicate that extensive absorption does occur and that elimination is slower than absorption. Thus, the conclusion is that bisulfite should either be reduced greatly in these fluids or eliminated entirely.

Bisulfite may be oxidized to sulfate to some extent during the autoclaving of dialysis fluids and during subsequent storage. The amount lost is variable, probably depending on the amount of air present in the container, the time of heating, and the quality of dextrose used. In samples of peritoneal solutions labeled to contain 500 mg. of sodium bisulfite, a range of 25–628 mg. of bisulfite has been found by titration with 0.05 *N* iodine. It may be possible to include bisulfite to protect the dextrose from discoloration, yet have most of it used up in the processing. If this procedure is used, it would appear proper to limit the amount of bisulfite in the finished product rather than restrict its use in the manufacturing process.

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