HYPOPROTEINEMIA AS PROTECTION AGAINST MERCURIC CHLORIDE INJURY IN DOGS*

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During the course of experiments designed to determine whether heavy metal poisoning is influenced by altering the plasma protein level, it was found that hypoproteinemia produced by repeated plasmapheresis rendered dogs more susceptible to uranium nitrate injury (3). Whereas the majority of normal adult dogs will survive the administration of 4.0 mg. of uranium nitrate per kg. (4), the dose had to be reduced to 2.0 mg. per kg. before a dog with depleted plasma proteins survived. The question naturally arose, would increasing the concentration of the blood plasma proteins protect against heavy metal injury. When the procedure was reversed, *i.e.* when the dog's protein level was increased from a normal of about 6.5 per cent to 9 or 10 per cent by repeated daily injections of plasma obtained from healthy donor dogs and the dose of uranium nitrate was increased from 2.0 to 5.0 mg. per kg. (a dose that proved fatal to 13 of 14 controls (5)), no protection was demonstrable. Instead acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, pulmonary artery, endocardium of the left auricle, etc.) were found when the dogs died from "uremia" with massive necrosis of the renal tubules 8 to 17 days after the injection of uranium nitrate (6). These arterial lesions were the subject under investigation at the time the findings reported in this paper were uncovered. As was to have been expected from the general similarity of action of uranium nitrate and mercuric chloride, *hyper*proteinemia likewise failed to protect against mercuric chloride poisoning; similar acute necrotizing arterial lesions were found in both dogs on which this procedure was tried (7). These experiments did not prepare the authors for the finding that hypoproteinemia completely protected dogs against doses of mercuric chloride which were uniformly lethal to dogs with normal blood proteins. Yet the data presented below support this conclusion.

None of the theories about the action of mercury in therapeutic or toxic doses satisfactorily explains all the actions of this heavy metal. Mercury is usually listed as a general protoplasmic poison, and many of its actions are attributable to the fact

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that as a heavy metal it can act as a protein precipitant. This concept hardly explains the curious coagulative necrosis of the proximal convoluted tubules of the kidney within 12 to 24 hours after intravenous administration and the subsequent stomatitis and gastroenteritis when presumably it would have an almost equal opportunity to precipitate every protein in the body. Nor does the explanation that it reaches an effective concentration—by reabsorption of water—in the kidneys and colon (sites of elimination) harmonize with recent chemical analyses (1) which show that most of the mercury is stored in the bones and in the liver. Inert complex salts have been postulated and some have been isolated (2) to reconcile the absence of demonstrable lesions at the sites of maximum concentration of the metal. Presumably these inert salts change as the metal is eliminated, but many gaps in our knowledge still exist.

Methods

All of the dogs used in these experiments were healthy adult mongrels ranging in weight from 4 to 19 kg. and in estimated age from 1 to 3 years (pups and old dogs were excluded). They were kept in individual cages and had free access to water at all times. The diet fed the experimental animals and an equal number of the controls consisted of: calves' liver (raw wet weight) 32 parts; cane sugar 25 parts; corn starch 25 parts; butter 12 parts; cod liver oil (commercial) 6 parts. Enough tomato juice was added to make a paste of which each gram contained 3 calories. 1 gm. of McCollum-Simmonds salt mixture (8) and 5 gm. of kaolin were thoroughly mixed with each day's diet. Essentially the diet is a low protein diet containing, on a caloric basis, 7 per cent protein (all derived from liver), 50 per cent carbohydrate, and 43 per cent fat. The diet was fed in amounts to furnish 75 calories per kg. per day. Accurate records were kept of food consumption. The remaining control dogs were fed the regular kennel ration of cooked meat scraps and uncooked bones with occasional feedings of purina dog chow. Some of the control animals received intravenous injections of trisodium citrate before and after the administration of the heavy metal. The procedure was similar to that used in published experiments with uranium nitrate (5). Since no difference could be detected clinically, chemically, or anatomically, all of these control dogs have been grouped together.

The methods for establishing the "standard hypoproteinemic state" have been published in detail (9). Briefly these consist of 1 week's fasting followed by the daily removal of approximately 20 to 25 per cent of the total blood volume of the dog and the reinjection of an equivalent amount of red blood cells suspended in a saline solution. With the dog being maintained on the standard low protein diet after the week of fasting, this procedure repeated daily 6 days per week for 2 to 3 weeks lowers the concentration of the blood plasma proteins from a normal of about 6.5 per cent to about 4 per cent and presumably exhausts all the "protein reserves."

Duplicate micro Kjeldahl analyses of total nitrogen, non-protein nitrogen (the filtrate from 10 per cent trichloroacetic acid precipitation), and albumin plus non-protein nitrogen (the filtrate from 22 per cent sodium sulfate precipitation by Howe's method) served as the basis for calculation of the plasma protein removed and blood level studies.

The mercuric chloride used in these studies was Baker's analyzed, lot 6939, and was made up in 0.1 per cent aqueous solution with distilled water. The injections

were made into the external jugular vein, each dog receiving 3.0 cc. (3.0 mg.) per kg. About 5 minutes were required for the injections. No immediate reactions such as vomiting were noted.

The experimental animals were kept under close observation, were sacrificed with ether if they were obviously moribund, and were necropsied promptly after death. Routine sections, taken from all organs and from many of the tissues, were fixed in 4 per cent solution of formaldehyde and Zenker's solution. In some of the control dogs, only sections from the liver and from both kidneys were studied. The tissues fixed in Zenker's solution were embedded in paraffin, sectioned at 6 microns, and stained routinely with hematoxylin and eosin. Appropriate special stains were used in selected cases.

EXPERIMENTAL OBSERVATIONS

The first procedure was to establish the minimum lethal dose of mercuric chloride. The literature on this subject is conflicting. The most definite statements are based on administration by the intravenous route. Sansum (10) states: "The minimum uniformly lethal intravenous dose of mercuric chloride in dogs was found to be 4 mg. per kg. body weight (1:1000 solution injected uniformly during 15–60 minutes);" and the efficacy of this dosage has been confirmed (11, 12). Havill, Lichty, and Whipple (13) found this dose too large and stated that the minimum lethal dose for dogs is 1.5 to 2.0 mg. per kg. As a starting point we decided to use 3.0 mg. per kg.; and as can be seen from Table I this dose proved uniformly fatal to 12 dogs with normal blood proteins in 4 to 11 days. All of the dogs became sick, refused food, and exhibited marked nitrogen retention. The kidneys of all of these dogs showed the classical picture of massive necrosis of the epithelium lining the proximal convoluted tubules (Fig. 1). Calcification was marked as early as 4 days after the injection and there was only slight evidence of regeneration as late as 11 days. Practically all of these dogs showed extensive necrotizing stomatitis and several had acute inflammatory lesions in the stomach and colon. The three dogs which were fed the standard diet did not differ from those which were fed the kennel diet.

In sharp contrast to these results the three dogs reduced to the standard hypoproteinemic state by bleeding and return of the washed red blood cells suspended in saline showed practically no effects following the administration of the same dose of the same standard solution of mercuric chloride (Table II). None of these dogs became sick, none refused food for a single day, none showed elevation of the blood non-protein nitrogen, and the kidneys both in the gross and microscopically appeared normal when they were examined 8, 14, and 45 days after the injection of the heavy metal (Figs. 2 to 4). None of the dogs had any "hemolytic reactions" during the period of plasmapheresis and none showed any pigment in the kidney on histological study. In other words, there is no evidence that the protective action of hypoproteinemia was in any way related to hemoglobin in the manner demonstrated by Havill, Lichty, and Whipple

TABLE I

Intravenous Administration of 3.0 Mg. of Mercuric Chloride per Kg. Uniformly Fatal to Dogs Regardless of Diet

Dog No.	Diet	Body weight	$HgCl_2$	Death after	Terminal N.P.N.	Kidney*	
						Necrosis	Calcification
		kg.	*mg./kg.*	*days*	*mg./100 cc.*		
41-88	Kennel	17.5	3.0	4	330	+++	+‡
41-96	"	12.0	3.0	11	692	++‡	++‡
41-97	"	14.8	2.0	9	531	++‡	+‡
B-30	"	18.4	3.0	5	207§	+++	++‡
B-31	"	19.2	3.0	10	320‖	+++	+++
B-32	"	8.5	3.0	5	295§	+++	+
B-33	"	7.5	3.0	5	220§	+++	+++
B-34	"	9.0	3.0	4	230¶	+++	
B-35	"	8.9	3.0	4	210¶	+++	++
42-2	Standard	5.7	3.0	6	436	+++	+
42-3	"	5.3	3.0	4	289	+++	+++
42-4	"	3.5	3.0	5	374	+++	

* The extent of the lesion has been graded as follows: + slight, ++ moderate, +++ marked.

‡ Slight evidence of regeneration.

§ N.P.N. 2 days before death.

‖ " 3 " " "

¶ " 1 day " "

TABLE II

*Intravenous Administration of 3.0 Mg. of Mercuric Chloride per Kg. without Effect in "Standard Hypoproteinemic Dogs"**

Dog No.	Body weight	No. of exchanges	Total plasma removed	Plasma protein concentration		$HgCl_2$	Highest N.P.N.	Sacrificed after	Kidney
				At start	At end				
	kg.		*cc.*	*gm./100 cc.*	*gm./100 cc.*	*mg./kg.*		*days*	
40-81	5.0	12	1078	7.3	4.6	3.0	20‡	45	Normal
41-90	7.4	18	1956	8.2	4.1	3.0	31	14	"
41-95	9.5	17	1949	6.7	4.3	3.0	22‡	8	"

* All three dogs maintained on standard diet.

‡ It is worth recording that the N.P.N. actually fell to 14 mg. per 100 cc. on the 10th day after the mercury in the case of dog 40-81, and to 16 mg. per 100 cc. on the 7th day in the case of dog 41-95. No such fall was observed in dog 41-90.

(13). The only findings that could be attributed to the mercury were small focal areas of stomatitis on the mucous membranes of the upper lip. In dog 41-95 the process was acute; in dog 41-90 the process was healing; and in dog 40-81 the process had healed.

A limited number of experiments have failed to demonstrate any protective action of plasmapheresis *after* the injection of mercuric chloride. The following protocol is illustrative of the results obtained:—

Dog 41-99. Male mongrel; estimated age 1 year. Kennel diet.

Nov. 27, 1941. Weight 5.58 kg. Control blood studies: hematocrit 35 per cent; non-protein nitrogen 33 mg. per 100 cc.; blood plasma proteins 7.1 gm. per 100 cc.; albumin: globulin ratio 0.83. 16.74 cc. of 0.1 per cent solution of mercuric chloride injected into external jugular vein 2:30–2:35 p.m., followed by 3 plasmaphereses as follows: 1st exchange (155 cc. bled; 60 cc. packed red blood cells—obtained from donor—suspended in saline injected) completed at 2:50 p.m.; 2nd exchange (125 cc. bled; 50 cc. packed cells injected) completed at 4:30 p.m.; 3rd exchange (80 cc. bled; 50 cc. packed cells injected) completed at 6:15 p.m.

Nov. 28, 1941. Weight 5.20 kg. Some retching and vomiting this a.m. 4th exchange (160 cc. bled; 72 cc. packed cells injected) completed at 11:00 a.m.; 5th exchange (125 cc. bled; 54 cc. packed cells injected) completed at 3:00 p.m.

Nov. 29, 1941. Weight 5.00 kg. No food consumed since injection of mercury. Obviously sick this a.m. Blood studies (sample at 8:45 a.m.): plasma faintly icteric; hematocrit 35 per cent; non-protein nitrogen 206 mg. per 100 cc.; blood plasma proteins 5.7 gm. per 100 cc.; albumin: globulin ratio 0.97. Died at 1:00 p.m.

Necropsy revealed extensive necrosis and calcification of the epithelium lining the proximal convoluted tubules (Fig. 5), moderate fatty change in the liver with early central necrosis, and focal hemorrhagic necrosis in the mucosa of the colon.

Replacement of about 125 per cent (5 exchanges totalling 645 cc.) of the total circulating blood volume with washed red blood cells suspended in saline within 24 hours after the injection of mercuric chloride failed to afford any protection against the heavy metal. Rather the typical coagulative necrosis and calcification of the renal epithelium appeared to be augmented and accelerated by the plasmaphereses. Similar results were obtained by Haskell, Hamilton, and Henderson (11) following "exsanguination-transfusion" in dogs after the intravenous administration of 4.0 mg. of mercuric chloride per kg.

DISCUSSION

The authors have no satisfactory explanation for the finding that standard lethal doses of mercuric chloride produce little or no effect in hypoproteinemic dogs. It would appear that hypoproteinemia cannot be used to combat mercuric chloride poisoning which is already under way. This is in harmony with the results of Haskell, Hamilton, and Henderson (11) who found "exsanguination-transfusion" ineffective in the treatment of mercuric chloride poisoning. Apparently the hypoproteinemia must *precede* the administration of the mercury. Much work remains to be done before the degree and duration of hypoproteinemia that must exist for this protective action—also the limit of this protective action—can be established. One possible therapeutic benefit

might result from the effort—mercurial diuretics such as salyrgan and novasurol might be used with greater assurance for the patient's safety in cases of nephrosis and nutritional edema.

The finding that hypoproteinemia protects against mercuric chloride injury, contrasts sharply with the results of similar experiments with uranium nitrate in which it has been shown that the hypoproteinemic state renders the animals more susceptible to uranium (3). The results with mercury, though based on a small number of animals, are uniform and very sharp, and warrant the hypothesis that the mode of action of the two heavy metals is different. At least a tool is presented by which this aspect of the subject can be investigated.

Another fact that seems to be clearly established is that reversal of the procedure—*hyper*proteinemia by plasma injections instead of hypoproteinemia by plasmapheresis—failed to demonstrate any difference between the two heavy metals. There was no protective action against either heavy metal and similar acute necrotizing arterial lesions were found after the administration of both uranium (6) and mercury (7).

While "normal" and "hyperproteinemic" dogs react similarly to uranium and mercury, a striking difference in the action of the two heavy metals is demonstrable in standard hypoproteinemic dogs with "reserve stores" of protein exhausted. Following uranium the hypoproteinemic dog quits eating, develops acidosis and marked nitrogen retention, and dies 6 to 17 days later with massive necrosis of the proximal convoluted tubular epithelium. The only difference between the hypoproteinemic and the normal dog is that in the former less uranium is required to produce these classical results. Following the intravenous administration of a dose of mercury, uniformly fatal to normal dogs, the hypoproteinemic dog fails to show any evidence of illness. Appetite and body weight were maintained in our animals, there was no nitrogen retention, and the kidneys both in the gross and histologically appeared normal when the dogs were sacrificed 8, 14, and 45 days later. There is no evidence—such as calcification, mitoses, or basophilic "flattened epithelium," emphasized by MacNider (12)—that the kidneys of these dogs would have shown any change if examined earlier than 8 days. And with the exception of small localized areas of healing stomatitis on the mucous membrane of the upper lips none of the other organs or tissues showed any changes that could be attributed to the heavy metal.

Four or five mechanisms come to mind that might explain findings, but the available data do not allow a satisfactory evaluation of any of them. The simplest purely theoretical explanation for the observed phenomena is that the establishment of the hypoproteinemic state depletes something (presumably fabricated or concentrated in the kidney cortex) and this substance is not available to be acted on by the heavy metal. Still other possibilities are: (1) The hypoproteinemic state fails to "hold" the mercury proteinate (this hypothesis involves differential solubilities in different concentrations of

proteins); (2) The acid-base balance may be upset and the pH of the medium so changed that it favors a shift from "toxic" to "non-toxic" mercurial compounds; (3) blood calcium may be altered and if mercury "follows" calcium as Young, Taylor, and Merritt (1) have shown, this may account for the failure of mercury to reach "effective concentration;" (4) the reducing powers of the blood—*e.g.* as that of the sulfhydryl groups—may be altered as postulated by Miller and Whipple (14, 15) to explain the increased susceptibility of standard hypoproteinemic dogs to chloroform anesthesia and the protective action of methionine and cystine against this increased susceptibility. Further speculation seems inappropriate at this time: the data had best rest on their own merits.

SUMMARY

Twelve control dogs receiving a single intravenous injection of mercuric chloride, 3.0 mg. per kg., all died within 4 to 11 days afterwards with marked nitrogen retention and extensive necrosis and calcification of the epithelium lining the proximal convoluted tubules.

Three dogs of comparable age and weight were reduced to a standard hypoproteinemic state by repeated plasmapheresis. Each dog then received the same dose of mercuric chloride as the controls. None of these dogs became sick, none showed any elevation of non-protein nitrogen, and the kidneys—both in the gross and histologically—appeared normal when they were examined 8 to 45 days later.

As tested thus far intensive plasmapheresis *following* the injection of mercuric chloride has been without effect in preventing the classical changes of mercuric chloride injury observed in the control dogs.

The simplest explanation for these phenomena is that mercuric chloride acts on a more or less specific substance (presumably fabricated or concentrated in the renal cortex) which is depleted in the standard hypoproteinemic state. Other possibilities are mentioned.

These findings are in sharp contrast to the results of similar experiments with uranium nitrate. The hypoproteinemic state appears to render the animals more susceptible to uranium injury (3). This probably indicates that the mode of action of the two heavy metals is different.

CONCLUSION

Lethal doses of mercuric chloride produce little or no effect in standard hypoproteinemic dogs.

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EXPLANATION OF PLATE 28

All sections of kidney fixed in Zenker's fluid and stained with hematoxylin and eosin.

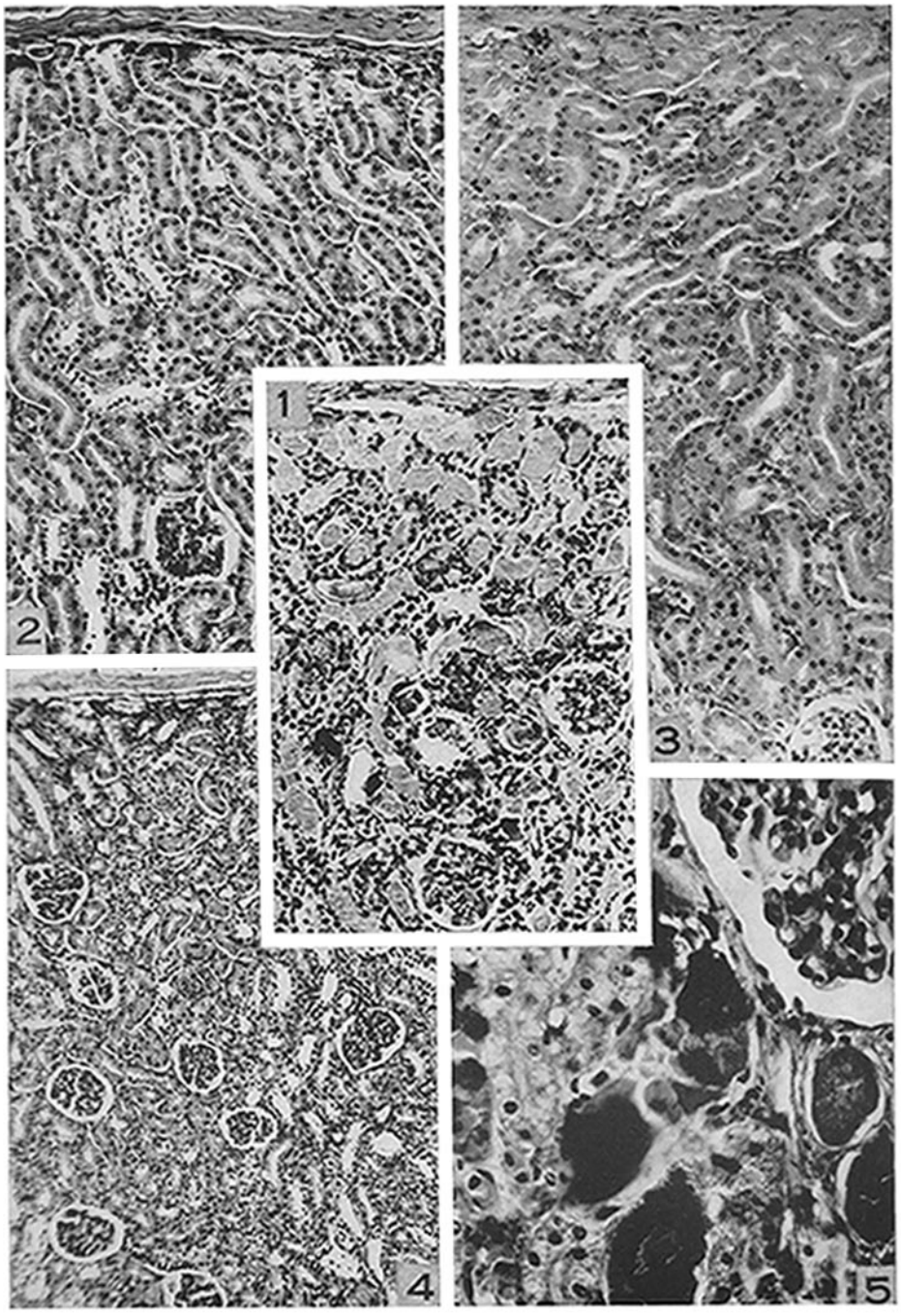
FIG. 1. Dog 42-2. Normal dog on "standard diet" for 6 weeks. 6 days after mercuric chloride. Typical coagulative necrosis of proximal convoluted tubules. × 145.

FIG. 2. Dog 40-81. Hypoproteinemic dog. Absence of any evidence of injury 45 days after mercuric chloride. × 148.

FIG. 3. Dog 41-95. Hypoproteinemic dog. Absence of any evidence of injury 8 days after mercuric chloride. × 170.

FIG. 4. Dog 41-90. Hypoproteinemic dog. Absence of any evidence of injury 14 days after mercuric chloride. × 88.

FIG. 5. Dog 41-99. Normal dog on kennel diet. Mercuric chloride *followed* by intensive plasmapheresis. Extensive necrosis with marked calcium deposition in proximal convoluted tubules 2 days after heavy metal. × 360.



(Holman and Donnelly: Hypoproteinemia and mercuric chloride injury)