Renal Localization, Excretion and Toxicity of Mercuric Chloride in Normal and "Nephrotic" Rats.* (23310)

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During studies on the toxicity of mercuric chloride we have tested rats with an experimental nephrotic syndrome. Rats given a course of injections of an aminonucleoside related to "puromycin" develop an increase in proteinuria, hypoproteinemia, edema and ascites. The clinical state and renal lesions have been described as resembling somewhat those present in the nephrotic syndrome in children(1). We have found that such "nephrotic" rats survive intravenous doses of mercuric chloride uniformly fatal to normal animals, although the mercury contents of the kidneys, collected urines and collected feces, as revealed by analysis 24 hours after injection, do not differ significantly between the normal rats and those with the experimental nephrotic syndrome.

Method. Sprague-Dawley males offered tap water and Purina Laboratory Chow were used. To compare the toxic effects of mercuric chloride in normal and "nephrotic" rats, 20 animals weighing between 142 and 200 g were injected subcutaneously once daily for 8 days with 0.003 ml per g of body weight of a 0.5% aqueous solution of 6-dimethylamino purine, 3-amino-d-ribose.† Plasma protein concentrations were determined(6) before the initial and 48 hours after the final injection. Within a few hours after the second blood sampling, 15 of the 20 rats were given mercuric chloride, 3 mg per kilo of (edematous) body weight, via the femoral vein. All 20 rats were then observed for 4 weeks or more. Survival rate of the mercuric chloride injected animals was compared with that of 12 mercuric chloride injected previously untreated normal rats on the same offered diet. Six additional rats weighing 165 to 195 g were given 12 daily subcutaneous injections of the aminonucleoside as above. Plasma protein concentrations were determined before the first and 48 hours after the final injection. Within a few hours after the second blood sampling the rats were given $Hg^{203}Cl_2$ intravenously, 3 mg per kg of (edematous) body weight and put into individual collection cages. Twenty-four hours after the injection they were weighed and killed. The kidneys were excised, weighed rapidly and placed in a tube for counting. Also assayed were aliquots of the diluted urine after washing of the cage, the total feces, the pledget used at the point of venepuncture and a block of tissue excised at the injection site. Aliquots of the diluted urine were analyzed also for protein(8). The remainder of the carcass, including all the fluid contents, was dried to constant weight in an oven between 80° and 90°C. The total solids content of 3 normal rats of the same weight group was also determined; using the mean percentage of body solids found in these 3, the "ideal" (nonedematous) body weights at the time of their sacrifice were calculated for the edematous rats. Six normal rats of appropriate weight also given intravenous radiomercuric chloride and similarly treated were used as controls. The $Hg^{203}Cl_2$ had been prepared with HCl from $Hg^{203}O$ and was of low activity, giving in our apparatus from 35,300 to 25,400 counts per mg of $Hg^{203}Cl_2$ per minute. Counting was done with a Model DS 3 Nuclear-Chicago well type scintillation counter and a Model 192 Nuclear-Chicago Ultrascaler. Counts were to at least 10,000 for backgrounds, 40,000 for standards, 10,000 for kidneys, 1,000 for urines, 4,000 for feces. The mean statistical errors of determination for urine and feces are, therefore, because of the low level of radiation and the low number of total counts, relatively high, being 10.5% for urine and 2.6% for feces. For kidneys the error is

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only 1%. No significant amounts of Hg^{203} were detected in the pledgets nor was leakage at the site indicated by the activity of any of the site areas excised.

Results. Toxicity of mercuric chloride in normal rats and rats with nephrotic syndrome. The mean plasma protein concentration of the 15 rats receiving stable $HgCl_2$ was 6.04 ± 0.76 g % before initial injection of the aminonucleoside and 4.60 ± 0.37 g % at the time of $HgCl_2$ administration. That of the 5 animals which received the aminonucleoside, but not mercuric chloride, was correspondingly 5.68 ± 0.78 g % and 4.59 ± 0.40 g %. All 20 appeared to have ascites on the tenth day. Of the 15 rats given $HgCl_2$, 14 were still alive and no longer ascitic 4 weeks after the injection. They had gained weight and were in apparent good health when killed. All 5 not given mercuric chloride were also in apparent good health at this time. One rat died 48 hours after mercuric chloride administration, having had the highest plasma protein (5.4 g %) of the group. This survival rate contrasts with that of normal untreated rats given 3 mg per kg $HgCl_2$ intravenously in which of 12 injected all died within 6 days, with diarrhea and hematuria.

Excretion and renal localization of $Hg^{203}Cl_2$ by normal rats and rats with nephrotic syndrome. Three uninjected normal rats were found to have solids contents of 31.0, 31.4 and 30.5% of the body weights. Therefore, the factor of 0.31 was used to convert the desiccated carcass weights of the "nephrotic" and normal animals given $Hg^{203}Cl_2$ to "ideal" weights at the time of sacrifice (desiccated weight divided by 0.31).

The data are summarized in Table I. The mean weights of the desiccated carcasses (actually, carcasses minus kidneys and injection sites) for the "nephrotic" and normal groups, respectively, were 44.9 and 42.2 g. These are convertible to mean "ideal" weights of 144.5 (range 127-160) and 136.2 (range 126-148) g at time of sacrifice for the "nephrotic" and normal groups, respectively, with mean excesses in body water content of 42.7 and 15.2 g, as compared with uninjected controls.

After radiomercuric chloride injection the normal group had diarrhea and hematuria, manifestations of acute mercury poisoning.

Discussion. Holman and Donnelly(3) and Martin and Reid(7) found that dogs made hypoproteinemic by plasmapheresis survived intravenous administration of 3 mg per kilo $HgCl_2$, a dose fatal to normal dogs. On the other hand, Havill, Lichty and Whipple(2) found that frequent injection of hemoglobin into dogs increased their tolerance for mercuric chloride, and Lippman and his associates reported that the toxic effects of mersalyl-theophylline, meralluride sodium (Mercuhydrin)® or mercuric chloride on the kidney in rats were reduced by the prior administration intraperitoneally of human or bovine serum albumin(4,5). The latter workers ascribed the protection to presence of albuminuria in their animals and suggested that both blockage of tubular reabsorption of mercury and an increase in urinary excretion of mercury due to binding by urinary protein might be the mechanisms operating.

Our rats with both hypoproteinemia and an increase in proteinuria clearly show an increase in resistance to the toxic effects of mercuric chloride. The dosage, 3 mg per kg, is higher than this when expressed in terms of the "ideal" weights. We have not analyzed the ascitic fluid for mercury, but ascitic fluid in human patients with hepatic cirrhosis given Mercuhydrin® contained mercury in only low concentration(9). We found no significant diversion of mercury from the kidney in the presence of the high excess of body water.

Our data show no significant differences between the "nephrotic" and normal groups with respect to the percentage of the administered Hg^{203} present at 24 hours in the kidneys ($P>0.1$), in the collected urine ($P>0.05$), and in the collected feces ($P>0.1$). The cause for the protection against mercuric chloride is not apparent. Failure to correlate such protection with amounts of mercury found in the kidneys was found also in rats in which an increase in resistance to the toxic effects of mercuric chloride was induced by feeding a diet of sucrose and vitamins(15). Although Lippman *et al.*, with rats protected by pretreatment with intraperitoneal serum albumin, interpreted their data as showing a reduction in the renal localization of Hg^{203}

TABLE I. Excretion and Renal Localization of Hg^{203} in Normal Rats and Rats with Experimental Nephrotic Syndrome, 24 Hr after Intravenous Injection of 3 mg/kg $Hg^{203}Cl_2$.

Initial wt, g	Wt at inj., g	Wt at sacrifice, g	Kidney wt, g	Dry wt, g	Body solids, %	Dosage* $Hg^{203}Cl_2$, mg/kg	Hg^{203} dose distribution (%)†			Plasma protein (g %)		Urinary protein, mg/24 hr
							Kidney	Urine	Feces	Initial	Final	
Rats with nephrotic syndrome												
186	216	204.1	3.07	45.3	22.54	4.43	15.9	14.7	5.5	5.37	4.39	118
166	194	158.8	2.76	39.5	25.32	4.57	17.4	17.7	1.2	7.00	4.91	302
195	190	167.1	2.23	41.6	25.23	4.25	20.2	10.5	2.7	6.27	4.15	97
198	220	201.3	2.93	46.1	23.24	4.44	19.2	21.8	4.2	5.71	<4.00	258
195	218	206.3	2.43	47.3	23.20	4.29	10.7	13.9	5.4	6.48	4.01	259
199	207	203.7	3.12	49.5	24.68	3.89	16.4	19.2	4.2	6.24	4.04	208
	Mean		2.76	44.9	24.04	4.31	16.6	16.3	3.9	6.18	4.25	207
	S.D.				±1.18	±.24	±3.3	±4.1	±1.7	±.57	±.34	±83
Normal rats												
	155	161.7	1.41	44.0	27.45	4.33	17.2	7.4	1.6			18
	140	138.3	1.29	38.9	28.39	4.70	18.8	9.7	6.5			35
	174	168.0	1.70	45.9	27.60	4.63	21.0	9.2	4.5			19
	149	134.4	1.33	39.2	29.45	4.78	16.3	16.2	2.0			42
	168	164.8	1.77	43.8	26.87	3.89	18.8	15.4	5.7			20
	164	150.5	1.75	41.5	27.89	4.53	19.5	5.5	1.2			37
	Mean		1.54	42.2	27.94	4.48	18.6	10.8	3.6			28.5
	S.D.				±.89	±.33	±1.9	±4.1	±2.3			±10.7

* mg $Hg^{203}Cl_2$/kg of "ideal" (nonedematous) body wt at sacrifice (see text).
† % of inj. dose found in kidney, urine and feces.

and an increase in its excretion(5), we have interpreted their data differently and have ourselves found a renal mercury content slightly greater in albumin-injected rats than in saline-injected controls when both sets were sacrificed 24 hours after mercuric chloride injection(11).

Summary. Rats given 8 or 12 daily injections of 6-dimethylamino purine, 3-amino-d-ribose, develop increase in proteinuria, hypoproteinemia, edema and ascites. Such rats survive an intravenous dose of mercuric chloride lethal to normal rats. The mercury contents of kidneys, collected urine and collected feces, 24 hours after intravenous injection of $Hg^{203}Cl_2$, are not significantly different in rats with the experimental nephrotic syndrome from those in normal rats given the same dose.

1. Frenk, S., Antonowicz, I., Craig, J. M., and Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

2. Havill, W. H., Lichty, J. A., Jr., and Whipple, G. H., *J. Exp. Med.*, 1932, v55, 627.

3. Holman, R. L., and Donnelly, G. L., *ibid.*, 1942, v76, 511.

4. Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 682.

5. Lippman, R. W., Finkle, R. D., and Gillette, D., *ibid.*, 1951, v77, 68.

6. Lowry, O. H., and Hunter, T. H., *J. Biol. Chem.*, 1945, v159, 465.

7. Martin, H. F., and Reid, N. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 863.

8. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry,* Vol. II. Methods. Williams & Wilkins Co., Baltimore, 1932, p683.

9. Ray, C. T., Burch, G. E., Threefoot, S. A., and Kelley, F. J., *Am. J. Med. Sci.*, 1950, v220, 160.

10. Surtshin, A., *Am. J. Physiol.*,

11. Surtshin, A., and Parelman, A. G.,

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Effect of Hypothermia on Ventricular Fibrillary Threshold. (23311)

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Cardiac arrhythmias occur with alarming frequency under conditions of experimental hypothermia(1,2). A hyperirritable ventricle brought about by the hypothermic process is believed responsible for these cardiac irregularities, of which ventricular fibrillation is the most common. Direct measurements of ventricular threshold thus far have failed to reveal any difference between excitability of the normo- and hypothermic heart (3,4). However, no information is available as yet on the actual threshold for ventricular fibrillation at various body temperatures. Thus quantitative data on ventricular fibrillary threshold during progressive hypothermia appear necessary to confirm or refute the hypothesis of a hyperirritable hypothermic heart. During our study, an attempt was made to measure ventricular fibrillary thresholds by determining the minimum electrical stimulus needed to induce a fibrillary state in dogs subjected to immersion hypothermia. In addition, the effect of pH and prolonged cold exposure on ventricular fibrillary threshold was evaluated. Previous studies have revealed that cold acclimatization(5) and maintenance of normal pH during general body cooling(6) significantly reduce the incidence of hypothermic ventricular fibrillation. Thus, ventricular fibrillary thresholds were measured in cold acclimatized normocapneic dogs during hypothermia to determine if these procedures exert their beneficial effect by direct action on the myocardium.

Method. Eight apparently healthy dogs, ranging 13 kg to 25 kg, were anesthetized with intravenous pentobarbital sodium (30 mg/kg). The left common carotid artery was exposed and cannulated for measurement of mean arterial blood pressure via a mercury manometer. Samples of venous blood were obtained from the left jugular vein at rectal

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