Protective Action of Certain Amino Acids on Toxicity of Mercurial Diuretics in Rats. (18543)

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Mercurial diuretics given in therapeutic doses with the intention of inducing diuresis rarely cause renal damage. Comparatively larger doses, however, will regularly produce severe renal injury in the experimental animal. Lippman(1) has shown that the administration of human albumin to rats prior to the administration of mercurial diuretics sharply reduced renal toxicity in the rat. It had been previously demonstrated that the severe necrotizing nephrosis induced by dl-serine in the rat can be favorably influenced by several amino acids and related compounds(2). In the present study a considerable protective action of various amino acids was established against the nephrotoxic effect of a mercurial diuretic.

Methods. More than 300 young female albino rats weighing approximately 150 g were used. The mercurial diuretic employed was Meralluride Sodium ("Mercuhydrin Sodium," Lakeside Laboratories Inc., Milwaukee, Wisc.). This preparation contains 39.0 mg mercury and 48 mg theophylline in 1 ml. The rats were given 0.01 mg mercury per g of body weight or approximately 2 ml of a 1 to 50 dilution of the original preparation intramuscularly. It was established in preliminary experiments that this amount produced regularly severe necrotizing nephrosis within 24 hours. The amino acids to be tested were dissolved in distilled water, adjusted to a pH ±7.2 if necessary and injected in amounts of 3 ml intraperitoneally 30 minutes before, simultaneously with, and 30 minutes after the mercurial was given. In some experiments (see Table I) a different schedule was used. The animals were killed after 24 hours and the kidneys fixed in formalin. Sections for microscopic study were stained with hematoxylin and eosin. Ten to 12 animals were used at one time. At least 2 animals served as controls. These were given the mercurial intramuscularly and corresponding amounts of physiological saline solutions intraperitoneally. The degree of renal damage was expressed as 0, ±1+, 2+, 3+, and 4+. In kidneys considered to show ± damage only very few necrotic tubules could be seen while in those with 1+ damage occasional ones appeared necrotic. The degree of protection given by a certain compound was judged by the percentage of animals which showed 0, ±, and 1+ lesions in this particular group. In order to further evaluate the degree of the renal injury, urinary protein excretion was measured. For this purpose rats were put in individual metabolism cages and 24 hour urine specimens collected. Protein was determined with Exton's method using a Leitz photocolorimeter(3). After protein excretion had been examined for 5 consecutive days, one group of 17 rats was injected with 1(+) arginine monohydrochloride and the mercury preparation while another group of 15 animals were injected with the mercurial only. Urine was collected for the following 7 days and the animals then killed and their kidneys prepared for microscopic study.

Results. Control animals. Severe necrotizing nephrosis restricted to the proximal convoluted tubules was regularly seen within 24 hours on microscopic sections (see Fig. 1). The appearance was identical with that observed following the application of mercury bichloride. With the amount of mercurial given only 1 out of 57 animals tested showed a 1+ lesion, while the damage of the kidneys of the remaining animals was graded from 2+ to 4+, with 42 rats revealing a 4+ involvement (Table II).

In 15 rats killed after 7 days there was the

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TABLE I. Summary of Results of Experiments to Determine Whether 1 or 2 Injections of 1(+) Arginine Monohydrochloride Given at Various Time Intervals Would Protect Rats Against Renal Injury Caused by Merullaride Sodium.

30 min. before	Simult.	30 min. after	60 min. after	90 min. after	Total No. rats	No. of rats showing lesions 0	±	1+	2+	3+	4+	Rats showing 0, ±, and 1+ lesions No.	%
One injection													
+	—	—	—	—	6	0	1	1	2	1	1	2	33.3
—	+	—	—	—	6	0	3	0	2	1	0	3	50
—	—	+	—	—	6	0	0	4	2	0	0	4	66.6
—	—	—	+	—	6	0	1	0	3	2	0	5	20
Two injections													
+	+	—	—	—	6	0	3	1	1	1	0	4	66.6
—	+	+	—	—	6	0	3	1	1	1	0	4	66.6
—	—	+	+	—	12	1	5	3	1	2	0	9	75
—	—	—	+	+	6	0	0	1	1	1	3	1	20

typical dilatation of many proximal convoluted tubules which were invested by flat regenerating epithelium. Calcification in damaged tubules was occasionally seen.

Influence of various amino acids given in 3 divided doses. The substances tested can be seen in Table II. Among the amino acids used 1(+) arginine monohydrochloride and glycine gave very considerable protection when three times 300 mg were administered. dl-alpha-alanine and 1(+) histidine monohydrochloride follow in their protective potency, while dl-methionine and 1(+) cysteine hydrochloride were only of moderate influence. With half the amounts given 1(+) arginine monohydrochloride was still quite effective while dl-alpha-alanine and glycine had only little effect. No protective action was seen from dl-threonine, dl-valine, dl-phenyl-alanine, dextrose and sodium chloride.

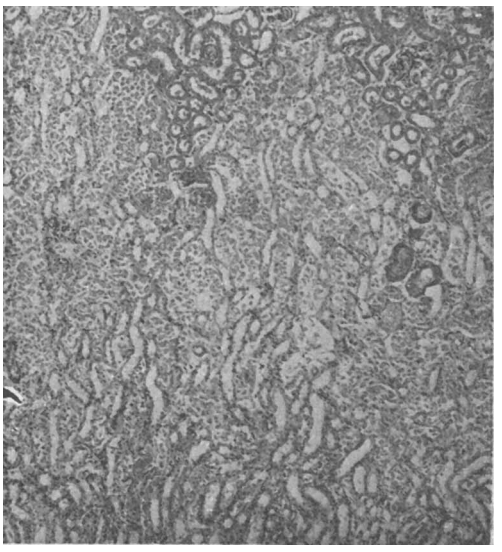

FIG. 1.
Section of kidney of a rat 24 hr after meralluride sodium (.01 mg mercury per g body wt). The renal damage is considered to be 4+. Hematoxylin-Eosin stain ×60.

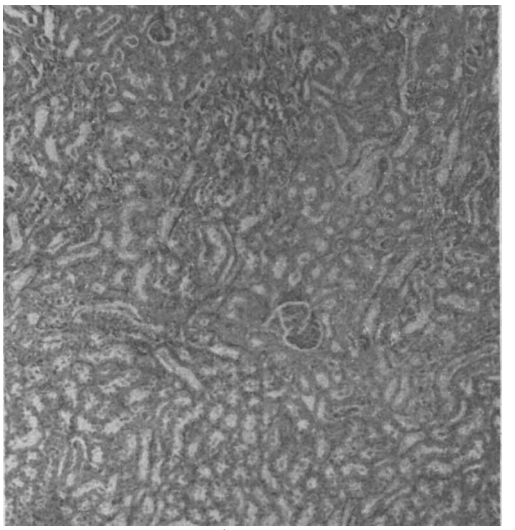

FIG. 2.
Section of kidney of a rat 24 hr after meralluride sodium (.01 mg mercury per g body wt) and protected by 3 inj. of 300 mg 1(+) arginine monohydrochloride. There is no renal damage seen. Hematoxylin-Eosin stain ×60.

Influence of 1 or 2 doses of 1(+) arginine monohydrochloride given at various time intervals. Table I shows the effect of 1 or 2 injections of 300 mg 1(+) arginine monohydrochloride upon the mercurial toxicity. Two injections given 30 minutes prior and

TABLE II. Summary of Results of Experiments to Determine Whether Various Amino Acids Protect Rats Against Renal Injury Caused by Merullaride Sodium.

Substance tested	Amt in mg, 3×	Total No. rats	No. of rats showing lesions 0	±	1	2	3	4	Rats showing 0, ±, and 1+ lesions No.	%
—	—	57	0	0	1	8	6	42	1	1.8
l(+) arginine monohydrochloride	300	19	3	10	5	1	0	0	18	94.7
l(+) arginine monohydrochloride	150	16	0	7	2	5	2	0	9	56.3
Glycine	300	13	1	9	2	1	0	0	12	92.3
"	150	8	0	0	1	4	2	1	1	12.5
DL-alpha-alanine	300	24	0	11	5	4	4	0	16	66.6
"	150	9	0	1	1	3	3	1	2	22.2
l(+) histidine monohydrochloride	300	6	0	3	1	0	1	2	4	66.6
l(+) cysteine hydrochloride	300	9	3	1	0	5	0	0	4	44.4
DL-methionine	150	8	0	1	2	0	2	3	3	37.5
DL-threonine	300	7	0	0	0	0	0	7	0	0
DL-valine	150	4	0	0	0	0	0	4	0	0
DL-phenylalanine	75	3	0	0	0	0	1	2	0	0
Dextrose	300	6	0	0	0	0	0	6	0	0
Sodium chloride	150	8	0	0	0	2	3	3	0	0

simultaneously with the drug or simultaneously and 30 minutes later, or 30 and 60 minutes later imparted considerable protection. Only little influence was seen if the two injections were given 60 and 90 minutes later. In the case of one injection an appreciable protective influence was demonstrable if the amino acid was administered simultaneously or 30 minutes after the mercurial had been given.

Proteinuria in animals protected and unprotected by l(+) arginine monohydrochloride. In agreement with the findings of others there was considerable individual variation in the amounts of protein excreted in the urine under normal conditions(4,5). In 32 rats the 24 hour excretions of protein averaged 3.7 mg and the average urine quantity 8 ml. Following the injection of mercuhydrin urinary protein excretion rose sharply (Fig. 3); the main increase took place in the first 72 hours following injection. The average excretion for 15 rats in this period amounted to 91.2 mg while the excretion of protein in 17 rats protected by 3 injections of l(+) arginine monohydrochloride was only 42.1 mg. The diuretic effect of mercuhydrin in both groups was not significantly different (Fig. 4).

On microscopic examination the degree of renal damage in the control group was con-

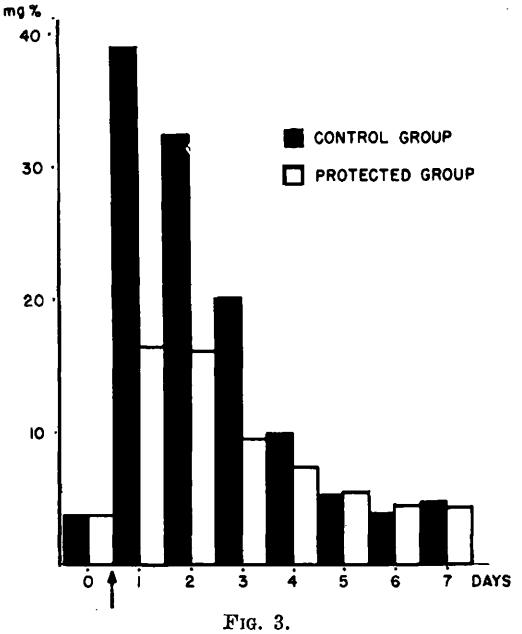

FIG. 3.

Average 24 hr urinary excretion of protein in a group of 15 rats before and following a single inj. of meralluride sodium (.01 mg mercury per g body wt) (dark column) and of a group of 17 rats protected with 3 inj. of 300 mg l(+) arginine monohydrochloride (white column).

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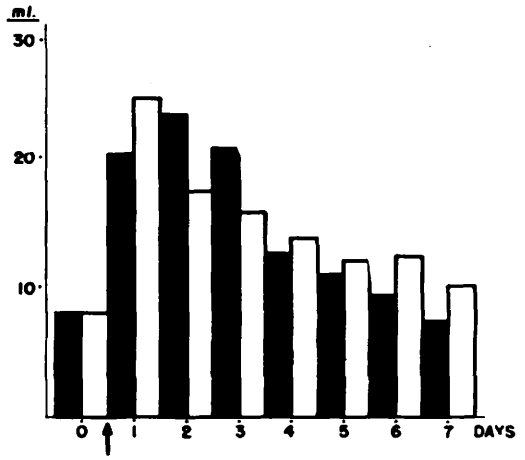

FIG. 4.
Average 24 hr excretion of urine in group of 15 rats before and following a single inj. of meralluride sodium (.01 mg mercury per g body wt) (dark column) and of 17 rats protected with 3 inj. of 300 mg 1(+) arginine monohydrochloride (white column).

sidered to be 1+ in one, 2+ in 3, 3+ in 4 and 4+ in 7 rats. There was therefore severe involvement in 93.4%. Among the 17 rats injected with 1(+) arginine monohydrochloride no renal damage was seen in 3. In the remaining 14 animals the lesion was graded as ± in 4, as 1+ in 2, as 2+ in 3, as 3+ in 4 and as 4+ in 1. Marked renal involvement was therefore found in 8 animals or 47%.

Comment. It is generally agreed that mercurial diuretics act by depressing tubular function and impair the reabsorption of sodium and water. In man they depress the reabsorption of glucose(6) as well as other functions ascribed to involve the proximal convoluted tubules(7). However, in the dog the tubular transfer of glucose and p-aminohippurate is not influenced by mercurials (8,9). The depression of tubular reabsorption of sodium is furthermore limited in the dog. According to Duggan and Pitts(9) this limitation is conditioned by the magnitude of the absorptive system which corresponds to the magnitude of the distal tubular absorption. The microscopic changes regularly seen with toxic doses in the rat are identical with those observed following mercury bichloride. With special technics the renal damage has been localized under the latter condition to the distal portion of the proximal convoluted tubules in the dog(10) and the median portion of the proximal convoluted tubules in the rat(11). The *microscopically* demonstrable influence of mercuhydrin sodium upon the renal cortex is therefore seen only in the proximal convolution even in highly toxic doses.

The experiments reported here show conclusively that certain amino acids given in large amounts are able to modify the nephrotoxic action of a mercurial diuretic. A similar protective effect of some amino acids has been established previously against the kidney damaging action of dl-serine in the rat(2). It was assumed that the beneficial effect of these amino acids was due to their competitive suppression of tubular reabsorption of the injurious d-isomer of the dl-serine. Competition for transport has not only been reported for amino acids but also for a number of compounds unrelated by chemical structure(12). The protective effect of human albumin upon the renal toxicity of mercurial diuretics was thus attributed by Lippman(1) in a similar manner to the inhibition of mercurial reabsorption when the tubules are saturated with protein. In those experiments no protection occurred if the protein was given simultaneously with the drug. In contrast 1(+) arginine monohydrochloride was found quite effective if given simultaneously or even 30 minutes later. This difference can be explained by the more rapid absorption of amino acids due to their smaller molecular size. There is a marked difference in the protective effect of certain amino acids against the toxic effect of dl-serine and that of mercuhydrin

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sodium. dl-threonine for instance was found to protect the kidney completely against dl-serine but is without effect on the toxicity of mercuhydrin sodium. These differences are at the present time unexplained.

Summary. Certain amino acids reduce considerably the nephrotoxic action of meralluride sodium in the rat. Among the amino acids examined l(+) arginine monohydrochloride and glycine were found to be most effective. The beneficial influence seen in microscopic sections as well as in the reduced proteinuria following a toxic dose of the mercurial diuretic is attributed to the competitive suppression of tubular reabsorption of the injurious mercury.

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Independent Biosynthesis of Different Hemin Chromoproteins—Cytochrome *c* in Various Tissues.* (18544)

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Since the same prosthetic group, iron protoporphyrin IX (structural type III porphyrin), is present in the different chromoproteins, hemoglobin, myoglobin, cytochrome *c* and catalase, it becomes of fundamental interest and importance to know whether the biosynthesis of the metalloporphyrin (protohemin) is confined to a localized site or whether porphyrin and hemin synthetic ability is a general property of cells of different tissues. Actually, very little information of decisive character is available as to the biosynthesis of chromoproteins other than hemoglobin. Experiments by Beinert *et al.*(1,2) on the incorporation of the radioactive iron isotopes in cytochrome *c* proved merely that if sufficient iron was supplied it would appear also in the prosthetic group of the widely distributed tissue pigment, cytochrome *c*, but left unsettled the question of relative degrees or rates of incorporation in the different chromoproteins, as the labelled iron was furnished in large doses during active growth from birth on, yielding rather similar very high levels of incorporation in all isolated pigment samples. On the other hand, in Theorell's laboratory, it has been shown with radioactive iron that the rate of incorporation of the iron in the hemin of liver catalase is significantly greater than in that of red cell catalase, from which it was deduced that the biosynthesis of the catalases of these respective tissues may be independent.‡ Work in our laboratory on the appearance of "new" cytochrome *c* in liver regenerating after partial hepatectomy(3-5) favored the inference that this chromoprotein was fabricated in tissues *in situ,* but the experiments were indecisive since the possibility of the mobilization of cytochrome *c,* its transfer to regenerating liver from tissues such as skeletal muscle, was not eliminated. A stronger deductive inference of the independent biosynthesis of cytochrome *c* could be drawn from the rapid changes in the cytochrome *c* content of various tissues after thyroidectomy and induced hyperthyroidism(6).

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† With the technical assistance of Anna May Dych, Jean Randall, Charlotte Glausser and Pauline A. Corneille.

‡ Personal communication from Professor Hugo Theorell, Director of the Biochemical Department, Medical Nobel Institute.

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