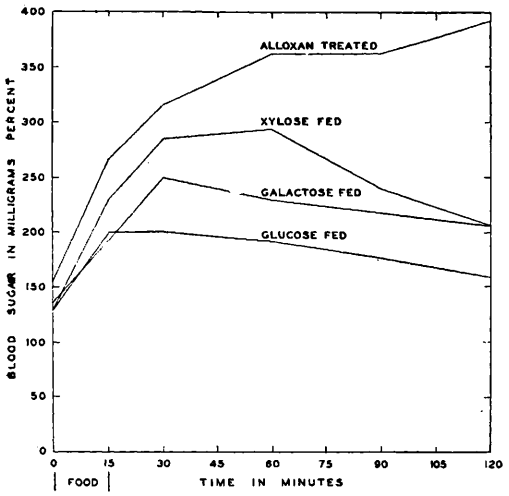

FIG. 1. Tolerance curves of individual rats from each group. Food was withheld for 12 hr. Blood was drawn (zero time), the animals were allowed access to their respective diets for 15 min, the food was again withheld, and blood samples were taken for analysis as shown.

galactose or xylose is injurious to the lens at a lower blood level.

Fig. 1 gives blood sugar tolerance curves for typical rats from each group.

Summary. Cataract was produced experimentally in weanling littermate rats by (1) alloxan treatment, (2) galactose feeding, and (3) xylose feeding. All groups showed greatly elevated blood sugar levels as compared with untreated controls receiving glucose. Cataract was exhibited by all of the xylose- and galactose-fed rats, and by nearly all of the alloxan-treated rats. However, the development of cataract was much slower in the alloxan-treated than in the other groups. It is concluded that not only the level of blood sugar, but also the specific configuration of the sugar concerned is a factor in its cataractogenic action.

Received August 13, 1951. P.S.E.B.M., 1951, v78.

Some Pharmacological Properties of Three New Mercurial Diuretics.* (19095)

CARROLL A. HANDLEY, DON CHAPMAN, AND JOHN H. MOYER.

From the Departments of Pharmacology and Medicine, Baylor University College of Medicine, Houston, Texas.

A preliminary appraisal of a new series of mercurial compounds with diuretic activity showed many in the group to have greater potency than diuretics currently employed(1). From stability data and other considerations, 3 of the agents were selected for further investigation. They are 3-chloromercuri-2-methoxypropylurea (1347Ex), 3-carboxymethyl-mercaptomercuri- 2 -methoxypropylurea (1353Ex), and 3-(α-carboxyethylmercaptomercuri)-2-methoxypropylurea (1431-Ex). The following data compare some of the pharmacological properties of these mercurials with meralluride (Mercuhydrin).

Methods. Diuretic activity and sodium excretion were studied in unanesthetized female dogs or in dogs lightly anesthetized with sodium pentobarbital (25 mg/kg, I.V.). The animals had not had access to food for 16-18 hours and water for 3-4 hours prior to the experiment. Urine, collected at 20 minute intervals from a retention catheter, was removed from the bladder as completely as possible by introducing air and using compression. Three or 4 control collections were made before the drugs were administered. Meralluride at several dosage levels was used as a standard, and the dosage of the other compounds was adjusted to give approximately equivalent responses. A Perkin-Elmer flame photometer was used for sodium analyses. Electrocardiograms, in dogs anesthetized with 30 mg per kg of pentobarbital sodium, recorded from lead II, were used to investigate the acute cardiac effects. After a control record, amounts of the 3 new mer-

* Aided by a grant from Lakeside Laboratories, Milwaukee, Wisc.

1. Nuhfer, P. A., and White, S., *J. Pharm. Exp. Therap.*, 1951, v101, 30.

curials equivalent to 250 mg of mercury dissolved in 50 ml of physiological saline were infused at a rate of 2 ml per minute into the external jugular vein. Electrocardiograms were taken at 5 minute intervals for the first hour, and then at half hour intervals for an additional 2-3 hours. In the chronic toxicity studies, changes in glomerular filtration rate (GFR) was used as a criterion for the development of renal damage, and the animals were carefully observed for other symptoms of mercury poisoning. In addition, the maximal rate of tubular reabsorption of dextrose (TmG) was used as a measure of tubular function in 7 and 3 dogs given 1347Ex and 1431Ex, respectively. The method of Lang and Nelson(2) was used for the determination of mercury in urine and feces.

Diuretic potency. Ten dogs were used for measuring the activity of each compound at each dosage level. All the assays of diuretic activity were made from a group of 20 animals of 12-16 kg. These animals were used for each mercurial, although none of the dogs was given a diuretic more frequently than once a week. No difference could be detected in the diuretic response between unanesthetized animals and those lightly anesthetized with sodium pentobarbital. The average control rate of urine formation in 10 unanesthetized dogs was 0.11 ml/min and the maximum rate after 4 mg/kg of meralluride mercury intravenously was 3.10 ml/min. Tests on the same animals several days later under the same conditions except that they were lightly anesthetized with 25 mg/kg of sodium pentobarbital intravenously gave corresponding figures of 0.15 and 3.42 ml/min. Diuretic activity of the 3 compounds, tested at different intravenous dosage levels, was 3 or 4 times that of meralluride as measured by the maximum output of urine and sodium per minute. Table I shows the average response of 10 dogs for each dosage. The development of maximal diuresis was usually more delayed than with meralluride, but the duration of action was approximately the same, namely 5 or 6 hours.

2. Lang, E. P., and Nelson, K. W., *J. Assn. Official Agr. Chem.*, 1942, v25, 399.

TABLE I. Comparison of Average Maximal Rates of Excretion of Urine and Sodium of 10 Dogs for Each Dose.

Compound	Dose, mg Hg/kg	Avg control urine, ml/min	Avg max rate of excretion	
			Sodium, mEq/min	Urine, ml/min
Meralluride	2	.07	.43	2.43
	4	.10	.64	3.10
1353Ex	.5	.12	.32	2.28
	1	.17	.56	3.67
1347Ex	.5	.10	.34	2.12
	1	.14	.43	3.22
1431Ex	.5	.11	.48	2.84
	1	.13	.61	4.29

TABLE II. Recovery of Mercury in Urine and Feces After Intravenous Administration of Doses Equivalent to 2 mg per kg of Mercury.

Compound	Avg of 12 dogs per compound (%)			
	24 hr	48 hr	Total	Range
Meralluride	87	4	91	75-98
Mercaptomerin	65	11	76	52-90
1353Ex	80	3	83	63-96
1347Ex	82	3	85	54-94
1431Ex	88	2	90	49-97

Recovery of mercury. Mercury determinations on 24 and 48 hour specimens of urine and feces showed that most of the mercury, from intravenous doses of the compounds equivalent to 2 mg per kg of mercury, was excreted within 24 hours. The amount of mercury recovered from the feces was usually less than 4% of the total mercury excreted. Table II compares the rates of excretion of the new mercurials with meralluride and mercaptomerin (Thiomerin). The rates of excretion approached that of meralluride and appeared to be somewhat greater than the rate of excretion of mercaptomerin. This difference was especially apparent at the end of 24 hours.

Acute cardiac effects. Six dogs, weighing 10-13 kg, were used for evaluating the cardiac effects of each compound. Five dogs given 1431Ex showed no significant cardiac changes during the infusion period, but one animal developed ventricular fibrillation at the end of 2 hours. None of the dogs receiving 1353Ex showed any evidence of cardiac effects. The QRS interval increased in all dogs infused with 1347Ex, and 2 developed ventricular fibrillation. All animals given

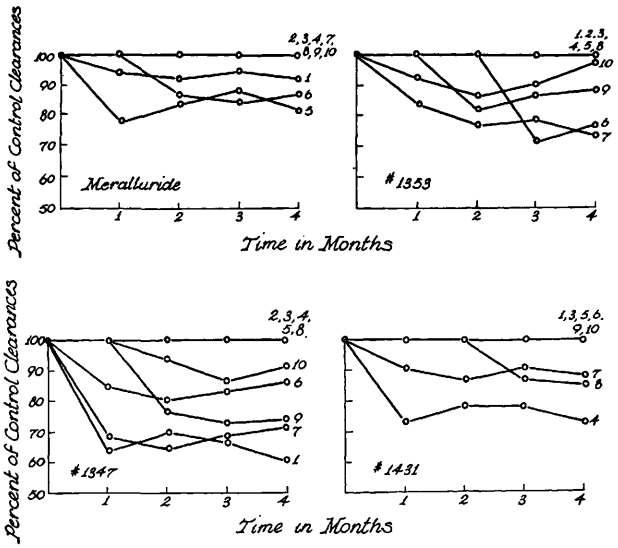

FIG. 1. Comparison of the effect of 3 mercurial diuretics with meralluride on glomerular filtration rate.

these large doses succumbed within 24-72 hours. The usual autopsy findings were pulmonary edema and gross hemorrhage of the gastro-intestinal tract.

Chronic toxicity. Chronic toxicity of the 3 compounds was compared to that of meralluride by determining changes in glomerular filtration rate at monthly intervals. The dogs were frequently examined for the appearance of dermatitis, and other symptoms of mercury poisoning. Ten dogs were used for each drug and were given an intravenous dose equivalent to 1 mg of mercury per kg 3 times weekly, except for the dogs in the meralluride group. Since meralluride is approximately one-fourth as active in producing diuresis in the dog as the other 3 compounds, it was administered in an intravenous dose equivalent to 4 mg of mercury per kg. These doses are several times the therapeutic level as used in man and were selected because they approach the maximum tolerated dose for the dog. Fig. 1 shows the effect of 4 months of such treatment on glomerular filtration rates. Each number in the graphs represents a curve for glomerular filtration rates of one dog determined at monthly intervals. The curves show that with these comparatively large and equivalent diuretic doses, there were no marked differences in renal toxicity among the three new compounds, or between the new agents and meralluride. Mild dermatitis developed in one dog in the meralluride group, 2 in the 1347Ex group, and one receiving 1353Ex. All animals developing dermatitis also showed a reduction in glomerular filtration rates. No diarrhea, stomatitis, or other symptoms of severe mercury poisoning that occur in dogs were noted in any of the animals.

Discussion. The greater diuretic activity of these compounds, considered in relation to the fact that they are excreted at rapid rates, should give them a safety margin equal to, or even greater than, mercurial diuretics currently used. Chronic toxicity data on dogs, receiving relatively large doses over a period of several months, indicate that there is little difference in renal toxicity, or other signs of mercury poisoning, when these agents are compared with an equivalent diuretic dose of meralluride. An extensive study has previously been made to show the changes in renal function associated with the development of chronic toxicity from several mercurial diuretics currently used therapeutically. The effects on glomerular filtration rate have been described(5) and the changes in renal tubular function, as measured by TmG and TmPAH, will soon appear in connection with

TABLE III. Comparison of Effect of Mercurial Diuretics on Glomerular Filtration Rate and Tubular Function.*

Dog No.	Wt, kg	Drug	Control GFR,† ml/min	Control TmG,† mg/min	Control GFR/TmG	1-4 mo of mercurial GFR,† ml/min	1-4 mo of mercurial TmG,† mg/min	1-4 mo of mercurial GFR/TmG
M-4	13	Meralluride	45	156	.29	22	87	.25
8	17	"	60	232	.26	45	170	.26
T-5	14	Mercaptomerin	55	207	.27	25	82	.31
7	19	1347Ex	67	240	.28	40	130	.31
1	17	"	83	275	.30	50	164	.30
4	12	1431Ex	53	169	.31	37	128	.29
M-2	15	Meralluride	64	208	.31	61	191	.32
3	17	"	76	273	.28	83	301	.28
1	15	1347Ex	58	215	.27	53	211	.25
2	16	"	54	203	.26	49	185	.27

* Arterial blood samples used for all analyses. Dogs unanesthetized.
† Average of 3 10-min periods.

a study of several types of agents having nephrotoxicity. The investigation included weekly determinations of the above renal functions in 20 dogs given daily injections of diuretics (4 mg/kg of mercury, I.V.) for one month. In addition, similar observations were made on 5 dogs given mercuric chloride (0.2 mg/kg I.V., daily). The data in Table III show representative examples taken from the previous study (M-4, -8, -2, -3 and T-5) and similar observations on 1347Ex and 1431Ex from the present investigation. The significant feature of the renal function studies is to demonstrate parallel changes in GFR and tubular function as may be seen by the constant GFR/TmG ratio. A similar correlation between GFR and TmPAH has been observed as nephrotoxicity develops from mercurial compounds. The logical conclusion to be derived from such data is that variable numbers of nephrons are completely inactivated as renal toxicity develops. De pressed renal functions develop either simultaneously with, but more frequently before, other signs of chronic mercury poisoning. In some animals, GFR and TmG returned to control values several weeks after discontinuing the drugs, while in others the damage appeared to be permanent. The GFR and TmG of dogs M-4 and T-5 in Table III had not improved significantly 2 months after discontinuing the drugs. Mercuric chloride produces similar chronic toxic effects as organic mercurial compounds, including the above mentioned renal changes. The last 4 dogs illustrate the absence of renal effects when no other signs or symptoms of chronic mercury toxicity were demonstrable.

Because of the proportional depression of GFR and tubular functions with the development of chronic toxic effects from mercurial diuretics, we have used GFR alone to indicate the development and progress of renal damage from these agents. Certain precautions must be observed if GFR is to be used as a measure of renal toxicity. Several hours of mercurial diuresis will frequently lower GFR and TmG considerably from dehydration(6). Recovery from extensive water loss is rapid, after the diuretic action of the drug disappears, and may be quickly eliminated by intravenous saline. All the data on renal functions reported were obtained on a day when no drug was given or prior to the administration of the diuretic. Routine hematocrit determinations also served to indicate the presence or absence of hemoconcentration.

Studies in this laboratory have indicated that doses of meralluride equivalent to 8 mg/kg of mercury, given intravenously to dogs, consistently produced electrocardiographic changes. In fact, 4 mg/kg is the minimum dose that may be administered without prolonging the QRS complex in some animals. The cardiac effects appeared

5. Handley, C. A., Sigafoos, R. B., Telford, J., and LaForge, M., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 201.

6. Handley, C. A., Sigafoos, R. B., and LaForge, M., *Am. J. Physiol.*, 1949, v159, 175.

promptly after a single injection of the above specified amounts of meralluride over a one minute period. Previous observations both in cats and dogs have yielded similar results(3,4).

Dogs tolerated large amounts of 1353Ex and 1431Ex without any indication of cardiac toxicity, which might have been anticipated since these two compounds are mercaptides (3). The chlorine substituted mercurial (1347Ex) appeared to have even less cardiac toxicity than meralluride, which is probably the least toxic of the non-mercaptide mercurials(3,4). No electrocardiographic changes occurred in any of the dogs given 1347Ex until the total amount infused was equivalent to 75 mg/kg of mercury and in 4 dogs, the QRS complex was not prolonged until about twice this amount of the compound had been given. These values are many times the amount of meralluride mercury required to produce similar electrocardiographic changes.

Summary. The compounds, 3-chloromercuri-2-methoxypropylurea (1347Ex), 3-carboxymethylmercaptomercuri - 2 - methoxypropylurea (1353Ex), and 3-(α-carboxyethylmercaptomercuri)- 2 -methoxypropylurea (1431-Ex), had 3 or 4 times the diuretic potency of meralluride in the dog. Twenty-four and 48 hour mercury excretion rates were comparable to that of meralluride at the same dosage level. Chronic toxicity studies indicated no essential difference between meralluride and the other compounds, if the greater diuretic potency of the new compounds was considered and the dosage adjusted accordingly. Large amounts of 1353Ex and 1431Ex were infused intravenously without disturbing cardiac function, but the acute cardiac toxicity of 1347Ex, although greater than the other 2 compounds, appeared to be less than meralluride.

Received July 23, 1951. P.S.E.B.M., 1951, v78.

3. Lehman, R. A., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 428.

4. Chapman, D., and Shaffer, C. F., *Arch. Int. Med.*, 1947, v79, 449.

Distribution of Phosphorus^{32} in the Embryo and Larva of the Frog.* (19096)

LOUIS A. HANSBOROUGH AND DAVID DENNY. (Introduced by A. H. Maloney.)

From the Department of Zoology, Howard University, Washington, D. C.

Radioactive substances have been employed in several studies on protein synthesis during development. Recently, Friedberg and Eakin (2) studied the incorporation of C^{14} of radioactive glycine into protein in the embryos and larvae of the Pacific tree-frog, *Hyla regilla*. They reported an increase in the uptake of glycine from the beginning gastrula through the early larval stages. This was interpreted as evidence of acceleration in protein synthesis accompanying differentiation and growth. Dent(1) observed the effects of phosphorus^{32} on regenerating amphibian tissues. He found that tadpoles of *Rana clamitans* immersed for 3 days in solutions of P^{32} (1000 microcuries) before amputation of the tail, regenerated a tail which was half as large as that of the control after 2 weeks. By introducing radioactive phosphorus into the hen's egg and analyzing chemical extracts of the tissues of the embryos, Hevesy, Levi and Rebbe(4) showed that the phosphatide molecule of the yolk was lower in specific activity than that of the embryo and con-

* This work was supported in part by a special grant from the Atomic Energy Commission under Contract No. AT(30-1)-949.

1. Dent, J. N., *Anat. Rec.*, 1949, v103, 439.

2. Friedberg, F., and Eakin, R. M., *J. Exp. Zool.*, 1949, v110, 33.

3. Hansborough, L. A., and Nicholas, P., *J. Exp. Zool.*, 1949, v112, 195.

4. Hevesy, G., Levi, H. B., and Rebbe, O., *Biochem. J.*, 1938, v32, 2147.

5. Holt, M. W., Cowing, R. P., and Warren, S., *Science*, 1949, v110, 326.