AG., Basle, and Burroughs Wellcome (Aust.) Ltd., for supplying lysergic acid diethylamide and ergometrine. The pharmacological testing of the LSD metabolites was carried out by Miss J. N. Pennefather, Pharmacology Department, University of Sydney.

The Fate and Excretion of Polythiazide[1] in the Dog

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Studies on the fate and excretion of polythiazide in the dog using doses in the range of 0.1 mg./kg. indicate that the compound is well absorbed. After either oral or intravenous administration, the drug is completely excreted within 5 days; 80–85% in the urine and 15–20% in the feces. The bulk (60–90%) is excreted in the first 24 hr. The time course of excretion of high doses (100 mg./kg.) is similar, a total of 63% being excreted over 5 days—35–45% in the urine and 20–30% in the feces. The polythiazide is excreted as a mixture of the unchanged drug with up to about 30% of a degradation product, 3-(methylsulfamyl)-4-amino-6-chlorobenzenesulfonamide (II). Another product of the formation of II, S-trifluoroethylthioglycolic acid, has been detected in the urine but the quantitative aspects of its formation and excretion have not been determined.

Several thiazide diuretics are currently in clinical use. Metabolic studies, however, have been reported only on the two simplest compounds, chlorothiazide and hydrochlorothiazide. In the species studied, dogs[2] and humans[3] in the case of chlorothiazide, and rats[4] and humans[5] for hydrochlorothiazide, both drugs proved to be metabolically stable and were completely excreted unchanged within 24 hr. following intravenous administration. Recoveries were somewhat less when the compounds were administered orally. Excretion

(1) Renese®.

(2) J. E. Baer, H. L. Leidy, A. V. Brooks and K. H. Beyer, *J. Pharmacol. Exptl. Therap.*, **125**, 295 (1959).

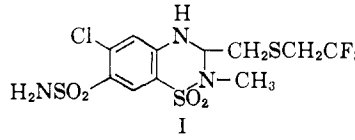
(3) H. R. Brettell, J. K. Aikawa and G. S. Gordon, *A.M.A. Arch. Internal Med.*, **106**, 57 (1960).

(4) H. Sheppard, T. F. Mowles, N. Bowen, A. A. Renzi and A. J. Plummer, *Toxicol. Appl. Pharmacol.*, **2**, 188 (1960).

(5) K. V. Anderson, H. R. Brettell and J. K. Aikawa, *A.M.A. Arch. Internal Med.*, **107**, 736 (1961).

of chlorothiazide in the bile has been demonstrated in nephrectomized dogs, and has been suggested for hydrochlorothiazide.

Polythiazide, 2-methyl-3,4-dihydro-3-(2,2,2-trifluoroethylthiomethyl)-6-chloro-7-sulfamyl-1,2,4-benzothiadiazine-1,1-dioxide (I) is a new diuretic of exceptional potency,[6] which has been shown to be useful in the management of a number of disease states.[7] This report is an account of a study of its fate and excretion in the dog.



I

Materials and Methods

Preparation of Materials: Polythiazide-2-C^{14}-methyl was prepared: Sodium hydride (92.8 mg., 2.0 mmoles) was added to a solution of 3,4-dihydro-6-chloro-7-sulfamyl-3-(2,2,2-trifluoroethylthiomethyl)-1,2,4-benzothiadiazine-1,1-dioxide[8] (860 .1 mg., 2.0 mmoles) in dry dimethylacetamide (20 ml.). An aliquot of the solution (2.0 ml., 0.2 mmole) was removed and transferred to a vacuum apparatus; methyl iodide-C^{14} (29 mg., 0.204 mmole) in dimethylacetamide (10 ml.) was then introduced dropwise with good stirring. Stirring was continued for 2 hr. At the end of this time, the solution was added dropwise to water (70 ml.) at 0°. Carrier polythiazide (14.2 mg.) was added to aid in crystallization. After 10 min., the turbid solution cleared; stirring was continued for 90 min. The product was recovered by filtration, washed with water and dried under vacuum at 80°. Yield was 41.4 mg. with a specific activity of 6.29 μc./mg. The radioscan of a paper chromatogram showed the desired product and some material identified as a product of dimethylation. Part of this mixture (19.3 mg.) was dissolved in acetone (0.1 ml.) and applied to Whatman No. 1 paper as a 15 cm. streak and the chromatogram developed in 3 passes in a chloroform–formamide system. A strip 6 mm. wide was removed from the paper and radio-scanned to determine the locations of the compounds. The polythiazide area was subsequently eluted; yield, 19.5 mg. (this includes a blank estimated to be about 1.8 mg.). The eluted material had a specific activity of 4.05 μc./mg. and appeared to be chemically and radiochemically homogeneous on paper chromatography.

4-Amino-6-chloro-3-(methylsulfamyl)benzenesulfonamide (II) has been described.[9,10]

4-Acetamido-6-chloro-3-(methylsulfamyl)benzenesulfonamide (III) was prepared by acetylation of II with a mixture of acetyl chloride and acetic anhydride.

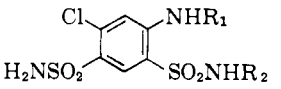
(6) A. Scriabine, B. Korol, B. Kondratas, M. Yu, S. Y. P'an and J. A. Schneider, *Proc. Soc. Exptl. Bio. Med.*, **107**, 864 (1961).

(7) R. V. Ford, *Curr. Therap. Res.*, **3**, 320 (1961).

(8) J. M. McManus, A. Scriabine and W. M. McLamore, *Federation Proc.*, **20**, 411 (1961).

(9) F. C. Novello, S. C. Bell, E. L. A. Abrams, C. Ziegler and J. M. Sprague, *J. Org. Chem.*, **25**, 970 (1960).

(10) W. J. Close, L. R. Swett, L. E. Brady, J. H. Short and M. Vernsten, *J. Am. Chem. Soc.*, **82**, 1132 (1960).



II $R_1 = H, R_2 = CH_3$
III $R_1 = COCH_3, R_2 = CH_3$
IV $R_1 = R_2 = H$

The acetyl derivative, recrystallized from aqueous methanol, had m.p. 215–217°.

S-Trifluoroethylthioglycolic Acid (VII).—Sodium methoxide (33.8 g.) was dissolved in methanol (200 ml.) with external cooling. Methyl thioglycolate (66.3 g.) was added, followed by trifluoroethyl iodide (131.8 g.). The solution was stirred under reflux for 3.5 hr., cooled, poured into water, and the alkaline mixture extracted with ether. Concentration of the ether extract, and subsequent distillation gave, after a small forerun (4.3 g.), *methyl S-trifluoroethylthioglycolate* (68.2 g.), b.p. 169–170°, $n^{24}D$ 1.4110.

Anal. Calcd. for $C_5H_7F_3O_2S$: C, 31.9; H, 3.7. Found: C, 32.3; H, 4.1.

A solution of sodium hydroxide (26.0 g.) in water (200 ml.) and methanol (50 ml.) was brought just to reflux, the apparatus being swept with nitrogen. After a 10 min. sweep at reflux, the above ester (66.7 g.) was added, and refluxing under a nitrogen atmosphere continued for a further 3.5 hr. The solution was poured into water and the alkaline solution extracted once with ether. The aqueous solution was acidified, and ether extraction and distillation gave *S-trifluoroethylthioglycolic acid* (29.8 g.), b.p. 89–92° (5 mm.), $n^{24}D$ 1.4251. *Anal.* Calcd. for $C_4H_5F_3O_2S$: C, 27.6; H, 2.9. Found: C, 27.9; H, 3.2.

Preparation of Polythiazide Solutions.—Although the compound is unstable to strong alkali, solutions of polythiazide can be prepared without measurable decomposition by rapidly dissolving 100 mg. in 1% sodium hydroxide solution (3 ml.) and diluting immediately with isotonic saline or water to a concentration of 0.2–0.5 mg./ml. Solutions prepared in this way have a final pH of 7–8 and are stable at room temperature for at least 24 hr.

Animals.—Mongrels or beagles of either sex were maintained in metabolism cages with free access to water and were fed the standard laboratory diet. The drug was administered as a solution either by stomach tube or by injection into the femoral vein. Urine and feces were collected from the cages, except that urine collections for periods of less than 24 hr. were usually made by catheterization.

Analytical Methods: Radiochemical.—All samples were assayed in duplicate by liquid scintillation counting and were corrected for counting efficiency by internal standards. Extraction recoveries were verified using biological materials to which known amounts of the compound had been added.

Urine.—Samples were assayed directly by dissolving 0.2 ml. of specimen in 15 ml. of a scintillator solution composed of 30% ethanol and 70% toluene and containing 0.3% diphenyloxazole and 0.01% *p*-bis-2-(5-phenyloxazolyl)benzene.

Feces.—The entire specimen was homogenized in 3 times its weight of water; potassium hydroxide solution (2 ml., 50%) was added to part of the homogenate (50 ml.) and the entire mass refluxed for 1 hr. This treatment converts any polythiazide present to II. The sample was then centrifuged, 5 ml. of the supernatant removed, and this aliquot acidified to pH 4–5 by the addition of 6 *N* hydrochloric acid followed by MacIlvaine buffer (pH 4, 10 ml.). The sample was then extracted with ethyl acetate (20 ml.), the organic phase washed with 0.2 *N* sodium hydroxide solution (10 ml.) and dried. An aliquot (2 ml.) was then evaporated to dryness and taken up in 15 ml. of toluene containing 0.3% diphenyloxazole for counting.

Chemical Assay.—The basic method is that of Baer, *et al.*,[2] suitably modified for polythiazide and related compounds in our laboratories. It involves hydrolysis of polythiazide to 4-amino-6-chloro-3-(methylsulfamyl)benzenesulfonamide (II) followed by diazotization and coupling of the free amino group to form a colored compound which is determined colorimetrically. For materials containing mixtures of both polythiazide and II the concentration of each can be determined by assaying first for II—omitting hydrolysis—and then for the total of the two compounds, including hydrolysis.

Reagents: A: pH 4 buffer; MacIlvaine solution (disodium phosphate-citric acid buffer). B: 0.1% sodium nitrite solution. C: 0.5% ammonium sulfamate solution. D: 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride solution. Reagents B, C, and D, should be stored in a refrigerator, and should be made up fresh each week or more frequently if reagent D turns brown.

Urine.—A 5 ml. sample of urine is pipetted into a 50 ml. centrifuge tube and the pH adjusted to 4–5 with dil. hydrochloric acid. Reagent A (4 ml.) and ethyl acetate (10 ml.) are added, shaken for 30 min. and centrifuged. An 8-ml. aliquot of acetate phase is transferred to a 10 ml. volumetric flask and evaporated to dryness in a stream of air. The residue is dissolved in 0.1 *N* sodium hydroxide and diluted to 10 ml. with water.

1. **For II.**—A 4 ml. aliquot of this solution is transferred to a 50 ml. centrifuge tube containing water (10 ml.) and 6 *N* hydrochloric acid (3 ml.). After mixing well, Reagent B (1 ml.) is added, the solution shaken for 1 min., and allowed to stand for 2 min. Reagent C (1 ml.) is added and the solution shaken for 1 min. Reagent D (1 ml.) is added, mixed well and read immediately in Beckman Model DU spectrophotometer at 510 mμ. The reference solvent is 0.1 *N* hydrochloric acid.

2. **For Polythiazide and II.**—A 4-ml. aliquot of the alkaline solution is transferred to a 50 ml. centrifuge tube containing 30% sodium hydroxide (5 ml.) and heated on a steam bath for 1 hr. The tube is cooled in an ice bath and 6 *N* hydrochloric acid (8 ml.) is added slowly. After mixing and cooling, Reagent B (1 ml.) is added and procedure 1 followed from this point.

Feces.—An ethyl acetate extract is prepared as described under Radiochemical Methods except that hydrolysis is carried out by heating the alkaline supernatant rather than the whole homogenate, and an 8 ml. aliquot assayed by procedure 1 of the method for urine. Note that all material in feces is thus converted by alkaline hydrolysis to II in the course of isolation.

Paper Chromatography: System I is a descending system on Whatman No. 4 paper treated with 40% formamide in methanol. The mobile phase is chloroform-ethyl acetate (3:1) saturated with formamide.

System II is a descending system on dry Whatman No. 4 paper. The mobile phase is chloroform–formic acid–95% (3A) ethanol (2:2:1). The components are mixed, allowed to stand 24 hr. and the lower phase used. Chromatographic data on polythiazide and related compounds are shown in Table I.

All compounds were detectable on the ultraviolet fluorescent screen scanner. Compounds having free aromatic amino groups (II and IV) also could be detected by spraying with nitrous acid and then 0.1% N-(1-naphthyl)-ethylenediamine in 95% 1-butanol.[11] The bicyclic compounds I and V react with this spray only after hydrolysis on the paper with a 1% sodium hydroxide spray followed by neu-

(11) W. H. Longenecker, *Anal. Chem.*, **21**, 1402 (1949).

TABLE I

CHROMATOGRAPHIC DATA ON POLYTHIAZIDE AND RELATED COMPOUNDS

Compound	System	Time, hr.	Approximate R_f
I	I	5	0.6
	II	1	0.9
II	I	15	0.3
	II	1	0.3
III	II	1	0.9
IV	I	15	0.05
	II	1	0.0
V	I	5	0.1
	II	1	0.6

tralization (1 *N* hydrochloric acid) and drying. The N-acetyl derivative III does not react even after treatment with the alkaline spray.

Vapor Phase Chromatography.—The urine sample, adjusted to pH 5 with hydrochloric acid, is shaken with half its volume of ethyl acetate for 45 min. The ethyl acetate layer is separated, and shaken with 1 *N* sodium hydroxide (5 ml.) for 30 min., and the aqueous phase is separated, acidified (pH 2) and shaken with ether (5 ml.) for 30 min. A sample of the ether solution is injected into a Burrell "Kromo-Tog" apparatus (column length 150 cm., diam. 4 mm., tristridecyl tricarballylate (20%) on "Fuoropak 80," temp. 150°, helium flow rate 55 ml./min.); under these conditions, S-trifluoroethylthioglycolic acid has a retention time of 11.5 min.

Results and Discussion

Although chlorothiazide and hydrochlorothiazide have been demonstrated to be metabolically stable, at least in the species studied, these compounds can both be degraded by alkaline hydrolysis to the same 4-amino-6-chlorobenzene-1,3-disulfonamide (IV). This reaction has been used as an initial step in chemical assay since it generates a free aromatic amino group which can be diazotized and coupled to form a colored substance.

As was anticipated, polythiazide undergoes the analogous reaction and, as also might be expected, undergoes it with much greater facility than the simpler thiazides. The ready formation of 4-amino-6-chloro-3-(methylsulfamyl)benzenesulfonamide (II) from polythiazide in either acidic or basic solution then becomes an important consideration in any approach to the metabolism of polythiazide. Approximate rates of hydrolysis of dilute aqueous solutions in 1 *N* hydrochloric acid, 0.5 *N* and 0.25 *N* sodium hydroxide, shown in Fig. 1, indicate that in the acid solution the drug is hydrolyzed to an extent of about 70% in 90 min. In the 0.5 *N* and 0.25 *N* sodium hydroxide, hydrolysis respectively is 50% and 20% complete in the

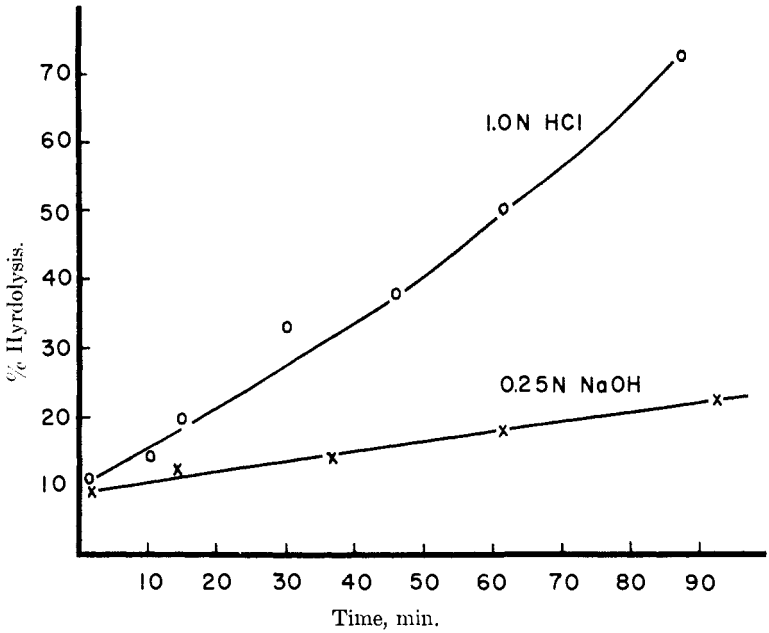


Fig. 1.—Hydrolysis of polythiazide in dilute aqueous solution.

same period. Figure 2 shows approximate rates of hydrolysis for solutions at various pH. Degradation, negligible at pH 7, amounts to 17% in 48 hr at pH 10. On the acid side, at pH 2 about 70% of the compound is hydrolyzed in 48 hr.

This easy degradation of polythiazide to II has several implications to the study of its metabolism. First, it appears highly likely that II will be found as a metabolite since at least small amounts of it could be expected to result from simple hydrolysis in body fluids even if the conversion were nonenzymatic. Second, this chemical lability can be turned to advantage in assaying biological material for both polythiazide and II (see Methods). Since the assay for polythiazide depends on its prior hydrolytic conversion to II the analysis will give the sum of both substances present. The concentration of II in the same sample is then obtained by direct assay without hydrolysis. It should be pointed out that this direct assay (without hydrolysis) of a solution which contains only polythiazide inevitably shows a small apparent concentration of II as an artifact of the assay, owing to hydrolysis in the strongly acid medium used for diazotization. This apparent amount is small (about 8%) and relatively constant, however, and appropriate correction can be made.

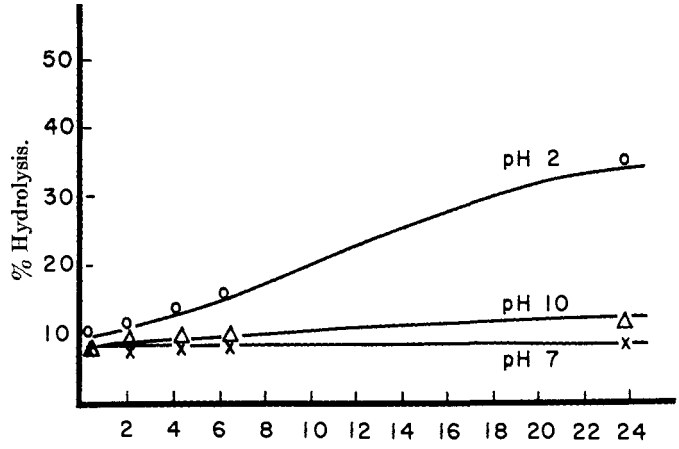
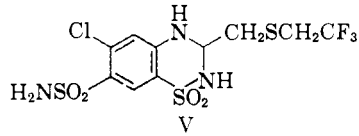


Fig. 2.—Hydrolysis of polythiazide at 37°.

Preliminary experiments with large doses of polythiazide in dogs indeed demonstrated the presence of II together with unchanged drug in the urine. Most significant to the experimental approach was the absence of any other compounds; especially the two products which would result from N-demethylation, IV and V. Reference samples of both were available and small concentrations of both were shown to be readily detectable in the chromatographic system used. N-Demethylation was considered to be a metabolic possibility requiring



V

careful scrutiny since the N-methyl groups of several methylsulfonamides are apparently readily cleaved *in vivo*. Maren,[12] for example, demonstrated that the administration to animals of the N-methyl derivative of acetazolamide, a compound devoid of carbonic anhydrase activity, produced a urine which inhibited carbonic anhydrase. Metabolic removal of the N-methyl substituent to give the potent carbonic anhydrase inhibitor acetazolamide was postulated as being responsible for the observed urinary activity. More recent studies have shown that in disulfonamidoanilines and sulfonamido-

(12) T. H. Maren, *J. Pharmacol. Exptl. Therap.*, **117**, 385 (1956).

1,2,4-benzothiadiazines,[13,14] alkylation of the free sulfonamide groups destroys all *in vitro* carbonic anhydrase inhibitory activity. However, these are apparently transformed, *in vivo*, to substances possessing activity, and an N-dealkylation step is offered in explanation. Work in our laboratories[15] has established that although the 2-methyl group in polythiazide is metabolically stable, this does not hold for certain of its congeners having N-methylsulfamyl groups in addition to the 2-methyl group. In these latter compounds, the additional N-methylsulfamyl group is smoothly removed *in vivo*, although the 2-methyl group is stable under all conditions. The fact that polythiazide was not similarly demethylated was important chiefly in that it made it possible to use the readily introduced N-methyl carbon atom as a C^{14} label without fear of loss of the radioactivity. The use of tracer techniques was, of course, essential for metabolic work at anything approaching therapeutic levels; the sensitivity of the chemical assay being adequate only for levels greatly in excess of those which produced the maximum diuretic effect.

The recovery of radioactivity following intravenous administration of polythiazide-2-C^{14} is shown in Table II. Recovery of administered activity was complete, 80–85% in the urine and 15–20% in the feces, entirely as a mixture of unchanged polythiazide and its previously mentioned metabolite, II. Exhaustive paper chromatographic examination of both urinary and fecal material failed to detect radioactivity associated with any other substance. The relative amounts of the two compounds as estimated from the relative areas of the radioscan of the chromatograms indicate that up to 30% of the administered polythiazide was excreted in the form of its metabolite, II. The bulk (60–90%) of the administered polythiazide was excreted during the first 24 hr., and excretion was complete after five days. The rise in activity excretion late in the experiment in the case of one dog remains unexplained.

Table III contains data from a similar experiment in which the polythiazide was administered orally. The fact that the excretion data, both with respect to rate and balance between urinary and fecal excretion, are essentially indistinguishable from those after intravenous administration indicates that oral absorption is complete. Polythiazide has a long duration of diuretic action (*ca.* 24 hr.), and from experiments using polythiazide-2-C^{14} in anesthetized dogs, evi-

(13) W. Kobinger, U. Katic and F. J. Lund, *Arch. Exptl. Pathol. u. Pharmakol.*, **240**, 469 (1961).

(14) F. J. Lund and W. Kobinger, *Acta Pharmacol. et Toxicol.*, **16**, 297 (1960).

(15) E. H. Wiseman, E. C. Schrieber and R. Pinson, Jr., *The Pharmacologist*, **3**, 81 (1961), and forthcoming publication.

TABLE II

EXCRETION OF RADIOACTIVITY BY THE DOG FOLLOWING ADMINISTRATION OF POLYTHIAZIDE-2-C^{14} (0.1 MG./KG.) INTRAVENOUSLY

Weight of dog, kg.	Period ending hr. post-dose	Specific activity, dpm./ml.	% Activity[a] in urine	% Activity[a] in feces	Total % excreted
8.5	6	10,700	23.2	—	23.2
	24	7,400	28.3	9.6	37.9
	48	3,600	15.9	6.1	22.0
	72	965	2.9	1.5	4.4
	96	310	0.9	0.2	1.1
	120	2,145	9.3	0.3	9.6
	144	0	0	0.2	0.2
	168	0	0	0	0.0
		Total	80.5	17.9	98.4
12.0	6	8,500	28.4	—	28.4
	24	9,000	33.6	11.2	44.8
	48	2,750	13.5	5.3	18.8
	72	620	1.9	0.8	2.7
	96	215	0.3	0	0.3
	120	330	1.0	0	1.0
	144	0	0	0	0
	168	0	0	0	0
		Total	78.7	17.3	96.0
16.0	6	9,415	26.8	—	
	24	10,680	47.3	16.7	90.8
	48	2,160	6.5	2.0	8.5
	72	451	0.9	0.8	1.7
		Total	81.5	19.5	101.0
13.9	6	9,630	19.3	—	
	24	8,945	41.6	11.2	72.1
	48	3,900	21.4	3.6	25.0
	72	693	2.6	0.1	2.7
		Total	84.9	14.9	99.8
		Average urine recovery		81.4%	
		Average fecal recovery		17.4%	
		Average total recovery		98.8%	

[a] Zeros are experimental values. Dashes indicate that no determination was made.

dence has been presented[16] that this is due to its low renal clearance, this being considerably lower than for other thiazide diuretics.

In addition to these experiments at dosage levels near the thera-

(16) A. Scriabine, E. C. Schreiber and M. Yu, *The Pharmacologist*, **3**, 58(1961).

TABLE III

EXCRETION OF RADIOACTIVITY BY THE DOG FOLLOWING ADMINISTRATION OF POLYTHIAZIDE-2-C^{14} (0.1 MG./KG.) ORALLY

Weight of dog, kg.	Period ending, hr.	Specific activity, dpm./ml.	% Activity in urine	% Activity in feces	Total % excreted
7.9	2	6790	8.0		8.0
	7	14750	15.1		15.1
	24	7540	33.3	15.4	48.7
	48	2415	13.7	4.8	18.5
	72	280	3.6	2.3	5.9
		Total	73.7	22.5	96.3
12.8[a]	2	3215	6.3		6.3
	6	10085	10.3		10.3
	24	9840	31.7	7.9	39.6
	48	3560	11.1	5.0	16.0
	72	625	3.5	1.1	4.6
	96	0	0		0
		Total	62.8	14.0	76.8

[a] Some material lost during administration.

peutic range, the disposition of very high doses (100 mg./kg.) was also studied. In these experiments, urinary and fecal concentrations were sufficiently high to be assayed by chemical methods. Excretion data following intravenous administration of 100 mg./kg. are summarized in Table IV. The fact that only about 63% of the dose was accounted for instead of the 100% obtained in the tracer experiments may be a reflection of the fact that the urine concentration on day 4 and 5 was approaching the lower limit of sensitivity of the analytical method. It is quite possible that the remaining material was excreted over a longer period at concentrations too low for detection.

The result of an analogous oral experiment is shown in Table V. Excretion of drug in this experiment was followed for only the first 48 hr., but for that period was comparable to that observed following intravenous administration and, it is reasonable to assume, followed the same course thereafter. In this experiment, the drug was administered in solution by stomach tube and was apparently well absorbed since only 6% of the dose was recovered from the feces in the 48 hr. period of observation.

None of these experiments gave evidence for the presence in urine or feces of any drug related compound except polythiazide and its degradation product II. It is not known whether the conversion of

TABLE IV

EXCRETION OF POLYTHIAZIDE AND 4-AMINO-6-CHLORO-3-(METHYLSULFAMYL)-BENZENESULFONAMIDE (II) BY THE DOG FOLLOWING ADMINISTRATION OF POLYTHIAZIDE (100 MG./KG) INTRAVENOUSLY

		Urine					
		II		Polythiazide			
Weight of dog, kg.	Day	Concn., μg./ml.	%	Concn., μg./ml.	%	Feces, %	Total %
10.2	1	55	3.6	430	19.0	19	41.6
	2	19	1.3	87	4.0	7	12.3
	3	0	0	29	1.3	2	3.3
	4			7	2.5	1	3.5
	5			7	2.3		2.3
	6			0	0		
	7			0	0		
		Total	4.9		29.1	29	63.0
12.7	1	56	5.0	390	24.0	20	49.0
	2	29	2.0	130	5.8	4	11.8
	3			32	1.8	0	1.8
	4			12	0.5		0.5
	5			7	0.3		0.3
	6			0	0		
	7			0	0		
		Total	7.0		32.4	24	63.4

polythiazide to II is an enzymatic process or a simple hydrolysis in body fluids. The compound II, the synthesis of which has been reported[9,10] had no diuretic effect at doses as high as 3.2 mg./kg. orally in the rat.[17]

The fact, brought out by the data on Table IV, that the urinary excretion of II ceases after 48 hr. while polythiazide excretion continues for 4 days leads to the conclusion that it is more rapidly eliminated than is the parent compound. Although II apparently was metabolically stable as judged by the absence of any radioactivity not associated with it or polythiazide in the urine of animals receiving the labeled drug, the excretion of II was studied by administering it intravenously to dogs (100 mg./kg.). Paper chromatographic examination of the urine and fecal extracts showed only II, with no evidence of either III from acetylation or IV from demethylation. Recoveries ranged from 50–80%, of which approximately 1% was in feces. The formation of III was considered as a possibility since N-acetylation is a known metabolic reaction of a number of *p*-aminobenzenesulfonamides. However, its absence was shown

(17) A. Scriabine, personal communication, 1960.

TABLE V

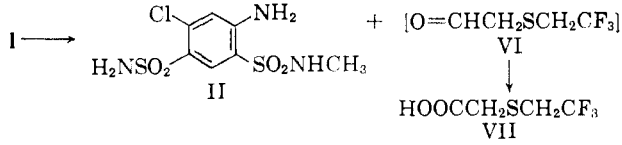
EXCRETION OF POLYTHIAZIDE AND 4-AMINO-6-CHLORO-3-(METHYLSULFAMYL)-BENZENESULFONAMIDE (II) BY THE DOG FOLLOWING ADMINISTRATION OF POLYTHIAZIDE (100 MG./KG.) ORALLY IN SOLUTION

		Urine					
		II		Polythiazide			
Weight of dog, kg.	Period, hr.	Concn., μg./ml.	%	Concn., μg./ml.	%	Feces, %	Total %
12.7	2	29	0.3	130	1.2		1.5
	4	39	0.4	190	2.0		2.4
	6	47	0.3	260	1.7		2.0
	24	49	1.6	180	5.5		7.1
	48	31	1.4	160	7.3	4.2[a]	12.9
		Total	4.0		17.7	4.2	25.9

[a] One stool passed in the 48-hr. period.

conclusively when an alkaline hydrolysis prior to assay failed to measurably increase the concentration of II.

The degradation of polythiazide to II necessarily produces an equimolar amount of a fragment derived from the trifluoroethylthiomethyl side chain since it seems highly unlikely that the side chain is removed piecemeal. On mechanistic grounds, it appeared most probable that either acid or base catalyzed hydrolysis yields first the aldehyde, VI, which has only a transitory existence before being oxidized to the corresponding acid VII.



That this pathway plays a part in the disposition of the side chain fragment was confirmed when the presence of VII in the urine of dogs receiving polythiazide was demonstrated by vapor phase chromatography of concentrated extracts of the acidified urine. The identity of VII was established both by the identity of its retention time using several different stationary phases and by the fact that it showed only a single peak after enrichment with an authentic sample. The possibility that VII might have been formed from polythiazide in the urine or as an artifact of the isolation procedure was ruled out by appropriate control experiments with pure polythiazide added to normal urine. The isolation was not quantitative, but the experiment does show that S-trifluoroethylthioglycolic acid (VII) is formed *in vivo* from that fraction of polythiazide which is degraded.

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Monoamine Oxidase Inhibitors. III. Structural Variations in 1-Alkyl and 1-Aralkyl-1(or 2)-acylhydrazines

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A number of sugar acyl, 4-hydroxybutyryl and D-pantoyl derivatives of benzylhydrazine, 4-dimethylaminobenzylhydrazine, and α-methylphenethylhydrazine were prepared by the reaction of the corresponding lactones with the substituted hydrazines. Most of these compounds had relatively low toxicity in mice as compared with other hydrazine compounds of comparable monoamine oxidase inhibitory activity. The reaction product of D-ribonolactone with benzylhydrazine was shown to be a N^1,N^2-hydrazine derivative. The most interesting compounds were 1-benzyl-2-(D-ribonoyl)hydrazine, and 1-benzyl-2-(D-pantoyl)hydrazine. Our variation in the structures of active monoamine oxidase inhibitors, such as 1-isopropyl-2-isonicotinoylhydrazine, 1-benzyl-2-(5-methyl-3-isoxazolylcarbonyl)-hydrazine, and 1-benzyl-2-picolinoylhydrazine, either eliminated or reduced MAO activity. In one case, 1-benzyl-2-picolinoylhydrazine, quaternization with methyl iodide or methyl bromide, increased response to 5-hydroxytryptophan.

Consideration of the clinical results obtained with isopropylisonicotinoyl-hydrazine[1] suggested modifications of this drug so as to obtain (a) a more rapid onset of action; (b) a lower toxicity; and (c) a more specific distribution of the drug in the different tissues. In previous papers,[2,3] we have described classes of compounds fulfilling, at least in part, the requirements (a) and (b), and in this paper are described some further compounds which have been investigated with respect to (c).

(1) Iproniazid (Marsilid®).
(2) T. S. Gardner, E. Wenis, and J. Lee, *J. Med. Pharm. Chem.*, **2**, 133 (1960).
(3) T. S. Gardner, E. Wenis, and J. Lee, *ibid.*, **3**, 241 (1961).