deposition activity in both Lederle and Upjohn Laboratories. Variations in potency relative to hydrocortisone can be reconciled by the formula: potency (Upjohn) = 1.3 (potency [Lederle])$^{1.4}$. Thus, results obtained in one laboratory become meaningful when compared to those in the other. Though the steroid potencies differ from laboratory to laboratory, the qualitative changes in activity induced by a given structural permutation are consistent in each laboratory.

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Renal Clearance of Polythiazide. (27677)

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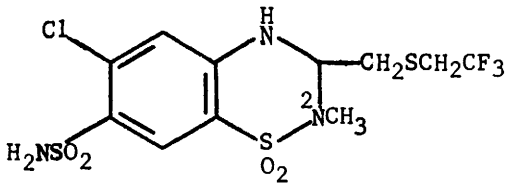
Polythiazide, 2-methyl-3-(β,β,β-trifluoroethyl-thiomethyl) - 6-chloro-7-sulfamyl-3,4-dihydro-1,2,4-benzothiadiazine, 1, 1-dioxide is one of the recently developed benzthiadiazine type diuretics(1,2,3). In rats and dogs this drug was found to be a highly potent diuretic and antihypertensive agent with prolonged duration of action. Clinical studies confirmed these observations(4,5,6). Further studies were recently undertaken to investigate the mechanism of renal excretion of polythiazide. The results of these studies are described here.



Methods. Polythiazide was labeled with C^{14} in 2 position as described by Pinson *et al.* (7). Renal clearance of C^{14} labeled polythiazide was studied in dogs anesthetized with sodium pentobarbital (25 mg/kg i.v.). The animals were infused with either 5% dextrose or 0.9% NaCl or 0.15M sodium bicarbonate at the rate of 0.4 ml/kg/min. The 5% dextrose and 0.9% NaCl solution was infused for an hour and one-half prior to the creatinine prime (0.2 g/kg, i.v. followed by creatinine infusion at 1.2 mg/kg/min). One hour later, 2 control creatinine clearance periods, 20 minutes each, were collected. C^{14} labeled polythiazide was then given intravenously followed by an infusion of solution containing drug. Two or more renal clearance periods of 20 minutes duration followed administration of the drug. The infusion of 0.15M sodium bicarbonate solution differs only from that of the 5% dextrose infusion in that a priming dose (10 ml/kg/min for 10 minutes) of 0.15M sodium bicarbonate was given at the beginning of experiment and was immediately followed by creatinine prime and infusion at the same dose (0.2 g/kg and 1.2 mg/kg/min respectively) and the same rate (0.4 ml/kg/min) as in experiments with dextrose solution. The concentration of C^{14} labeled polythiazide

in urine and plasma was measured with a liquid scintillation counter and corrected by means of an internal standard.

To study the plasma protein binding, blood samples were withdrawn from dogs 30 minutes after intravenous administration of either polythiazide or chlorothiazide. Plasma samples were obtained and dialyzed through cellulose dialysis tubing against a pH 7.4 phosphate buffer for 22 hours at 37°C. In experiments with C^{14} labeled polythiazide the drug concentrations were determined with a liquid scintillation counter. In experiments with non-labeled polythiazide, the drug concentrations were determined by a modification of the method of Baer *et al.*(8) as described by Pinson *et al.*(7). In another series of 4 *in vitro* experiments dog plasma inside the cellulose bag was dialyzed against a pH 7.4 phosphate buffer containing polythiazide (0.2 mg/ml).

The technic of stop-flow studies was essentially that described by Malvin *et al.*(9) and modified by Pitts *et al.*(10). Surgical and perimental procedures were the same as previously described(11). The labeled polythiazide was given i.v. at 0.1 mg/kg followed by an infusion at 0.15 mg/kg/hr. Twenty minutes after the primary dose 2 control renal clearance periods of 5 minutes duration were taken. The catheter from the left ureter was then clamped for 6 minutes. During the clamping period blood sample was taken from the jugular vein and at the end of clamping period 200 mg inulin was injected i.v. After release of the ureter clamp, 20 urine samples, 1.0 to 1.2 ml each, were collected in the succeeding 2½ to 3½ minutes. At the end of this procedure 2 control clearance periods, each of 5 minutes duration, were taken again.

Creatinine determinations were in accordance with the Folin method(12) and PAH was determined according to Smith *et al.*(13). Lipid solubility of polythiazide was estimated from partition coefficients in diethyl ether/buffer system. They were determined after equilibration of 25°C for 24 hrs. Citric acid-Na_2HPO_4 system was used; it was buffered at pH 7.4.

Results. Clearance of C^{14} Labeled Polythiazide. The renal clearance of labeled polythiazide was studied in 9 anesthetized dogs. The clearance of polythiazide was found to be considerably lower than creatinine clearance. The average ratio $\frac{\text{polythiazide clearance}}{\text{creatinine clearance}}$ of a total of 30 clearance periods in 9 experiments was 0.14 with a standard error of ± 0.01. With the increase in the dose of polythiazide the drug clearance decreased further (Table I).

Plasma protein binding. Plasma protein binding of polythiazide was found to be considerably greater than that of chlorothiazide. In 10 dogs which received 100 mg/kg of either chlorothiazide or polythiazide i.v., the average percent binding for polythiazide determined chemically was 85.3 with standard error of ± 2.3; the value for chlorothiazide was 39.4 ± 4.1. Plasma protein binding at lower drug concentrations, 0.25 μg/ml *in vivo* and 100 μg/ml *in vitro,* determined with C^{14} labeled polythiazide was between 79 and 83%.

Urine pH and polythiazide clearance. Urinary pH was dependent on the infusion fluid. Urine pH was lowest (6-7) during infusion with dextrose solution, almost neutral during infusion with NaCl solution and elevated during infusion with sodium bicarbonate solution. The polythiazide clearance was lower in dogs infused with dextrose and higher in animals infused with sodium bicarbonate solution. Attempts were made to increase the urine pH in the same experiment by changing infusion fluid. Slight increase in urine pH was usually accompanied by a slight increase in polythiazide clearance (Table II).

Urine flow and polythiazide clearance. In the experiments with labeled polythiazide changes in drug clearance appeared to be independent of the relatively small changes in urine flow.

Lipid solubility of polythiazide. The partition coefficients in diethyl ether/citric acid-Na_2HPO_4 buffer system for chlorothiazide, hydrochlorothiazide, trichlormethiazide, and polythiazide were estimated as 0.02, 0.40, 3.06, and 8.38 respectively.

Effect of probenecid on renal clearance of polythiazide. Probenecid (25 mg/kg i.v. prime followed by 35 mg/kg/hr infusion)

TABLE I. Renal Clearance of C^{14} Polythiazide in Female Dogs Anesthetized with Sodium Pentobarbital, 25 mg/kg i.v. Infusion rate at 0.4 ml/kg/min. Clearance periods of 20 min duration. In all experiments 5% dextrose solution was infused except Exp. 3, where 0.9% NaCl solution was used.

	Urine flow, ml/min	Creatinine clearance, ml/min	Drug conc., μg/ml Plasma	Drug conc., μg/ml Urine	Drug clearance, ml/min	Drug clearance / Creatinine clearance
Exp. 1; dog, 18.7 kg; polythiazide, .1 mg/kg prime followed	4.0	70	.24	.34	5.7	.08
by .15 mg/kg/hr infusion	4.0	59	.31	.69	8.9	.2
	2.5	52	.38	1.20	7.9	.2
Exp. 2; dog, 10.1 kg; polythiazide, 10 mg/kg prime followed	1.7	35	38.8	15.9	.68	.02
by 15 mg/kg/hr infusion	1.3	24	43.1	19.5	.60	.03
	1.5	26	50.9	27.9	.80	.03
Exp. 3; dog, 22 kg; polythiazide, .1 mg/kg prime followed	8.7	66	.21	.34	14.1	.21
by .15 mg/kg/hr infusion	6.3	61	.28	.87	19.4	.32
	9.7	106	.34	1.20	28.5	.27
Exp. 4; dog, 17.0 kg; polythiazide, .1 mg/kg prime and	7.9	138	.53	.52	7.7	.06
.15 mg/kg/hr infusion	8.1	103	.54	.74	11.0	.11
	7.7	103	.54	.89	12.7	.12
Exp. 5; dog, 14.5 kg; polythiazide, .1 mg/kg prime and	2.1	28	.25	.32	2.6	.09
.15 mg/kg/hr infusion	2.1	31	.34	.46	2.9	.09
	2.2	29	.37	.58	3.4	.12
Exp. 6; dog, 15.7 kg; polythiazide, 10 mg/kg prime and	9.1	60	36.1	8.5	2.2	.04
15 mg/kg/hr infusion	7.9	52	40.5	20.1	3.9	.08
	7.7	55	54.1	30.3	4.3	.08

sharply reduced the renal clearance of polythiazide to well below 50% of control values. A typical experiment is shown in Table II.

Stop-flow experiments with the labeled polythiazide. In an attempt to define further the renal excretion mechanism of polythiazide, 3 stop-flow experiments with labeled polythiazide were performed. All 3 experiments were consistent in indicating decreases in $\frac{\text{u/p drug}}{\text{u/p creatinine}}$ ratio to well below free flow values. This was most marked in the area corresponding to the upper part of the distal tubule. The stop-flow pattern in the region characteristically associated with secretion of organic acids suggested a small secretory peak (Fig. 1).

Discussion. In comparison with other benzthiadiazines the renal clearance of polythiazide was found to be considerably lower. The ratio $\frac{\text{drug clearance}}{\text{creatinine clearance}}$ for chlorothiazide was reported to be equal to 3.0 and for hydrochlorothiazide 4.0(14). Our average value for polythiazide clearance is 0.14 with standard error of ± 0.01. The previously described prolonged duration of polythiazide

TABLE II. Effects of Changes of Infusion Fluid from Dextrose to Sodium Bicarbonate and of Probenecid on Renal Clearance of C^{14} Polythiazide.

Urine flow, ml/min	Urine pH	Creatinine clearance, ml/min	Drug concentration, μg/ml Plasma	Drug concentration, μg/ml Urine	Drug clearance, ml/min	Drug clearance / Creatinine clearance
Dog, female, 11.6 kg; polythiazide, .1 mg/kg prime followed by .15 mg/kg/hr infusion in 5% dextrose solution						
2.3	7.22	37	.24	.48	4.5	.12
2.2		38	.31	.93	6.4	.17
Dextrose replaced by .15M sodium bicarbonate solution after prime infusion of sodium bicarbonate at 10 ml/kg/min for 10 min. Drug continued.						
4.6	7.9	42	.40	.78	8.9	.21
4.5		43	.44	1.07	10.9	.26
Probenecid, 25 mg/kg prime followed by 35 mg/kg/hr infusion. Sodium bicarbonate and polythiazide continued.						
5.4	7.8	48	.56	.43	4.1	.09
5.8		43	.68	.18	1.5	.04

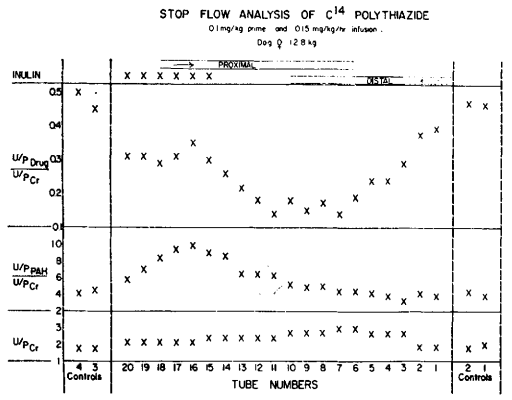


FIG. 1. Stop-flow analysis of C^{14} labeled polythiazide, 0.1 mg/kg prime and 0.15 mg/kg/hr infusion. Female dog, 12.8 kg. Sodium pentobarbital anesthesia, 25 mg/kg i.v. Symbols for inulin represent presence of inulin in the samples.

action(11) can be explained by its low renal clearance.

These studies indicated further that approximately 85% of polythiazide is bound to plasma proteins and that only about 15% is, therefore, available for filtration at the glomerulus. The protein binding of polythiazide is, however, not the only factor determining its low renal clearance. Differences in physicochemical properties of benzthiadiazines may greatly influence their tubular reabsorption. Unlike chlorothiazide which behaves as a dibasic acid with pK values of 6.7 and 9.5, polythiazide has only one pK value which is higher than 11. It exists, therefore, largely in the undissociated form within the pH range encountered in the renal tubule. In addition, studies in our laboratories indicate that the lipid solubility of polythiazide is very much greater than that of chlorothiazide or hydrochlorothiazide. Both of these properties would favor the relatively greater reabsorption and therefore lower clearance of polythiazide.

Tubular secretion of probenecid was demonstrated by Weiner *et al.*(15). The ability of probenecid to reduce the clearance of polythiazide may, therefore, indicate that polythiazide is, like other benzthiadiazines, secreted by the same mechanism as probenecid. The results of the stop-flow experiments also suggest secretion in the proximal segment as well as considerable reabsorption distal to this.

Summary. The renal clearance of C^{14} labeled polythiazide, a new and highly potent diuretic agent, was studied in anesthetized dogs. The $\frac{\text{drug clearance}}{\text{creatinine clearance}}$ ratio for polythiazide was found to be considerably lower than for other benzthiadiazines. The low clearance of polythiazide appears to be determined by the high degree of drug binding by plasma proteins, and by extensive reabsorption in the distal segment. The experimental data indicate that polythiazide is also secreted by the renal tubule.

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