of sialic acid and hexosamine(6), so that the genetically controlled differences may involve either amino acids or carbohydrates. The nature of the multiplicity of transferrin from cows homozygous at the transferrin locus also remains an unsolved problem. It is possible that the bands are composed of the same protein with different numbers of sialic acid moieties attached. It has been found that treatment of human transferrin with neuraminidase results in the appearance of 5 bands whose intensity varies with the concentration of the enzyme(18,19). Direct evidence bearing on these questions, however, must await further purification of these proteins.

Summary. A ratio of 3 volumes 0.4% rivanol to one volume serum was used to partially purify cattle transferrin. After treatment, the supernatant fraction contained predominantly transferrin and γ-globulin. No albumin was detectable by starch gel or paper electrophoresis. Fractionation of serum from cattle of phenotypically different transferrin types yielded transferrin with the same pattern and mobility in starch gel electrophoresis as in the original serum.

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Pharmacological Studies with Polythiazide, a New Diuretic and Antihypertensive Agent.* (26780)

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The search for orally effective and safer diuretic agents led to the introduction of benzthiadiazines as a new class of diuretics (1,2).

Recently attempts have been made to develop more potent benzthiadiazines with a

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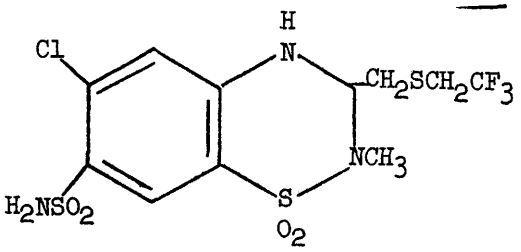
greater specificity in their natriuretic action, lesser effect on excretion of potassium or on glomerular filtration rate. Such efforts culminated recently in synthesis and development of polythiazide, 2-methyl-3-(β,β,β-trifluoroethyl - thiomethyl) - 6 - chloro - 7 - sulfamyl-3,4-dihydro-1,2,4 benzothiadizine, 1, 1 dioxide.



Material and methods. Polythiazide occurs as a white crystalline substance with a molecular weight of 439.9 and a melting point 214-215°C. The compound is insoluble in water but dissolves readily in alkaline solution.

To study the diuretic activity of polythiazide in rats the method of Lipschitz *et al.*(3) was used. Each animal was placed in a separate metabolism cage for a 5-hr period. Concentrations of Na^+, K^+ and Cl^- were determined in each collected urine sample. Results were expressed as $\frac{T\text{-}C}{5\ hr}$ where T is urine volume or electrolyte concentration in the urine sample collected from a treated animal and C is average control value for 8 animals. A total of 112 rats was used in this study.

The saluretic activity of polythiazide was evaluated in 8 trained female dogs during water diuresis. To produce water diuresis the animals received orally a prime of 40 ml of water per kg of body weight and 20 ml/kg hourly thereafter. The dogs were trained to remain without anesthesia or restraint on trough shaped boards for 6-7 hours. Urine was collected by retention catheter continuously at hourly or at 10-30 minute intervals. Four experiments were made at each dose level of each drug. Polythiazide was given orally or intravenously. The increase in sodium or chloride concentrations in the urine was used to measure the saluretic effectiveness of the drug.

To study the duration of action of polythiazide over a 24 hour period 6 female mongrel dogs were deprived of food and water during the night prior to and throughout the experimental period. The animals received drugs orally. Urine samples were collected by repeated catheterizations. The effects of the diuretics were expressed as T-C, where T is average urine volume or amount of electrolytes excreted by 6 dogs after treatment and C is average control value for the same dogs.

Renal clearance experiments were performed on dogs lightly anesthetized with sodium pentobarbital, 25 mg/kg I.V. Isotonic dextrose or NaCl solutions were infused at the rate of 0.4 ml/min/kg for 2½ hours prior to and during the experiment.

Experimental metabolic acidosis was produced in 4 female mongrel dogs by dietary supplement of ammonium chloride, 0.5 g/kg/day for 5 days prior to the test. On the test day 5% dextrose solution was infused at 0.4 ml/min/kg for 2½ hours prior to and throughout the experiment. Experimental metabolic alkalosis was produced in female mongrel dogs anesthetized with sodium pentobarbital (25 mg/kg I.V.) by infusion of a solution containing 0.075M $NaHCO_3$ and 2½% dextrose (1 ml/kg/min for 10 minutes and 0.4 ml/min/kg for one hour).

Sodium and potassium in urine and plasma were determined with the Process Instruments flamephotometer. Urinary chlorides were determined by a method of Sendroy(4) and chlorides in plasma by the method of Schales and Schales(5). Determinations of total CO_2 in urine and plasma and calculation of bicarbonate concentration were done according to Peters and Van Slyke(6). Determinations of creatinine were made by the Folin method(7) and PAH was determined according to Smith *et al.*(8). Determinations of ammonium concentration in urine were done by the Conway method(9).

The changing pH method of Philpot and Philpot(10) was employed for estimation of carbonic anhydrase inhibitory activity as previously described(11).

To study the effect of chronic administration of polythiazide on blood pressure of hypertensive dogs systolic and diastolic pres-

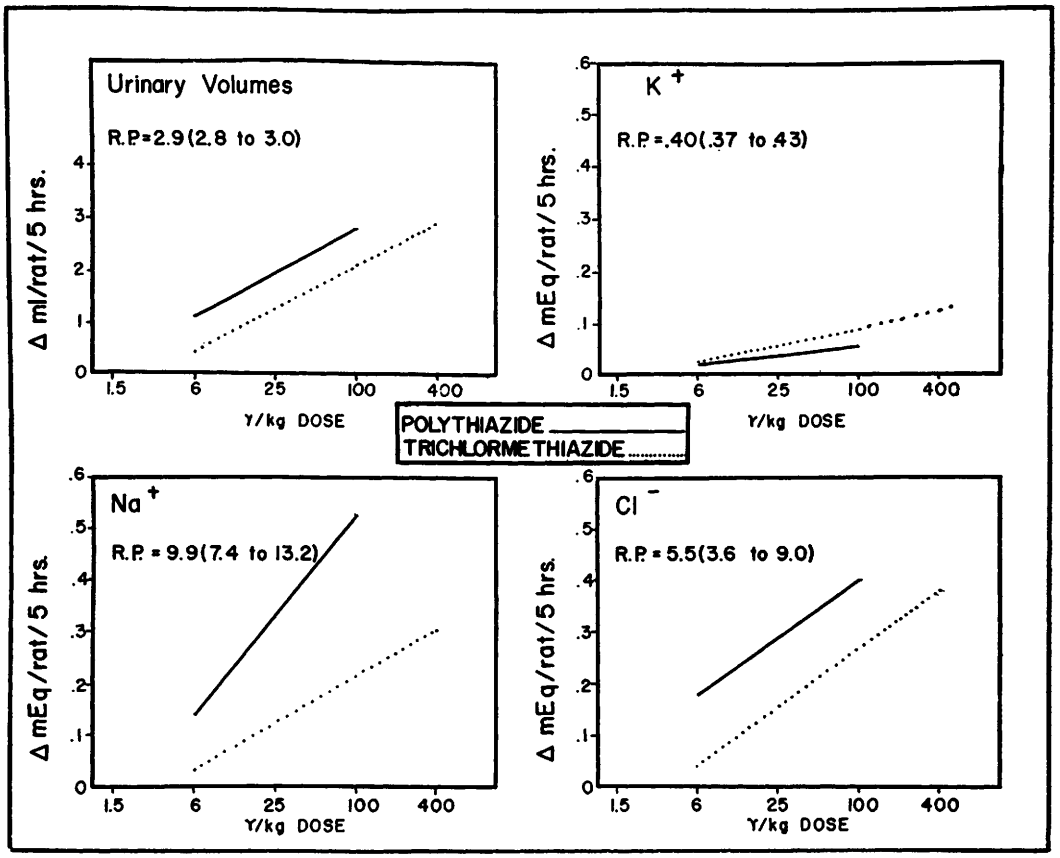


FIG. 1. Dose-response regression lines for polythiazide and trichlormethiazide orally in rats. Effects on urinary flow, Na^+, K^+ and Cl^- excretion are expressed as differences in excretion between an animal under test and an avg value for 8 control rats. Regression lines were calculated on the basis of 56 observations for polythiazide and 72 observations for trichlormethiazide. R.P. = Relative potencies with 95% confidence limits. Potency of trichlormethiazide is considered equal to 1.

sures were measured in 8 hypertensive unanesthetized dogs by the method of Prioli and Winbury(12). Three types of hypertensive dogs were used: (a) 4 renal hypertensive dogs prepared by the method of Goldblatt *et al.*(13); (b) 2 neurogenic hypertensive dogs prepared by the method of Grimson (14); and (c) 2 dogs exhibiting a profound and sustained spontaneous hypertension. The dogs were randomly divided into 2 groups of 4 dogs per group. One group received daily oral dose of 400 μg/kg of polythiazide (by stomach tube) for 5 days; the other group served as controls. Systolic and diastolic arterial pressure measurements and heart rate determinations were made twice daily over the experimental period.

The antihypertensive activity of polythiazide was also evaluated in renal hypertensive rats prepared by the method described by Grollman(15) and treated with oral doses of 50, 100, 200 and 400 μg/kg, twice a day over a 3 or 3½ day period. Eight rats were used at each dose level. One group served as the solvent control. The drug solutions were prepared fresh daily. Blood pressure measurements in these animals were made without anesthesia by the oximetric technic(16) each morning prior to and 4 hours after drug administration.

Results. Diuretic and saluretic effects in rats. In rats polythiazide by oral administration was 2.9, 9.9, 5.5 and 0.4 times more potent than trichlormethiazide on the basis of the increase in urine volumes, Na^+, Cl^-, K^+ excretion, respectively (Fig. 1). The 95%

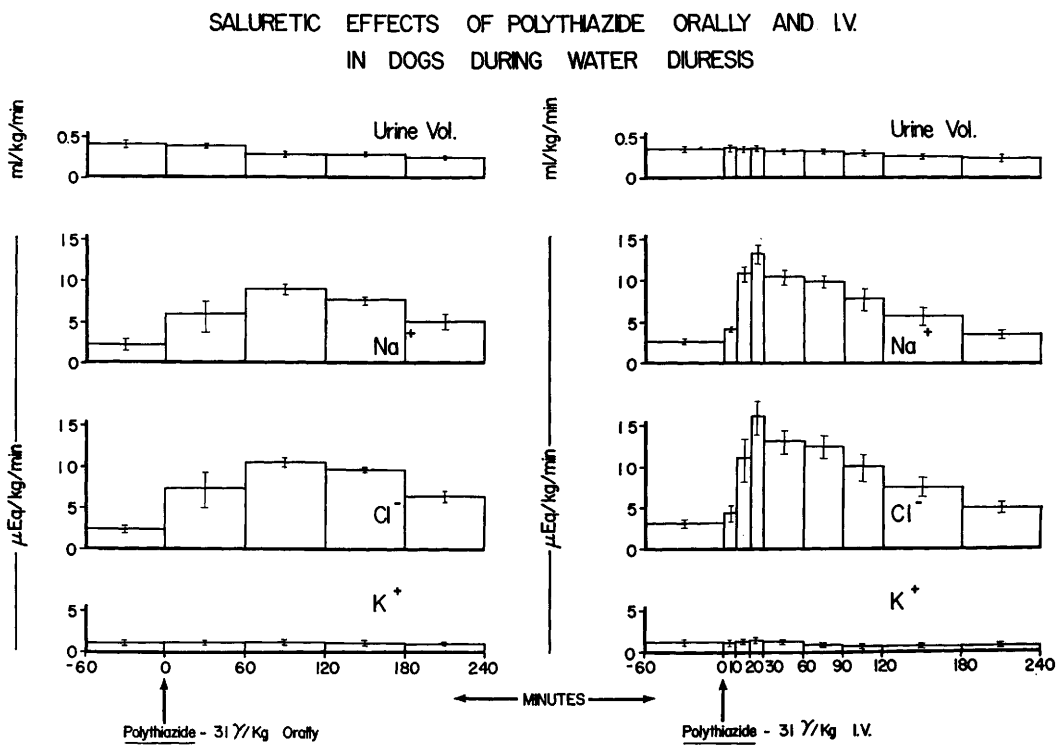


FIG. 2. Saluretic effects of polythiazide 31 μg/kg by oral and intrav. administration to trained dogs during water diuresis. Avg values for 4 dogs. Brackets indicate stand. errors.

confidence limits of the relative potency indicated the statistical significance of the differences in potency. On the basis of K^+ excretion polythiazide was less potent than trichlormethiazide whereas on the basis of Na^+ excretion it was considerably more potent.

Comparison of dose-response regression lines for polythiazide with those for benzthiazide and chlorothiazide(11) revealed that polythiazide is about 40.0 times as potent as benzthiazide or 400-600 times as potent as chlorothiazide in its natriuretic and chloruretic effects.

Saluretic effects in dogs during water diuresis. Under the conditions of water diuresis urine volumes and K^+ excretion are usually not appreciably influenced by benzthiadiazines and the effectiveness of these drugs can be evaluated only on the basis of increase in excretion of sodium and chloride. Polythiazide produced a pronounced increase in excretion of both. This increase became apparent in the first hour after oral and 20 minutes after intravenous administration of the drug (Fig. 2). In control experiments there was no increase in excretion of electrolytes during the same period.

Duration of action studies. The duration of diuretic and saluretic effects of polythiazide and trichlormethiazide is compared in Fig. 3. The difference in duration of action of polythiazide and trichlormethiazide (each at 125 μg/kg orally) became apparent in 6-24 hours after oral administration of drugs. During that period trichlormethiazide produced a rebound phenomenon—*i.e.*, a decrease in urinary excretion of Na^+ and Cl^- when polythiazide effect was still evident. The long duration of polythiazide action was observed at 31 μg/kg dose level as well as at 125 μg/kg.

Renal clearance experiments. a. *During infusion of 5% dextrose solution.* Polythiazide is an effective natriuretic agent under conditions of these experiments (Table I). Intravenous injection of 50 μg/kg followed by infusion at the rate of 75 μg/kg/hr produced a 6-8 fold increase in rate of Na^+ and Cl^- excretion. This increase was much greater than the increase in urine volumes so

TABLE I. Effect of Polythiazide i.v. on Renal Function and Electrolyte Excretion in Dogs during Infusion of Isotonic Dextrose Solution.

Avg values for 4 female dogs
Avg body wt, 17.2 kg
Anesthesia: Sodium pentobarbital, 25 mg/kg i.v.

Period	Duration, min.	Urine flow, ml/min.	Glomerular filtration rate, ml/min.	Renal plasma flow, ml/min.	Plasma conc., meq/l Na⁺	K⁺	Cl⁻	HCO_3^-	pH Urine	pH Plasma	Rate of excretion, μeq/min. Na⁺	K⁺	Cl⁻	HCO_3^-	NH_4^+
					Polythiazide, 50 γ/kg i.v. followed by 75 γ/kg/hr infusion										
1	20	4.1	54	144	132	2.8	98	16	6.05	7.34	42	20	38	4	17
2	20	4.0	51	135	131	2.5	99	15	5.97	7.35	43	19	43	5	19
					Polythiazide, 50 γ/kg i.v. followed by 75 γ/kg/hr infusion										
3	20	4.5	51	135	128	2.4	96	16	6.01	7.34	225	36	256	6	18
4	20	4.7	50	124	131	2.3	96	15	5.89	7.35	280	38	289	4	19

that concentrations of Na^+ and Cl^- in the urine increased greatly. Excretion of K^+ was also increased. The increase was less than 1/10 of that of sodium excretion. There was no effect on excretion of bicarbonate, ammonium or on urinary pH. Polythiazide had no effect on glomerular filtration rate (creatinine clearance). Slight secondary decrease in renal plasma flow (clearance of sodium p-aminohippurate) was observed during the second clearance period after treatment. It is of questionable significance and may not be related to drug action.

b. *During infusion of isotonic NaCl solution.* Table II summarizes 4 experiments in which 10 μg/kg of polythiazide was given intravenously followed by infusion at 15 μg/kg/hr. In these experiments the rate of Na^+ excretion prior to the drug was relatively high (400-500 μEq/min) and the saluretic effects of polythiazide were less pronounced. The average increase in rate of Na^+ excretion was 143 μEq/min; that of chloride, 153 μEq/min. There was only a slight and questionable increase in urinary pH and in rates of bicarbonate, potassium and ammonium excretion. In these experiments polythiazide produced a slight increase in glomerular filtration rate and renal plasma flow. Plasma electrolytes and plasma pH were not affected by the drug. At dose levels as high as 10 mg/kg i.v. the effects of polythiazide under

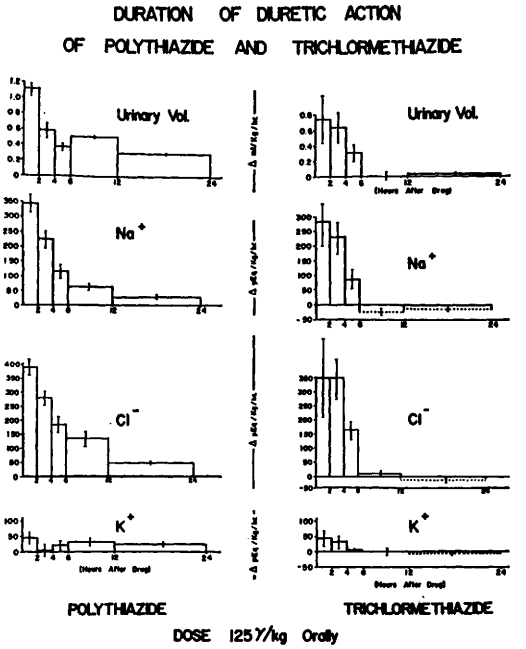


FIG. 3. Effects of polythiazide and trichlormethiazide each at 125 μg/kg orally on urinary volumes, Na^+, K^+ and Cl^- excretion over a 24-hr period in dogs deprived of food and water. Effects are expressed as differences in excretion after drug and control values for the same dogs. Avg values for 6 animals. Vertical lines represent stand. errors. Note long duration of polythiazide action.

TABLE II. Effect of Polythiazide i.v. on Renal Function and Electrolyte Excretion in Dogs during Infusion of Isotonic NaCl Solution.

Avg values for 5 female dogs
Avg body wt, 16.2 kg
Anesthesia: Sodium pentobarbital, 25 mg/kg i.v.

Period	Duration, min.	Urine flow, ml/min.	Glomerular filtration rate, ml/min.	Renal plasma flow, ml/min.	Plasma conc., meq/l				pH		Rate of excretion, μeq/min.				
					Na^+	K^+	Cl^-	HCO_3^-	Urine	Plasma	Na^+	K^+	Cl^-	HCO_3^-	NH_4^+
Avg of 1–2	20	2.7	52	188	142	3.4	108	20	7.12	7.33	486	60	466	51	8
Polythiazide, 10 γ/kg i.v. followed by 25 γ/kg/hr infusion															
3	20	3.3	60	212	141	3.4	102	—	7.26	7.31	629	73	619	—	9
4	20	2.9	55	205	146	3.2	101	19	7.20	7.34	580	61	550	60	10

same experimental conditions were not greatly different (Table III). At 10 mg/kg the natriuretic and chloruretic effects of polythiazide were not considerably greater than at lower dose levels. Even at such high doses, the effects of polythiazide on rates of potassium and bicarbonate excretion were not pronounced. The glomerular filtration rate in these experiments was not depressed even by massive doses of polythiazide.

Experimental metabolic acidosis and alkalosis. In 3 dogs with experimental acidosis, 0.4 mg/kg of polythiazide followed by infusion at 0.6 mg/kg/hr produced a pronounced increase in excretion rate of sodium and chloride, slight increase in excretion of potassium, and no effect on rate of ammonium excretion. There was a slight and questionable decrease in urinary pH and in rate of bicarbonate excretion. A typical experiment on an acidotic dog is presented in Table IV.

In dogs with experimental alkalosis, polythiazide at 0.4 mg/kg followed by infusion at 0.6 mg/kg/hr produced a pronounced increase in rate of Na^+ and Cl^- excretion. There was a slight increase in excretion rate of K^+. Under conditions of metabolic alkalosis polythiazide produced a considerable increase in rate of bicarbonate excretion (Table V).

Carbonic anhydrase inhibitory activity in vitro. The *in vitro* molar concentration of polythiazide required for 50% inhibition of carbonic anhydrase was estimated as 5×10^{-7}M and that for chlorothiazide 1×10^{-6}M.

Cardiovascular effects of polythiazide. Anesthetized normotensive dogs. In normotensive anesthetized dogs, polythiazide was tested at dose levels of 1 to 50 mg/kg, intravenously. The animals were observed for at least 4 hours following drug administration. There were no significant changes in blood pressure or heart rate which could be attributed to the drug action. Slight potentiation of the pressor response of adrenaline was noted occasionally.

Unanesthetized hypertensive dogs. In 4 dogs with experimental or spontaneous hypertension, daily oral administration of polythiazide (400 μg/kg/day for 5 days) caused decrease in both systolic and diastolic pressure, the maximum effect seemed to appear on the fifth day of treatment (Fig. 4), when the decrease in systolic and diastolic pressure was significantly ($P<0.05$) lower in the treated than in the control group of 4 other hypertensive dogs. Heart rate recorded concomitantly with arterial pressure deter-

TABLE III. Effect of Polythiazide on Glomerular Filtration Rate and Electrolyte Excretion during Infusion of Isotonic NaCl Solution.

Dog ♀, 11.4 kg
Anesthesia: Sodium pentobarbital, 25 mg/kg i.v.

Period	Duration, min.	Urine flow, ml/min.	Glomerular filtration rate, ml/min.	Plasma conc., meq/l				pH		Rate of excretion, μeq/min.			
				Na^+	K^+	Cl^-	HCO_3^-	Urine	Plasma	Na^+	K^+	Cl^-	HCO_3^-
1	20	1.0	28	145	3.9	120	25	7.12	7.17	149	21	158	29
2	20	.9	28	151	3.8	118	23	7.19	7.27	145	20	146	27
			Polythiazide, .1 mg/kg prime followed by .1 mg/kg/hr infusion										
3	20	1.8	—	150	3.9	120	—	7.20	7.26	340	50	305	—
4	20	1.4	33	151	3.7	120	22	7.20	7.24	217	32	228	39
			Polythiazide, 1 mg/kg prime followed by 1 mg/kg/hr infusion										
5	20	1.8	—	145	4.0	120	—	7.30	7.27	331	48	305	—
6	20	1.6	33	151	3.7	122	21	7.20	7.28	390	39	267	51
			Polythiazide, 10 mg/kg prime followed by 10 mg/kg/hr infusion										
7	20	2.4	—	146	3.5	120	—	7.30	7.25	463	62	407	—
8	20	2.0	34	152	3.8	120	20	7.40	7.25	374	56	345	75

minations was not significantly changed by the polythiazide treatment.

Unanesthetized hypertensive rats. Daily oral administration of 100 μg/kg, or more, of polythiazide produced a statistically significant ($P<0.05$) lowering of mean arterial blood pressure in hypertensive rats (Fig. 5). The antihypertensive effect did not become apparent until the second or third days of treatment.

Discussion. Our studies in rats and dogs on the pharmacological activities of polythiazide indicated that this compound is a potent, orally active diuretic and antihypertensive agent. The long duration of action, increase in excretion of sodium and chloride in equivalent amounts, effectiveness in metabolic acidosis and alkalosis characterized the diuretic activity of polythiazide. Almost equal activity by oral and intravenous administration indicated complete absorption. The potency of polythiazide was estimated on the basis of a 5 hour urine collection in rats. Because of the prolonged duration of polythiazide action its actual potency is probably considerably higher than these data indicated. But even on the basis of a 5 hour observation period polythiazide is one of the most potent diuretics known. The exceptionally long duration of polythiazide action shown in this study was confirmed in recent clinical investigation(17). Polythiazide was found to be effective in man for as long as 48 hours after oral administration.

Polythiazide produced a pronounced increase in sodium and chloride excretion in dogs infused with a 5% dextrose solution. Glomerular filtration rate in these experi-

TABLE IV. Effect of Polythiazide i.v. on Excretion of Electrolytes in a Dog with Experimental Metabolic Acidosis.

Dog ♀, 11.5 kg
Anesthesia: Sodium pentobarbital, 25 mg/kg i.v.

Period	Duration, min.	Urine flow, ml/min.	Glomerular filtration rate, ml/min.	Plasma conc., meq/l				pH		Rate of excretion μeq/min.				
				Na^+	K^+	Cl^-	HCO_3^-	Urine	Plasma	Na^+	K^+	Cl^-	HCO_3^-	NH_4^+
Avg of 1–2	20	2.1	30	145	2.8	108	11	5.3	7.15	36	12	92	.4	38
			Polythiazide, 0.4 mg/kg i.v. followed by infusion at 0.6 mg/kg/hr											
3	20	2.1	29	143	2.6	105	13	5.1	7.13	57	14	164	.2	36
4	20	1.9	30	149	2.6	95	12	5.0	7.13	158	24	222	.2	38
5	20	1.5	27	141	2.6	103	13	4.8	7.18	123	21	178	.1	37

TABLE V. Effect of Polythiazide i.v. on Excretion of Electrolytes in a Dog with Experimental Metabolic Alkalosis.

Dog No. 6086 ♀, 22.2 kg
Anesthesia: Sodium pentobarbital, 25 mg/kg i.v.

Period	Duration, min.	Urine flow, ml/min.	Glomerular filtration rate, ml/min.	Plasma conc., meq/l				pH		Rate of excretion, μeq/min.			
				Na^+	K^+	Cl^-	HCO_3^-	Urine	Plasma	Na^+	K^+	Cl^-	HCO_3^-
1	20	4.5	60	148	2.6	99	28	7.7	7.46	88	35	34	98
2	20	4.2	56	147	2.6	97	28	7.7	7.48	90	31	40	93
			Polythiazide, 0.4 mg/kg followed by 0.6 mg/kg/hr infusion										
3	20	5.4	60	150	2.7	97	31	7.8	7.45	438	44	237	214
4	20	5.8	59	149	2.7	99	30	7.8	7.47	465	48	252	243
5	20	6.3	61	153	2.7	96	32	7.8	7.47	504	53	265	286

ments was not increased. The inhibition of carbonic anhydrase which is observed *in vitro*, was not generally evident during the polythiazide diuresis seen in normal animals, even at doses as high as 10 mg/kg i.v. Only in experimental alkalosis was there a marked increase in bicarbonate excretion after polythiazide, attributable to the carbonic anhydrase inhibitory activity of the drug.

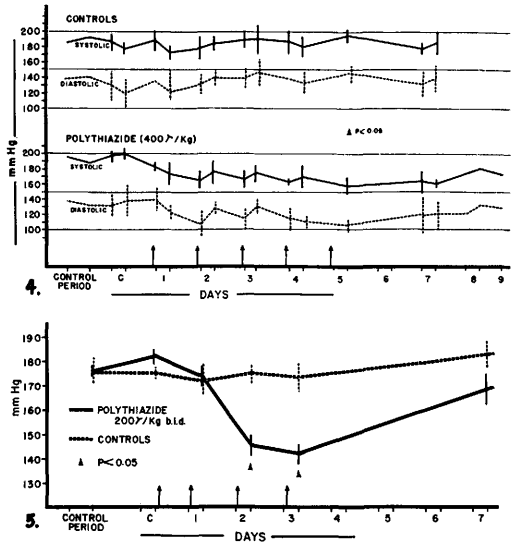


FIG. 4. Effect of polythiazide, 400 μg/kg orally, on blood pressure and heart rate of unanesthetized, hypertensive dogs. At the arrow polythiazide was administered; C is control. Avg values for 4 dogs. Vertical bars represent stand. errors of mean. Where indicated (▲) there is a statistically significant difference between control and treated animals.

FIG. 5. Effect of oral treatment with 200 μg/kg of polythiazide twice daily on blood pressure in unanesthetized hypertensive rats. At the arrow polythiazide was administered; C is control. Avg for 8 rats. Vertical bars represent stand. errors of mean. Where indicated (▲) there is a statistically significant difference between control and treated rats.

The demonstration of antihypertensive activity of polythiazide in animals indicated the possible usefulness of this drug in treatment of hypertension. The sustained effect should be a desirable property of polythiazide used as an antihypertensive agent. The mechanism of antihypertensive action of benzthiadiazines cannot yet be clearly defined. The reduction of blood volume or decrease in reactivity of blood vessels as a result of blood volume reduction(18) are still favored explanations for the mechanism of the benzthiadiazines action in hypertension. The changes in intracellular concentrations of Na^+ or K^+ in the arterial walls may, on the other hand, also explain the antihypertensive activity(19). It was recently postulated(20), that ischemic kidneys of rats may produce a substance which would tend to increase selectively the intracellular K^+ concentration in the arterial walls of hypertensive rats. It is conceivable that a drug like polythiazide may antagonize the effect of this hypothetical substance on redistribution of tissue electrolytes.

Summary. Polythiazide was found to be a highly potent, orally active diuretic agent in rats and dogs. Duration of polythiazide action was prolonged. The diuretic effect was demonstrated as late as 12 to 24 hours after oral administration of the drug. Onset of polythiazide action was rapid. After intravenous administration to dogs, the maximal saluretic effect was obtained in 10 to 20 minutes. The onset of action after oral administration was less than one hour; maximal ef-

fect was obtained either during the first or second hour. Polythiazide produced an almost equal increase in excretion of sodium and chloride. The increase in excretion of potassium was approximately 1/10 of that of sodium. In acute experiments on rats polythiazide was found to be 10 times more potent than trichlormethiazide in its natriuretic effect but only 0.4 times as potent in its kaliuretic effect. Polythiazide at dose levels as high as 10 mg/kg i.v. did not depress glomerular filtration rate. Polythiazide is a carbonic anhydrase inhibitor *in vitro*. *In vivo*, polythiazide produced no or only minimal increases in excretion of bicarbonate in animals with normal acid-base balance. The drug was effective as saluretic agent in dogs with experimental metabolic acidosis or alkalosis. Antihypertensive activity of polythiazide was clearly demonstrated in rats and dogs with experimental hypertension. The antihypertensive effect had a slow onset and became evident on the second day of treatment.

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Demonstration of Countercurrent Diffusion Exchange in the Vasa Recta of the Renal Medulla.* (26781)

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Man and certain animals are capable of producing urine hypertonic to plasma as a means of conserving body water. It is currently held that, under the influence of antidiuretic hormone, the collecting ducts become more permeable to water and probably to urea. Urine passing through the collecting duct equilibrates with the hypertonic interstitium of the renal medulla and papilla, becoming concentrated with respect to plasma (1). Not only is the medulla hypertonic, but a gradient of osmolality(2) and solute concentration (sodium, urea, chloride, creatinine) has been demonstrated, increasing from the base of the medulla to the papillary tip(1,3,4). Micropuncture studies have

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