regard.¶ Further studies are indicated to determine the factor or factors in raw pancreas which induced salivary gland hypertrophy in rats and the *modus operandi* of this effect.

Summary. Rats fed a stock diet supplemented with graded amounts of raw pancreas exhibited retarded growth and a marked hypertrophy of the salivary glands, effects which were proportional to the level of pancreas fed. These effects did not occur when heated pancreas or other raw tissues were fed.

¶ Ershoff, B. H., Levin, E., unpublished data. Isoproterenol also induced salivary gland enlargement when administered orally by incorporating it at a 1% level in the stock ration but the increment in salivary gland weight was less than one third that induced by intraperitoneal injection.

1. Selye, H., Veilleux, R., Cantin, M., *Science,* 1961, v133, 44.

2. Levin, E., Finn, R. K., *Chem. Eng. Progress,* 1955, v51, 223.

Received April 2, 1962. P.S.E.B.M., 1962, v110.

Acute Effects of Chlorothiazide on Plasma Electrolytes and Acid-Base Pattern of Nephrectomized Rats and Dogs.* (27486)

RICHARD W. ERBE AND JOHN M. WELLER
Department of Internal Medicine, University of Michigan, Ann Arbor

Chlorothiazide administration initially enhances excretion of sodium, chloride and water(1). Within a week after starting treatment with this drug, hypertensive patients show a decreased plasma volume and cardiac output, and a fall in blood pressure (2). After a month of therapy, plasma and extracellular fluid volumes and exchangeable sodium return to pretreatment levels, but the reduction in blood pressure is maintained(2, 3). Chlorothiazide, given intravenously to acutely nephrectomized dogs, was reported by Beavers to produce an extrarenal effect consisting of a significant immediate reduction of plasma K concentration persisting for at least 2 hours(4). To investigate this, 2 studies were done, using rats and dogs, respectively. In each study experimental and control groups were used, the experimental group receiving chlorothiazide and the control group receiving an equal amount of sulfamethoxypyridazine (SMP), another sulfonamide derivative with pK_a of 6.7, equal to that of chlorothiazide, and molecular weight differing by only 5% (295.74 for chlorothiazide and 280.32 for SMP), but lacking known antihypertensive action. In addition the effects of a non-natriuretic antihypertensive thiazide, diazoxide, and of 5% glucose solution alone were determined.

Method and materials. Rats. Twenty male Sprague-Dawley rats weighing 250 to 300 g were anesthetized with intramuscular sodium pentobarbital (40 mg/kg). Bilateral lumbar nephrectomies were performed. The left femoral vein was cannulated and 200 units of heparin sodium injected. The left femoral artery and right femoral vein were similarly cannulated. Just prior to drug injection 0.45 ml arterial blood was collected in a Wintrobe tube under 0.1 ml mineral oil. Chlorothiazide† was dissolved in distilled water to give a concentration of 10 mg/ml and a pH of 9.6. SMP was dissolved in distilled water, brought to pH 12-14 with 1.0 N NaOH, then titrated to pH 9.6 with 0.5 N HCl, the final concentration of SMP being 10 mg/ml. Then 20 mg/kg body weight drug were injected into the left femoral vein and an equal quantity of drug plus 0.1 ml 5% glucose solution was infused over a 30-minute period. Chlorothiazide was given to half of

* Supported in part by grants from Am. Heart Assn. and Mich. Heart Assn.

† Chlorothiazide kindly supplied as Lyovac Diuril by Dr. J. E. Baer, Merck Inst. for Therapeutic Research, SMP as Midicel by Dr. K. O. Courtney, Parke, Davis and Co., and diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide) by Dr. J. Black, Schering Corp.

TABLE I. Rat Plasma K Concentrations (meq/l).

Rat No.	Pre-drug	Post-drug 15 min.	45 min.	1¼ hr	1¾ hr	2¼ hr
Chlorothiazide						
1	4.36	4.28	4.34	4.88	5.20	6.40
2	4.49	4.28	5.00	6.02	9.38	—
3	5.71	5.61	5.71	5.71	6.12	9.28
4	5.59	5.32	5.61	6.02	9.69	—
5	5.10	5.25	5.08	5.17	5.55	6.52
6	4.71	4.82	4.50	4.69	4.95	5.82
7	5.80	5.86	6.00	5.97	6.15	6.27
8	5.12	5.28	5.30	5.52	5.56	5.66
9	5.06	5.10	5.10	5.30	5.32	5.20
10	5.10	5.15	5.20	5.41	5.71	5.82
Mean	5.10	5.10	5.18	5.47	6.36	6.37
S.E.M.	±.16	±.16	±.16	±.15	±.54	±.44
SMP						
1	4.59	4.79	4.59	4.79	5.00	5.30
2	6.02	5.92	5.71	5.60	7.75	5.81
3	5.10	5.14	5.20	5.00	5.81	8.21
4	5.10	5.61	5.81	5.50	6.53	6.58
5	5.41	5.51	5.61	6.22	9.08	—
6	6.07	5.53	5.51	5.81	6.72	6.57
7	5.94	5.63	5.93	6.12	6.64	7.75
8	6.12	6.17	5.73	6.12	6.47	8.45
9	5.14	5.28	5.10	5.09	4.95	5.36
10	5.13	5.27	5.12	4.94	5.30	5.50
Mean	5.46	5.49	5.43	5.52	6.42	6.61
S.E.M.	±.17	±.10	±.13	±.17	±.31	±.32

the animals and SMP to the others. Arterial blood was drawn 15 min, 45 min, 1¼ hr, 1¾ hr, and 2¼ hr after initial drug injection, centrifuged under oil 20 minutes, per cent red cells recorded, and plasma separated. Na and K determinations were done by flame photometry.

Dogs. Twenty-four mongrel dogs, ranging in weight from 12.1 to 23.5 kg, were anesthetized with sodium pentobarbital, 35 mg/kg.‡ Bilateral nephrectomy was done. The left femoral vein was exposed and 2,000 units heparin sodium injected. The right femoral artery and vein were cannulated. Fifty-nine minutes after the renal vessels were ligated, predrug arterial blood samples were collected under oil. Chlorothiazide was dissolved in 5% glucose solution. SMP was dissolved in 5% glucose solution by bringing to pH 12-14 with 1.0 N NaOH and titrating with 0.5 N HCl to the pH of the chlorothiazide solution, 9.6. Eight animals received chlorothiazide and 8 SMP. Exactly one hour after ligation of renal vessels, 20 mg/kg body weight of either chlorothiazide or SMP, in 10 ml 5% glucose solution, were injected into the left femoral vein and a 30-minute infusion of an equal amount of drug in 50 ml 5% glucose solution was begun. Four dogs received diazoxide (2.6 or 3.0 mg/kg body weight) titrated to pH 11.0 with 0.1 N HCl. Half of the diazoxide, diluted to 10 ml with 5% glucose, was injected intravenously and the remainder, diluted to 50 ml with 5% glucose solution, was infused over the subsequent 30 minutes. Four dogs were injected with 10 ml

‡ The authors wish to express appreciation to Dr. Paul Rondell, Dept. of Physiology, for assistance with these studies.

TABLE II. Mean Determined and Calculated Electrolyte and Acid-Base Values of Chlorothiazide and SMP Groups of Dogs.

	Group	Pre-drug	Post-drug 15 min.	1 hr	1½ hr	2 hr
Plasma Na (meq/l)	Chlor.	141.2 ± 1.7	141.1 ± 1.1	138.9 ± 2.7	140.1 ± 1.4	138.0 ± 2.6
	SMP	142.4 ± 1.6	142.4 ± 1.5	141.7 ± 1.1	142.5 ± 1.8	142.2 ± 1.5
Total plasma CO_2 (mM/l)	Chlor.	20.96 ± .94	20.59 ± 1.17	19.56 ± 1.30	19.25 ± 1.27	18.78 ± 1.25
	SMP	22.35 ± .53	22.46 ± .46	21.48 ± .43	21.55 ± .97	20.66 ± .90
Plasma pH (units)	Chlor.	7.23 ± .05	7.22 ± .05	7.20 ± .04	7.18 ± .04	7.18 ± .04
	SMP	7.24 ± .06	7.24 ± .05	7.23 ± .04	7.23 ± .04	7.22 ± .05
P_{CO_2} (mm Hg)	Chlor.	49.0 ± 5.8	49.4 ± 6.7	49.1 ± 6.1	50.3 ± 5.8	46.3 ± 6.8
	SMP	51.8 ± 6.9	52.7 ± 6.7	50.2 ± 4.7	49.0 ± 3.7	50.3 ± 5.2
Whole blood buffer base (meq/l)	Chlor.	40.4 ± 1.9	40.0 ± 1.9	38.6 ± 1.8	37.8 ± 2.0	37.7 ± 2.1
	SMP	41.3 ± 2.0	41.2 ± 1.8	40.3 ± 1.4	41.0 ± 1.5	39.8 ± 2.2
Plasma HCO_3^- (meq/l)	Chlor.	19.7 ± .9	19.4 ± 1.1	18.4 ± 1.0	17.8 ± 1.2	17.4 ± 1.2
	SMP	20.8 ± .5	20.9 ± .5	19.9 ± .4	20.1 ± .9	19.2 ± .8
Hematocrit	Chlor.	.52 ± .02	.53 ± .01	.53 ± .02	.56 ± .02	.56 ± .02
	SMP	.51 ± .02	.52 ± .02	.52 ± .02	.55 ± .02	.55 ± .01

All values are means of groups ± stand. errors of mean.

5% glucose intravenously, followed by 50 ml 5% glucose over 30 minutes. Fifteen minutes after initial drug or glucose injection and at the midpoint of the infusion, an arterial blood sample was drawn under oil. At 45 minutes another 2,000 units of heparin sodium were given. Arterial blood samples similarly were drawn 1, 1½, and 2 hours after initial drug or glucose injection. Each blood sample was centrifuged 20 minutes, per cent cells recorded and plasma separated under oil. Total CO_2 content was measured by the method of Van Slyke and Neill(5), pH with a Cambridge research model pH meter, and Na and K with a flame photometer. Whole blood buffer base, Pco_2 and bicarbonate concentration were calculated from measured values for plasma total CO_2 content and pH, using the nomogram of Singer and Hastings (6).

Results. Plasma K concentrations of rat experiments are shown in Table I. Potassium rose in both groups over the 2-hour period. No statistically significant differences between chlorothiazide and SMP groups were present at any sample time. Mean values for plasma Na, total plasma CO_2 content, pH, Pco_2, whole blood buffer base, plasma bicarbonate and per cent cells of dog experiments are shown in Table II. None of the differences at any time between chlorothiazide and SMP groups with respect to these variables is statistically significant. Table III gives plasma K values for all dog experiments. Differences in mean plasma K between the 4 groups at any one sample time were not significant. There were also no statistically significant differences between pre-drug and post-drug mean plasma K concentrations. Plasma Na levels, including those of diazoxide and glucose groups which are not presented in the Table, also showed no statistically significant differences.

Discussion. These results clearly differ from those reported by Beavers(4). The direction and magnitude of changes in plasma K after chlorothiazide in 5% glucose solution were comparable to those seen with 5% glucose solution alone. No extrarenal effect of chlorothiazide on plasma electrolytes was found. This agrees with Blackmore who failed to find depression of plasma K with either benzydroflumethiazide or hydrochlorothiazide(7). The non-natriuretic thiazide, diazoxide, also showed no effect on plasma K. Mean plasma K in all groups of animals showed a decrease at 15 minutes followed by a slow rise, so that the 2-hour value was considerably higher than at the onset. The decrease in plasma K at 15 minutes following administration of 5% glucose solution with or without drug is at least in part related to synthesis of glycogen by liver with accompanying influx of K(8). Entry of K into muscle cells also occurs(9). The rise in plasma K after 15 minutes undoubtedly is ac-

TABLE III. Dog Plasma K Concentrations (meq/l).

Dog No.	Pre-drug	Post-drug 15 min.	Post-drug 1 hr	Post-drug 1½ hr	Post-drug 2 hr
Chlorothiazide					
1	5.00	4.75	4.90	5.00	5.32
2	5.92	5.49	5.71	5.23	6.12
3	5.00	4.63	5.18	5.30	5.33
4	4.60	4.45	4.50	4.30	4.40
5	3.80	3.70	3.70	3.80	4.00
6	5.63	5.45	5.80	6.20	6.30
7	4.00	3.75	3.45	3.53	3.50
8	4.20	3.80	4.18	4.35	4.45
Mean	4.77	4.50	4.68	4.71	4.93
S.E.M.	±.22	±.26	±.31	±.31	±.35
SMP					
1	4.81	4.56	5.43	5.45	5.57
2	5.10	4.85	5.89	6.38	7.40
3	5.53	5.09	5.61	5.65	5.50
4	5.11	4.63	5.30	5.08	4.83
5	4.00	3.65	3.90	4.05	4.05
6	3.75	3.70	3.55	3.60	3.90
7	4.55	4.45	4.82	5.15	5.18
8	4.68	4.30	4.32	4.36	4.26
Mean	4.69	4.40	4.85	4.96	5.09
S.E.M.	±.21	±.18	±.30	±.32	±.40
Diazoxide					
1*	5.50	5.40	5.90	5.90	6.13
2	4.59	4.51	4.59	4.60	4.69
3	4.20	3.90	4.10	4.20	4.35
4	4.65	4.20	4.60	4.90	4.90
Mean	4.74	4.50	4.80	4.90	5.02
S.E.M.	±.18	±.33	±.38	±.36	±.38
Glucose					
1	3.70	3.40	3.90	4.40	4.85
2	3.89	4.00	4.10	4.20	4.31
3	4.09	3.40	4.23	4.35	4.50
4	4.95	4.60	4.68	4.90	5.25
Mean	4.16	3.85	4.23	4.46	4.73
S.E.M.	±.26	±.29	±.14	±.19	±.08

* Received 2.6 mg/kg body wt; others in group received 3.0 mg/kg.

centuated by the trauma of surgery in these animals lacking normal excretory pathways.

Summary. Chlorothiazide (a natriuretic antihypertensive drug), sulfamethoxypyridazine (another sulfonamide derivative lacking antihypertensive effect), and diazoxide (a sodium-retaining antihypertensive thiazide) were given to nephrectomized animals. The effects of these drugs on plasma electrolytes and acid-base pattern did not differ from that of 5% glucose solution alone. No acute extrarenal action of chlorothiazide on plasma electrolytes or acid-base balance was evident.

1. Beyer, K. H., *Ann. N. Y. Acad. Sci.,* 1958, v71, 363.

2. Conway, J., Lauwers, P., *Circulation,* 1960, v21, 21.

3. Lauwers, P., Conway, J., *J. Lab. and Clin. Med.,* 1960, v56, 401.

4. Beavers, W. R., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 711.

5. Van Slyke, D. D., Neill, J. M., *J. Biol. Chem.,* 1924, v61, 523.

6. Singer, R. B., Hastings, A. B., *Medicine,* 1948, v27, 223.

7. Blackmore, W. P., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 681.

8. Fenn, W. O., *J. Biol. Chem.,* 1939, v128, 297.

9. Kamminga, C. E., Willebrands, A. F., Groen, J., Blickman, J. R., *Science,* 1950, v111, 30.

Received April 2, 1962. P.S.E.B.M., 1962, v110.

Influence of Steroidal Structure on Glucuronoside Formation by Mouse Liver and Kidney.* (27487)

DAVID L. BERLINER, WALTER STEVENS† AND THOMAS F. DOUGHERTY
Department of Anatomy, University of Utah College of Medicine, Salt Lake City

It has been shown previously that liver and kidney can conjugate Δ^4-3-keto steroids as well as ring A reduced steroids(1,7). The amount of Δ^4-3-keto corticosteroids conjugated by the kidney is quite small(1). Berliner and Dougherty(2) showed that biological half-lives for various steroids and sterols vary according to their molecular structure. The mouse liver and kidney conjugate corticosterone to a greater extent than cortisol(1). It has been postulated that the hydroxyl group at position C-17 in cortisol was influencing its conjugation(3). This study was undertaken to determine whether the presence or absence of the 17α-hydroxyl group will influence the glucuronoside conjugation of ring A reduced steroids and Δ^4-3-keto steroids *in vitro.*

Methods. Normal male CBA mice 10-14 weeks of age with body weights ranging from 20-30 g were used as a source of tissue. The animals were sacrificed by cervical dislocation. Kidneys and livers were removed and placed in iced buffer.

The tissues were finely minced with a razor blade. One gram of tissue was placed in a 125-ml Erlenmeyer with 20 ml of 0.1 M phosphate buffer (pH 7.4). Fifty mμM of each of the following steroids were incubated: cortisol-4-C^{14}, corticosterone-1,2-H^3, pregnane-3α, 17α-diol-11,20-dione-4C^{14} (compound XIII), and pregnane-3α,ol–11,20-dione-4C^{14} (compound X). All the flasks contained corticosterone-1,2-H^3 and one of the carbon-14 labelled steroids. Two flasks contained compound X only and two contained compound XIII only. Using this (double label) technic two steroids can be incubated simultaneously with the same cells in the same flask. The kidney and liver minces were incubated separately for 3 hours at 37.5°C. Four flasks, 2 containing boiled kidney and 2 containing boiled liver, were prepared in the above manner and were incubated for 3 hours. These are the zero controls.

Reactions were stopped by adding 20 ml of acetone to each flask and the contents immediately extracted 4 times with equal volumes

* Supported by USPHS grant.

† This work has been done in partial fulfillment of the requirements of the University of Utah for the Ph.D. degree.