mals not treated with corticoids. The results were therefore not totally adrenal-dependent.

Summary. The anabolic steroid methandrostenolone partially antagonized the growth inhibiting effects of glucocorticoids in rats and also, depending on the type of experiment, augmented or added to the anti-inflammatory activity of these steroids. Similar activity was found with methyltestosterone and testosterone, whereas norethandrolone and progesterone were inactive. The action of methandrostenolone was independent of the adrenal. Methandrostenolone itself showed no other signs of glucocorticoid-like activity.

The authors wish to express their appreciation to Dr. Robert Gaunt for advice and encouragement and to Mrs. Hanna Sylwestrowicz for statistical evaluation. Thanks are due also to Nancy Howie, Margot Stein, Patricia Reilly and Viola Dube for technical assistance.

1. Desaulles, P. A., Krähenbühl, C., Schuler, W., Bein, H. J., *Schweiz. med. Wschr.,* 1959, v89, 1313.

2. Desaulles, P. A., *Helv. med. Acta,* 1960, v27, 479.

3. Liddle, G. W., Burke, H. A., *ibid.,* 1960, v27, 504.

4. Foss, G. L., *Brit. Med. J.,* 1960, v1, 1300.

5. Wynn, V., Landon, J., *ibid.,* 1961, v1, 998.

6. Clark, G. M., Mills, D., *Arthritis Rheumat.,* 1962, in press.

7. Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 328.

8. Robert, A., Nezamis, J. E., *Acta endocr. (Kbh.),* 1957, v25, 105.

9. Meier, R., Schuler, W., Desaulles, P. A., *Experientia,* 1950, v6, 469.

10. Jasmin, G., Bois, P., Mongeau, A., *ibid.,* 1960, v16, 212.

11. Renzi, A. A., Chart, J. J., Gaunt, R., *Toxicol. Appl. Pharmacol.,* 1959, v1, 406.

12. Chart, J. J., Howie, N., Krauser, F., *Fed. Proc.,* 1961, v20, 177.

13. Gaunt, R., Gross, F., Renzi, A. A., Chart, J. J., *Hypertension, The First Hahnemann Symposium on Hypertensive Disease,* Ed., J. Moyer, W. B. Saunders Co., Philadelphia, 1959, p219.

Received March 30, 1962. P.S.E.B.M., 1962, v110.

An Orally-Active, Heat-Labile Factor in Pancreas Which Induces Salivary Gland Hypertrophy in Rats. (27485)

BENJAMIN H. ERSHOFF AND EZRA LEVIN

Western Biological Laboratories, Culver City, Calif., and the VioBin Corp., Monticello, Ill.

During an investigation of the comparative nutritive value of raw and heated animal tissues, it was observed that rats fed a stock diet supplemented with raw pancreas exhibited retarded growth and a marked enlargement of the salivary glands. These effects did not occur when heated pancreas or other raw tissues were fed. The present study was undertaken to confirm and extend these earlier observations.

Procedure and results. Twenty-four male rats of the Holtzman strain were selected at an average body weight of 43 g (range 41 to 47 g) and were divided into 4 comparable groups of 6 each. One group was fed a basal natural food stock ration;* the remaining groups were fed the basal ration plus 2%, 3% or 4% desiccated and defatted raw pancreas† respectively, the latter being incorporated in the diet in place of an equal amount of stock ration. Animals were placed in metal cages with raised screen bottoms (3 rats per cage) and were provided the above diets and water *ad libitum.* The animals were weighed weekly; and after 26 days of feeding were killed and examined for gross pathology. Findings indicate that increment in body weight was retarded in all rats fed the desiccated and defatted raw pancreas-containing diets, the growth retardation being proportional to the level of pancreas fed. In addition to growth retardation, rats fed the desic-

* Rockland Rat Diet in meal form, A. E. Staley Mfg. Co., Decatur, Ill.

† Viokase Powder 4 N.F. Pancreatin, VioBin Corp., Monticello, Ill.

TABLE I. Comparative Effects of Raw and Heated Pancreas and Other Raw Tissues on Weight Increment and Submaxillary Gland Weight of Immature Male Rats (6 Animals/Group).

Dietary group	Body wt (g) Initial	Final	Submaxillary wt (mg) Avg	Range	Submaxillary wt/100 g body wt (mg)
Exp. 1					
Basal	43	191	458	431- 480	241
Basal + following supplements:					
2% desiccated raw pancreas	43	165	883	751- 962	540
3% " " "	43	157	1277	1014-1605	827
4% " " "	43	150	1271	1073-1498	860
Exp. 2					
Basal	53	108	237	193- 280	221
Basal + following supplements:					
2½% desiccated raw pancreas	53	94	360	313- 441	382
5 % " " "	53	86	466	358- 536	547
10 % " " "	53	70	523	339- 733	744
2½% heated desiccated pancreas	53	104	255	240- 270	248
5 % " " "	53	101	227	200- 263	226
10 % " " "	53	99	233	203- 287	235
Intact raw pancreas	53	77	510	330- 645	675
" " " —activated	53	82	453	328- 560	551
10% desiccated raw liver	53	104	259	237- 283	249
10% " " duodenum	53	105	256	223- 305	244
10% " " spleen	53	102	258	205- 314	254

cated and defatted raw pancreas-containing diets also exhibited a marked enlargement of the salivary glands (parotid, submaxillary and sublingual). The average and range of submaxillary gland weights of rats in the various groups is indicated in Table I, Exp. 1. No other gross pathologic effects were noted.

Since the desiccated and defatted raw pancreas employed in the above experiment was a processed material, studies were undertaken to determine whether intact raw pancreas and raw pancreas treated to convert trypsinogen to trypsin would have a similar effect. Studies were also undertaken to determine the effects of heat treatment of the desiccated and defatted raw pancreas and of feeding other desiccated and defatted raw tissues (liver, duodenum and spleen) on weight increment and submaxillary gland weights of immature rats. Seventy-two male rats of the Long-Evans strain were selected at an average body weight of 52 g (range 42 to 62 g) and were divided into 12 comparable groups of 6 each. Group I was fed the basal natural food stock ration;* Groups II, III and IV were fed the basal stock ration plus 2½%, 5% and 10% desiccated and defatted raw pancreas† respectively; Groups V, VI and VII were fed the basal stock ration plus 2½%, 5% and 10% desiccated and defatted raw pancreas respectively which had been heated for 3 hours in an oven at a temperature of 98° to 100°C; Group VIII was fed a supplement of intact raw hog pancreas at a level corresponding to 10% of the diet on a dry weight basis;‡ Group IX was fed a similar amount of intact raw hog pancreas which was activated to convert trypsinogen to trypsin;§ Groups X, XI and XII were fed the basal stock diet plus 10% desiccated and defatted raw liver, duodenum and spleen respectively.‖ The various supplements indicated above

‡ The diet fed Group VIII was prepared by mixing 650 g fresh ground raw pancreas with 900 g basal stock ration.

§ The trypsinogen was activated by enterokinase. This product was identical to the material from which desiccated and defatted raw pancreas (Viokase Powder) was prepared.

‖ Jayron Powder (raw liver substance), Viodenum Powder (raw duodenum substance) and Spleen Powder (raw spleen substance), VioBin Corp., Monticello, Ill. These materials as well as the Viokase Powder (raw and defatted pancreas) were desiccated and defatted at low temperature by azeotropic solvent extraction by the process of Levin and Finn(2).

were incorporated in the diets in place of an equal amount of stock ration. Rats were killed after 10 days of *ad libitum* feeding, body weights recorded, and the submaxillary glands dissected out and weighed. The latter were placed immediately thereafter in Bouin's solution, prepared for paraffin embedding in the routine manner, sectioned at 7 μ in thickness, and stained with hematoxylin and triosin.

In agreement with the findings reported above, desiccated and defatted raw pancreas when incorporated at graded levels in a stock ration caused a significant retardation in growth and enlargement of the salivary glands of rats, effects which were proportional to the level of pancreas fed. These effects did not occur in animals fed similar amounts of desiccated and defatted raw pancreas which had been heated for 3 hours at 98° to 100°C, nor did they occur in rats fed diets containing 10% desiccated and defatted raw liver, duodenum or spleen. That the effects obtained with desiccated and defatted raw pancreas were not due to processing procedures is indicated by the fact that fresh hog pancreas (either untreated or activated) when fed in amounts corresponding on a dry weight basis to a supplement of 10% desiccated and defatted pancreas resulted in a similar retardation in growth and increment in salivary gland weight. The above findings are summarized in Table I, Exp. 2. Histologically the submaxillary glands of rats fed the desiccated or fresh raw pancreas exhibited marked enlargement of the alveoli with the cytoplasm appearing mucoid in appearance (Fig. 1) in contrast to the submaxillary glands of rats fed the stock ration which had smaller alveoli and a coarsely granular cytoplasm (Fig. 2). Heating the desiccated and defatted raw pancreas or feeding desiccated and defatted raw liver, duodenum or spleen resulted in submaxillary glands which were comparable in microscopic appearance to those of rats fed the stock ration.

No data are available as to the factor or factors in raw pancreas responsible for the above effects. A hypertrophy of the salivary glands similar to that observed in the present

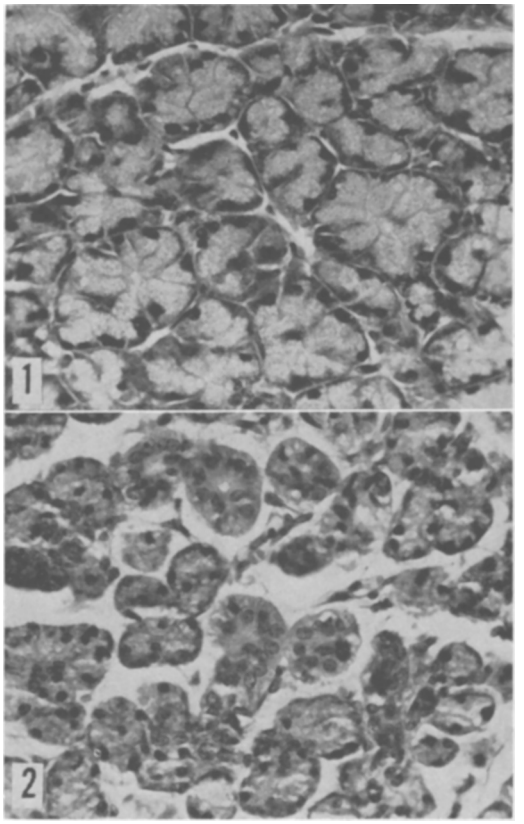

FIG. 1. Submaxillary gland from a rat fed 10% desiccated and defatted raw pancreas. Alveoli are greatly distended and cytoplasm is mucoid in nature. Haem. and Tr. × 300.

FIG. 2. Submaxillary gland from a rat fed basal stock ration. Alveoli are uniform in size and the cytoplasm of the alveolar cells appears coarse in nature. Haem. and Tr. × 300.

experiment has been reported by Selye *et al.* (1) in rats chronically treated with the catecholamine isoproterenol. Not all catecholamines, however, cause enlargement of the salivary glands of rats. Thus neither dl-amphetamine phosphate dibasic or ephedrine sulfate when fed for 10 days at levels of 1 g and 5 g per kg of diet respectively induced salivary gland hypertrophy in rats although both supplements were ingested in sufficient amounts to result in loss in body weight. Similarly intraperitoneal injections twice daily of ¼ cc adrenalin chloride solution (1:1000 adrenalin) were also ineffective in causing salivary gland enlargement although twice daily injections of 25 mg isoproterenol in ¼ cc saline solution had marked activity in this

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.