androgenic and anabolic activities of the compounds, must be responsible for the differences in the effect on various organs.

Summary. Injection of androgens such as testosterone propionate or Norethandrolone in normal and castrated male rats promotes growth of the m. levator ani even on protein-free diets. Based on these and other experiments, it is assumed that the growth of this muscle is a sex-linked function. It is, therefore, concluded that the hormone-induced growth of this muscle is not an appropriate index for the general "myotropic," *i.e.* anabolic effects of steroid compounds.

1. Geiger, E., and El Rawi, I., *Metabolism,* 1952, v1, 145.

2. Borun, R., and Geiger, E., *J. Clin. Invest.,* 1956, v35, 1109.

3. Eisenberg, E., and Gordan, G. S., *J. Pharm. Exp. Therap.,* 1950, v99, 38.

4. Hershberger, L. G., Shipley, E. G., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 175.

5. Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.,* 1924, v46, 2066.

6. Samuels, L. T., *Progress in Clinical Endocrinology,* Greene and Stratton, N. Y., 1950, p. 509.

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Early Development of Glycogen Infiltration in Duct Epithelium of Dog Pancreas after Growth Hormone Administration. (23026)

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The original hypothesis concerning hydropic change in the pancreas advanced by Allen(1) attempted to achieve a common pathogenetic denominator for vacuolization of B cells and duct cells. B cell hydropic degeneration was considered to be due to "exhaustion of an internal secretory function" while that occurring in duct epithelium was thought to be due to "exhaustion of a proliferative function." Most subsequent workers(2,3,4) emphasized B cell hydropic degeneration as a characteristic lesion of the pancreas in experimental diabetes, resulting from "exhaustion" with the duct epithelial vacuolization as a more or less unexplained concomitant. The pathogenetic implication of the duct epithelial lesion is usually not mentioned and the exhaustion theory accepted uncritically. Toreson(5) demonstrated that hydropic degeneration in the pancreas in diabetes consists actually of the deposition of glycogen. It was suggested that the glycogen accumulation might represent merely one component of the widespread pathologic glycogen deposits found in diabetes or that it might be indicative of proliferative activity of duct epithelium with deranged islet regeneration. More recently it was shown that in cortisone treated rabbits the glycogen infiltration of duct epithelium occurs prior to and even in the absence of that in B cells(6). This observation is at variance with the exhaustion hypothesis of hydropic change unless the B cell and duct epithelium lesions are thought of as being distinctly separate and of different etiologies. However, the known facts concerning their development would negate this latter idea. It was therefore theorized that the pancreatic glycogen deposition was similar to that observed in other organs in diabetes. It was considered of importance to determine whether the time sequence observed in the cortisone-treated rabbit also occurs in other forms of experimental diabetes. The present study was designed therefore to investigate whether ductular glycogen infiltration occurs prior to or in the absence of that in B cells in the growth hormone treated dog.

Material and methods. The study was carried out on 27 mongrel dogs of either sex weighing between 12-15 kg. Nineteen of

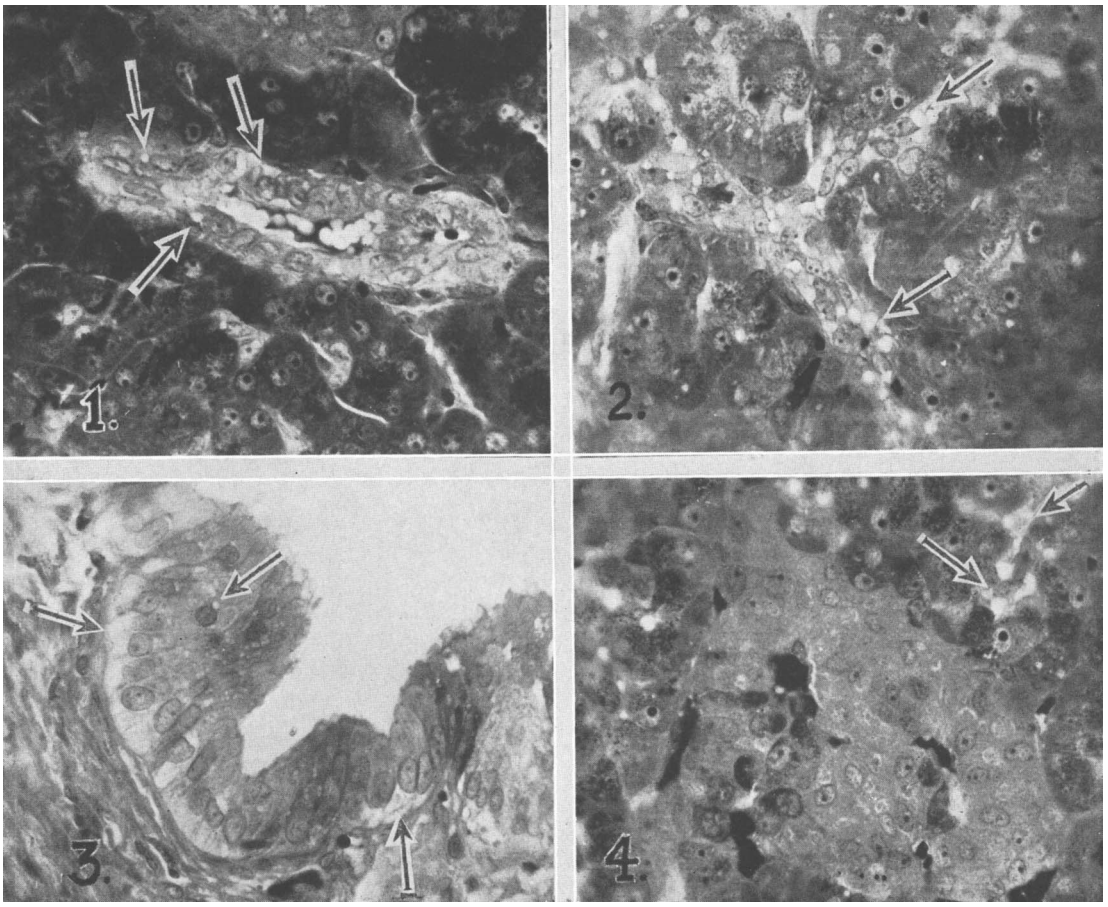

FIG. 1. Pancreas of normal dog showing intralobular duct. The arrows point to vacuoles in the cytoplasm of the epithelial cells. Masson trichrome stain. X 390.

FIG. 2. Pancreas of dog treated for 6 days with growth hormone showing increased vacuolization of intercalated duct epithelium and occasional small vacuoles in acinar epithelium. Masson trichrome stain. X 390.

FIG. 3. Pancreas of dog treated with growth hormone for 8 days showing a large duct. There is moderately increased sub- and supra-nuclear vacuolization (arrows). Masson trichrome stain. X 390.

FIG. 4. Pancreas of dog treated with growth hormone for 6 days showing intercalated duct in close contiguity (arrows) with an islet. The ductular epithelium is vacuolated. The B cells (grey in photo) are degranulated but not vacuolated. Masson trichrome stain. X 390.

these animals each received 3 mg/kg of purified anterior pituitary growth hormone* once a day by subcutaneous injection. One animal each was killed on the 1st and 2nd days, 3 on the 3rd day, and 2 each on the 5th, 6th, 7th, 9th and 11th days. Four animals died inadvertently and were not autopsied. Eight untreated dogs were used as controls. All animals received a high carbohydrate diet consisting of mashed potatoes, meat and meat gravy, and 5% glucose solution *ad libitum* instead of drinking water. The animals were killed by overdosage with intravenous nembutal and autopsies were performed immediately after death. Tissue from the tail, body and head of the pancreas was removed for histologic study and fixed in Zenker-formol solution (20%). The blocks were embedded

* The growth hormone was Armour & Co. Lot #208 provided through courtesy of Dr. Irby Bunding and Somar-A. Armour, gift of the Nat. Inst. Health.

in paraffin and were stained by the aldehyde-fuchsin(7) and the Masson trichrome methods(8). The periodic acid-Schiff method(9) controlled by diastase digestion was utilized for the demonstration of glycogen.

Results. A) *Normal dogs*: In sections stained by the trichrome method there were occasional small sharply defined vacuoles noticeable in the epithelium of the interlobar and intralobular ducts (Fig. 1) and also in the epithelium of the intercalated ducts. In the larger ducts with columnar epithelium they were subnuclear in position. In the smaller ducts or in the ductules the vacuoles were centrally located adjacent to the nuclei. Using the periodic acid-Schiff technic small deposits of glycogen were noted in ductular epithelium which corresponded with the cytoplasmic vacuolar changes. There was no glycogen present in the B cells of the islets.

B) *Growth hormone treated dogs*: In general the degree of vacuolization of the intralobular and of the intercalated ducts increased with duration of treatment. This increase first became apparent in an animal treated for 2 days and was usually present in animals at the 5th day and thereafter (Fig. 2). The columnar epithelium of the large interlobar ducts showed modest increase in subnuclear vacuolization (Fig. 3). B cell vacuolization was present in only 3 animals and was minimal as compared with the ductular vacuolization. The priority and preponderance of duct vacuolization was evident in many instances by the contiguity of vacuolated ducts with non-vacuolated islet tissue (Fig. 4). In several instances small vacuoles also appeared in acinar cells. P.A.S.-positive material which was removed by diastase was present in these vacuoles. Mild B cell degranulation was apparent in an occasional animal treated for 2 days. Thereafter several dogs showed very marked degranulation whereas others, even after prolonged treatment, showed little or no degranulation. Mitotic figures were sometimes found in the epithelium of ducts of all sizes and in centroacinar cells. B cell mitoses were quite rare.

Discussion. The present studies demonstrate that vacuolization due to glycogen deposits is sometimes present in the ductular epithelium in the normal adult canine pancreas. This finding is in accord with the previous observation that glycogen is present in the ductular epithelium of the human fetus (10) and indicates that its appearance in the diabetic pancreas is a quantitative rather than a qualitative change. It is interesting in this connection that many animal species such as the rabbit do not normally show glycogen in pancreatic ductular epithelium(6).

Our studies demonstrate that the growth hormone treated dog pancreas behaves similarly to the cortisone treated rabbit pancreas, in that there is increased ductular vacuolization due to glycogen infiltration prior to and generally in the absence of B cell vacuolization. These facts make it unlikely that a peculiarity of B cell metabolism is responsible for this glycogen infiltration. These observations also make questionable the concept that this histologic lesion is an expression of a degenerative process.

This process more logically seems to constitute merely one facet of the ubiquitously increased cellular glycogen occurring in diabetes. It is interesting in this connection that glycogen infiltration of renal tubular epithelium was considered a degenerative process until Ehrlich(11) demonstrated that the vacuoles contained glycogen. At the present time renal tubular glycogen deposits are considered a reversible metabolic phenomenon (12) which is an expression of a high urinary glucose concentration. Furthermore, the increased cardiac glycogen observed in both human and experimental diabetes is accepted as a mere reflection of the elevated blood sugar concentration(13). Similar mechanisms most probably account for the pancreatic glycogen deposition. A possible explanation accounting for glycogen in ductular epithelium at early stages would be that these cells absorb glucose from the lumen. This assumption seems to be borne out by the direct relationship between glucose concentration in blood and that in external pancreatic secretion(14).

Summary. The sequential development of hydropic degeneration (glycogen infiltration)

in the dog pancreas during administration of purified anterior pituitary growth hormone has been studied. Fifteen mature normal mongrel dogs each received 3 mg/kilo of growth hormone daily subcutaneously. Two each were sacrificed on alternate days for 14 days. Eight animals were used as controls. It was found that sometimes glycogen is normally present in ductular epithelium but not in B cells. Increased vacuolization (glycogen infiltration) of intralobular and intercalated ducts developed prior to and generally in the absence of the appearance of glycogen in the B cells of the islets. These findings are taken to support the hypothesis that hydropic degeneration of B cells is not a degenerative lesion resulting from "functional exhaustion" but is rather a morphologic expression of hyperglycemia similar to that seen in other organs.

1. Allen, F., *J. Metab. Res.*, 1922, v1, 5.
2. Richardson, K. D., *Proc. Roy. Soc., London,* 1940, v128, 153.
3. Ham, A. W., and Haist, R. E., *Am. J. Path.,* 1941, v17, 787.
4. Duff, G. L., *Am. J. Med. Sci.,* 1945, v210, 381.
5. Toreson, W. E., *Am. J. Path.,* 1951, v27, 327.
6. Lazarus, S. S., and Bencosme, S. A., *Am. J. Clin. Path.,* 1956, v26, 1146.
7. Gomori, G., *ibid.,* 1950, v20, 665.
8. Bencosme, S. A., *A.M.A. Arch. Path.,* 1952, v53, 87.
9. Lillie, R. S., *Histopathologic Technique and Practical Histochemistry,* Blakiston Co., 1954.
10. Ohohashi, Y., Quoted by Toreson, W. E., *Am. J. Path.,* 1951, v27, 327.
11. Ehrlich, P., *Z. f. Klin. Med.,* 1883, v6, 33.
12. Robbins, S. L., *Am. J. Med. Sci.,* 1950, v219, 376.
13. Cruickshank, E. W. H., *Phys. Rev.,* 1936, v16, 597.
14. Babkin, B. P., *Secretory Mechanism of the Digestive Glands,* Paul B. Hoeber, 1944.

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Effect of Dietary Lipid on Rat Serum and Liver Cholesterol and Tissue Mast Cells.*† (23027)

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It has been shown repeatedly in man(1-2) that increase in proportion of calories from dietary lipid causes elevation of concentration of serum cholesterol. Recent reports indicate that this effect does not follow when lipid consists of certain vegetable oils(3). Moreover certain oils rich in linoleic acid have been reported to produce a significant decrease of serum cholesterol concentration in man(4-8). In view of differences in cholesterol metabolism between man and rat, the present study on the rat is concerned first with the effect of different fat-diets on cholesterol concentration in serum lipoproteins and in liver.

Since heparin has a "clearing" action on lipemic serum, consideration was also given to the possibility that heparin might serve to some degree as a physiological regulator of blood lipid. Tissue mast cells contain heparin, and the question is whether their number, intact or undergoing disruption, is influenced by nature and concentration of blood lipid. This possibility was examined by counting mast cells in external ear tissue of rats maintained on various diets for study of serum and liver cholesterol.

Methods. Adult male albino rats of Sprague Dawley strain were used. The age-

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