GLYCOGEN INFILTRATION (SO-CALLED HYDROPIC DEGENERATION) IN THE PANCREAS IN HUMAN AND EXPERIMENTAL DIABETES MELLITUS *

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In 1901 Weichselbaum and Stangl [1] described unique changes in the islets of Langerhans of 18 pancreases from comatose diabetic human subjects. Having found the affected islet cells uncolored by methods designed to demonstrate hyaline and mucoid forms of degeneration, they [2] later interpreted the lesion as a manifestation of cytoplasmic vacuolation and liquefaction, and applied the term hydropic degeneration to indicate its presumably aqueous character. Allen [3] accomplished the first experimental production of a similar lesion in the islets and ductules of the pancreatic remnants of dogs made diabetic by resection of nine-tenths of the pancreas. Homans [4,5] demonstrated that in the islets of such experimental material, degranulation and vacuolation developed only in the beta cells. Both experimenters failed to find fat, mucin, or glycogen in the affected cells; each concluded, as Weichselbaum did, that the vacuoles represented aqueous fluid. Richardson,[6] Ham and Haist,[7] Lukens and Dohan,[8,9] and Lukens, Dohan, and Wolcott [10] described the pancreases of dogs and cats treated with anterior pituitary extract; these authors apparently accepted the idea of aqueous vacuolation as the basis of "hydropic degeneration" in their material.

Kennedy and Lukens,[11] Duff, McMillan, and Wilson,[12] and Ogilvie [13] reported "hydropic degeneration" in the pancreas of alloxan diabetes of rabbits, and Goldner and Gomori [14] observed it in alloxan diabetes of dogs. Duff, McMillan, and Wilson did not find fat, mucin, or glycogen in the vacuolated islet and ductule cells, while Ogilvie attributed the lesion to artifactual loss of excessive intracytoplasmic accumulations of serous fluid. Dohan and Lukens [15] produced permanent diabetes and pancreatic islet "hydropic degeneration" in cats by repeated intraperitoneal injections of glucose. Kobernick and More [16] recently observed a severe diabetic state in rabbits given repeated injections of cortisone. In the pancreases of some of these animals extreme "hydropic" appearances were evident in the beta islet cells, ductular epithelium, and centro-acinar cells.

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Pathology textbooks generally attribute the cytoplasmic vacuolation of "hydropic degeneration" to an exaggerated process of aqueous imbibition qualitatively similar to the supposed mechanism of cloudy swelling. Except for the presence of glycogen in fetal pancreatic duct epithelium, as reported by Ohohashi,[17] the normal human pancreas has not been reported to contain histologically demonstrable glycogen. Warren [18] has observed glycogen deposits in the cuboidal and columnar epithelium of pancreatic ducts in human diabetes mellitus, but he has never found it in any other pancreatic cells. He apparently regarded these deposits as simply another occasional site of abnormal glycogen infiltration comparable to the others often encountered in various tissues of diabetic subjects.

The vacuolated islet cells of partially depancreatized diabetic dogs, of dogs and cats rendered diabetic by injections of anterior pituitary extract, and, according to Weichselbaum,[19] of diabetic human beings, gradually disintegrate and disappear. However, the affected ductular cells of diabetic dogs and both the islet and ductular cells of diabetic rabbits persist in the vacuolated state for many weeks or months. Such cells may be restored to normal appearance by appropriate manipulation of the carbohydrate content of the diet,[9,20] by poisoning with phloridzin,[10] or by the provision of exogenous insulin.[8,9,12,21,22] This capacity for prolonged persistence in a remarkably abnormal morphologic state which is nevertheless reversible would seem to be incompatible with the generally accepted presumption that the cytoplasmic vacuoles contain nothing but excessive quantities of water. Furthermore, the presence of mitochondria, as noted by Homans,[4,5] and the Golgi apparatus and macular area, as demonstrated by Bencosme,[23] in "hydropic" islet cells, is irreconcilable with the aqueous imbibition hypothesis. The structural features of various cells known to contain large quantities of cytoplasmic glycogen (*e.g.*, vaginal epithelial cells, parathyroid "wasserhelle" cells, heart muscle fibers adjacent to areas of infarction, renal tubular epithelial cells in Armanni's lesion, and liver cells in von Gierke's disease) are similar to those of pancreatic islet, ductular epithelial and centro-acinar cells affected by so-called hydropic degeneration. It is the purpose of this paper to report that these "hydropic" pancreatic cells actually do contain glycogen in amounts demonstrable by appropriate histologic methods.

Materials and Methods

Large numbers of blocks of pancreatic tissue were obtained from 20 domestic albino rabbits made permanently diabetic by single intra-

venous injections of alloxan, as described by Duff, McMillan, and Wilson.[12] Some of these animals had received repeated daily injections of protamine zinc insulin.[22] The pancreases of 2 dogs which had received repeated daily injections of crude alkaline extract of fresh frozen ox anterior pituitary glands,* prepared and used according to the methods of Young,[24] were studied. Pancreatic remnants were available also from 2 dogs subjected to extensive resection of the pancreas in the manner of Allen's [3] and Homans' [5] experiments. Drs. Kobernick and More [16] kindly allowed me to study sections of pancreas fixed in Zenker-formol (20 per cent formalin in Zenker's base) from a cortisone-treated rabbit. Some of these tissues were taken for biopsy, but the majority were secured at autopsy performed immediately after killing each animal. Small thin slices of pancreas were immersed in freshly prepared Helly's fluid (5 per cent formalin in Zenker's base), allowed to fix for 8 to 24 hours, washed in tap water for 8 to 24 hours, dehydrated in alcohol, cleared in toluol, and embedded in paraffin. Multiple sections were cut at 3 to 5 μ, and mounted on glass slides with gelatin.

The human material was obtained from autopsies performed between the years 1926 and 1950 on 26 cases of diabetes mellitus in which death occurred in coma. These tissues had been fixed in 10 per cent formalin and embedded in paraffin. New sections were cut at 5 μ. One case provided material which had been fixed in acetic acid-Bouin's fluid and embedded in paraffin. For this material, a thin film of celloidin was applied to the sections after they had been mounted on glass slides, according to the suggestion of Lillie.[25]

Blocks of rabbit kidney from alloxan diabetic animals, fixed in Helly's fluid and embedded in paraffin, provided "known-positive" control material for glycogen staining reactions and enzymatic digestion tests. Digestion was carried out for ½ to 1 hour at 37°C. in buffered 1 per cent malt diastase U.S.P.[25] All other technical manipulations of control and test sections were identical and simultaneous.

The animal tissues were stained by the following methods: hematin, phloxine, saffron; a modification of Gomori's chromic alum hematoxylin in which the ponceau mixture of Masson's trichrome was substituted for phloxine; Best's carmine; Mayer's mucicarmine; aqueous periodic acid-Schiff's reagent routine of McManus [26]; the alcoholic version of the same test described by Hotchkiss [27]; the Bauer chromic acid-Schiff's reagent technic as described by Lillie [25]; and Mancini's [28] iodine reactions. The human tissues were submitted to most of the same battery

* The pituitary glands were made available to me by the courtesy of Dr. John R. Mote, Armour & Company, Chicago, Illinois.

of stains except that efforts to demonstrate islet cell cytoplasmic granules were not made routinely.

The glycogen content of the pancreas of an alloxan diabetic rabbit in which severe "hydropic change" was present was determined chemically * by the Good, Kramer, and Somogyi method as utilized by Venning, Kazmin, and Bell.[29]

Observations

In sections stained by the hematin, phloxine, and saffron method and by the Gomori and Masson technics (Figs. 5, 13, 16, and 19), pancreatic cells affected by so-called hydropic degeneration appeared slightly to extremely swollen, being apparently distended by accumulation in their cytoplasm of a substance not visible in such preparations. The cytoplasmic membranes were sharply defined and the nuclei had well preserved, apparently normal, structural patterns. In most instances the nuclei were situated in the centers of the cells or were only slightly displaced toward the periphery; they never appeared compressed in the semilunar fashion of cells containing fatty cytoplasmic masses. Often the demonstrable cytoplasm was represented by occasional delicate wisps of lacy, cobweb-like material extending in radial fashion from the nucleus to the cytoplasmic membrane. However, there was present sometimes a more or less vesicular cytoplasm devoid of demonstrable specific beta granules but containing mitochondria, Golgi apparatus, and macular area.[23] In some cells one or several small, round or irregular, homogeneous bodies (the Körner of Weichselbaum) were evident.

Mucin was encountered frequently in epithelial cells lining the large and intermediate pancreatic ducts. The Gomori method demonstrated this material in the same situations and quantities as did the Mayer mucicarmine technic, but depicted it as fine, closely packed, blue granules. Both the McManus and the Hotchkiss versions of the periodic acid-Schiff's reagent technics colored mucin less brilliantly red than they did glycogen. Mucin-filled columnar cells of ducts often were found together with cells having vacuolated cytoplasm. In no instance was mucin detected in cells of the islets (Fig. 12). Exposure to diastase did not remove mucin.

When the pancreases of alloxan-treated rabbits were stained by the several technics capable of demonstrating glycogen, this substance was always found in the cells affected by so-called hydropic degenera-

* I am indebted to Dr. E. H. Venning of the University Clinic, Royal Victoria Hospital, for this analysis.

tion (Fig. 10). It was present not in traces but in abundance, and its location coincided accurately with the cytoplasmic vacuolar change in beta cells of the islets, ductular epithelium, and centro-acinar cells. Ordinarily there was an obvious ipsilateral shift of the material referable to the artifact attributed to the diffusion current of the fixative. Although the material was often represented as granular deposits of variable dimensions, in many cells the whole cytoplasmic area was occupied by a single, homogeneous mass which partially obscured the nucleus. In some instances there appeared to be partial loss of glycogen so that the remnant occupied only a fraction of the cytoplasmic area of the affected cells. This appearance was not more frequent than the similar and more familiar aspect of glycogen infiltration of vaginal epithelium, myocardial fibers, and renal tubular epithelium (Figs. 7, 8, and 9). Pancreatic tissue from dogs treated with anterior pituitary extract and from partially pancreatectomized dogs showed glycogen infiltration of beta islet cells, ductular epithelium, and centro-acinar cells similar in every qualitative respect to the appearance of the lesion in alloxan diabetic rabbit tissue (Fig. 14). However, the lesions observed in these experiments were of mild degree.

The presence of glycogen in the vacuolated pancreatic cells was displayed in flamboyant manner in the preparations from the cortisone diabetic rabbit (Fig. 17). The effect was exaggerated comparatively by the presence of more numerous beta cells than appeared in alloxan diabetic rabbits, so that the islet lesion, as well as the ductular one, was obvious at a glance.

If allowance be made for the poor technical quality of the available human material, the appearance of vacuolation of islet cells in it closely resembled the experimental lesions (Fig. 19). In this material, however, the glycogen appeared much more granular and was less strictly confined to cytoplasmic vacuoles (Fig. 20). Some of it appeared to have been deposited upon the islet rather than within swollen beta cells. Ductular epithelium was relatively uninvolved, even in the presence of marked infiltration of islets. Among the 26 human cases studied, "hydropic degeneration" was apparent according to ordinary stains in 11 (42.3 per cent). Glycogen was demonstrable in abundance in one case (acetic-Bouin fixation), in moderate amounts in 2, and in small amounts in 6. No positive staining reaction could be elicited in the remaining 2 instances. In no case was glycogen found in the absence of definite cytoplasmic vacuolation.

Exposure of control sections to diastase always removed the demonstrable substance from the various cells (Figs. 11, 15, 18, and 21) in

which it could regularly be demonstrated if the tissues were not exposed to diastase (Figs 10, 14, 17, and 20). Mucin in columnar duct epithelium remained demonstrable despite diastase treatment.

The pancreas of the alloxan diabetic rabbit contained 0.0025 gm. of glycogen per gm. of tissue (= 0.25 per cent).

Discussion

The lesion as described is qualitatively similar in every morphologic respect in all of the tissues studied without regard to species or mode of production of diabetes. Modifications of it are known to occur in cats, dogs, and probably in human beings, referable to gradual pyknotic and lytic changes in the affected cells terminating in numerical atrophy of beta cells. In the alloxan treated rabbit very few islets contain many glycogen-filled cells because of the preceding necrosis of the beta cells. The cortisone treated rabbit provides the most abundant and flagrant lesions; in this instance, however, the details of the development, progress, and termination of the lesion are not yet available. The amount of glycogen determined by chemical analysis is small, but it is similar to the amounts found in human kidneys in glycogen nephrosis.[18]

Artifactual removal of the abnormal content of glycogen in the cytoplasm of swollen pancreatic islet, ductular epithelial, and centro-acinar cells showing "hydropic degeneration" is probably more responsible for the characteristic vacuolation apparent in ordinary histologic preparations than is the concomitant presence of excessive intracytoplasmic water. The granular appearance of glycogen in chemically fixed preparations and the ipsilateral disposition of more homogeneous crescentic masses of intracytoplasmic glycogen represent fairly constant artifacts.[28] Fixation by freezing, dehydration *in vacuo*, and subsequent direct embedding in paraffin permit demonstration of glycogen evenly distributed throughout the cytoplasm of cells of various types which normally contain or store it.[28] Microscopic demonstrations of glycogen prepared by other technics inevitably misrepresent the amounts and locations of the material to some extent. Because in the normal process of glycogen storage in the liver each gram of glycogen stored is accompanied by the simultaneous entrance of 1.16 to 2.33 cc. of water into the liver cells,[30] it is reasonable to suppose that a similar imbibition may occur when abnormal deposits of glycogen accumulate in the pancreatic islet, ductular epithelial, and centro-acinar cells. Nevertheless, this lesion of the pancreas would be more distinctively

designated "glycogen infiltration," in keeping with customary usage for pathologic accumulations of glycogen in other tissues.

Although so-called hydropic degeneration in the pancreas has been accorded unique status as the histopathologic common denominator of human and experimental diabetes ever since Allen [3] first produced the experimental lesion, certain recognition of its presence in diabetic human material has always posed a difficult diagnostic problem. Post-mortem autolytic phenomena may produce simulated vacuolar appearances in islet cytoplasm; on the other hand, shrinkage referable to fixation artifact may so distort cytologic detail that actual vacuolar effects may become obscured. Warren [18] found the incidence of the lesion to be no greater in material from the pre-insulin era than in that obtained subsequently. However, in experimental alloxan diabetes of rabbits, provision of exogenous insulin, even in amounts insufficient to affect appreciably the level of hyperglycemia and causing only moderate diminution of glycosuria, may prevent the development of the lesion or restore affected cells to normal structural appearance.[22] Thus, apparently anomalous observations on routinely prepared human material are to be expected. Weichselbaum [19] reported vacuolated islet cells in 67 of 183 pancreases of diabetic patients (36.6 per cent), whereas Warren [18] found them in only 22 instances among 484 autopsies (4.5 per cent). As reported by various authors,[31] the incidence has varied from 48 per cent to zero. The true incidence of the lesion in human material is probably insusceptible to exact analysis. However, utilization of histologic technics for demonstrating glycogen in suspected lesions should permit an improved accuracy of diagnosis. The presence of histologically demonstrable glycogen in human islets apparently affected by characteristic cytoplasmic vacuolation constitutes positive morphologic evidence of diabetes mellitus. Failure to find glycogen in characteristically vacuolar islet cytoplasm need not necessarily mean the absence of diabetes; such failure might result also from either post-mortem glycolysis or from imperfections of fixation and staining.

The significance of accumulation of glycogen in various pancreatic cells remains obscure. Possibly it represents merely one component of the more widespread pathologic deposits found in subjects whose diabetes has been poorly controlled. One alternate explanation which may be suggested tentatively is that in the diabetic pancreas, glycogen infiltration may be an indication of deranged regeneration of islets. This idea derives from the occurrence of the material in the islet cells,

centro-acinar cells, and ductular epithelium. These latter cells are the principal histogenetic source of differentiated islet cells in fetal and probably also in extra-uterine life. Furthermore, the pancreatic duct epithelium of the human fetus has been reported to contain glycogen [17] and it is known that embryonic tissues in general contain more chemically determinable glycogen than their mature derivatives. On the other hand, mitotic activity as an indicator of proliferative regeneration is not a common feature of diabetic pancreases showing glycogen infiltration except in animals treated with anterior pituitary extract.

A second alternative is suggested by the renal lesion known as glycogen nephrosis. Renal tubular epithelial cells normally resorb and transmit glucose from the glomerular filtrate, but they do not normally contain histologically demonstrable glycogen. When their functional capacity to handle glucose is exceeded, as in severe spontaneous or experimental diabetes mellitus and in phloridzin diabetes, these renal cells accumulate glycogen in their cytoplasm. Allen [3,20] considered the pancreatic islet lesion a regressive morphologic response to prolonged excessive functional stimulation; because of its presumed aqueous character and for want of analogous phenomena in other organs or tissue, he thought so-called hydropic degeneration was unique. The demonstration of glycogen in pancreatic cells in diabetes establishes a degree of parallelism between the morphologic response of the overstrained pancreatic islets and the renal tubular cells. However, a normal physiologic process provides a basis for the development of the renal lesion, whereas no analogous pancreatic function has been determined.

Fixation in Helly's fluid offers several advantages for the histologic demonstration of glycogen infiltration in the pancreas. As with other chrome-containing solutions, comparable amounts of glycogen are found in the central and in the peripheral regions of blocks fixed in Helly's fluid. Use of celloidin for embedding, or application of a celloidin film to paraffin-embedded sections mounted on slides is unnecessary. Furthermore, such fixation of pancreatic tissue not only permits utilization of methods recommended for histologic demonstration of glycogen and enzymatic digestion of control preparations, but also provides excellent material for concurrent assessment of islet cytoarchitecture with respect to cytoplasmic granulation and relative numbers of cell types. Although the periodic acid-Schiff's reagent routine colors many substances other than glycogen, its reproducibility, simplicity, and brilliant differentiation make it especially valuable for screening old material of doubtful technical quality. Confusion of glycogen with mucin, fibrin, hyalin, basement membranes, and other

substances colored by the periodic acid-Schiff's reagent reaction can be circumvented, at least partially, by enzymatic digestion of control sections. Even so, anomalous results occur with all technics employed in this study; therefore, careful utilization of sections known to contain glycogen as controls for both the staining and digesting processes is essential to accurate interpretation.

SUMMARY

In so-called hydropic degeneration of the pancreatic islets and ductular epithelium of experimental and human diabetes, glycogen was demonstrated in the vacuolated cells by common histologic technics.

The characteristic vacuolar appearance of swollen pancreatic cells affected by "hydropic degeneration" was shown to be referable to artifactual removal of intracytoplasmic accumulations of glycogen rather than to excessive quantities of water or serous fluid.

This pancreatic lesion would be more precisely and distinctively designated by the term glycogen infiltration.

Failure to demonstrate glycogen in suggestively vacuolated islet cells does not necessarily preclude the possibility of its having been present at the time of death.

Glycogen infiltration of human islets of Langerhans may constitute positive morphologic evidence of diabetes mellitus.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 56

Figs. 1 to 6. The swollen, vacuolar appearance of various types of cells after artifactual removal of glycogen from their cytoplasm by routine histologic methods.

FIG. 1. Human vaginal epithelium. Formalin fixation; hematoxylin and eosin stain. × 530.

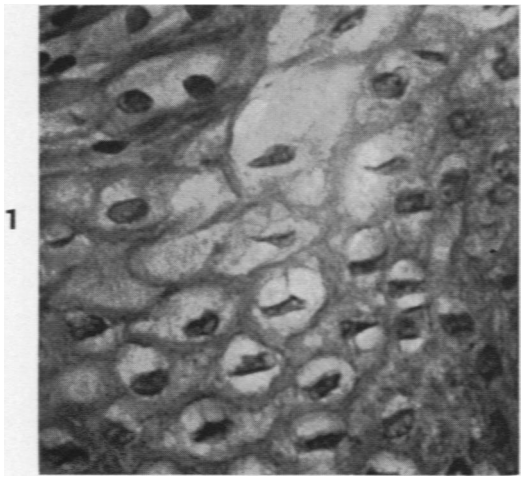
FIG. 2. Human parathyroid adenoma. Formalin fixation; hematoxylin and eosin stain. × 530.

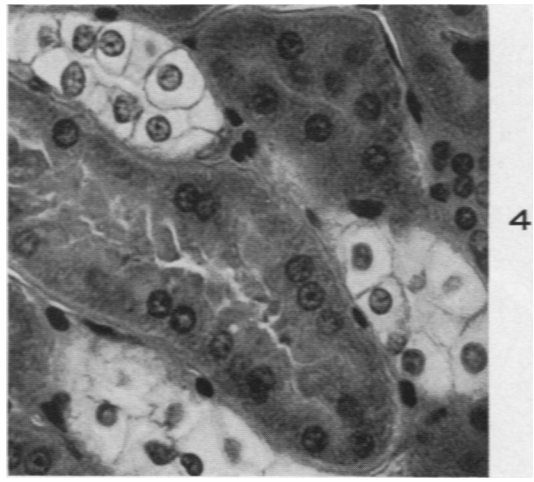
FIG. 3. Human heart muscle fibers adjacent to an area of infarction. Formalin fixation; hematoxylin and eosin stain. × 530.

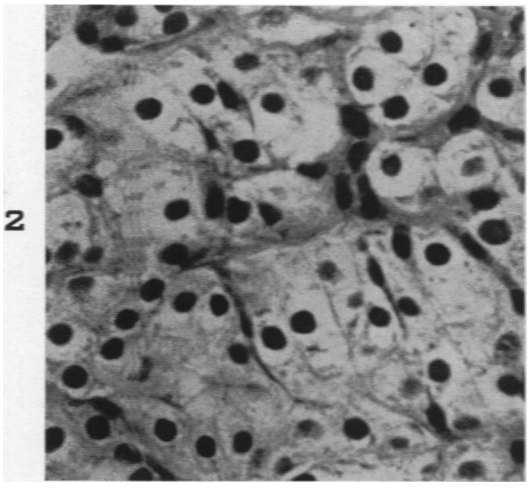
FIG. 4. Armanni lesion in renal tubular epithelium of the rabbit in permanent, severe alloxan diabetes. Helly's fluid; hematin, phloxine, and saffron method. × 530.

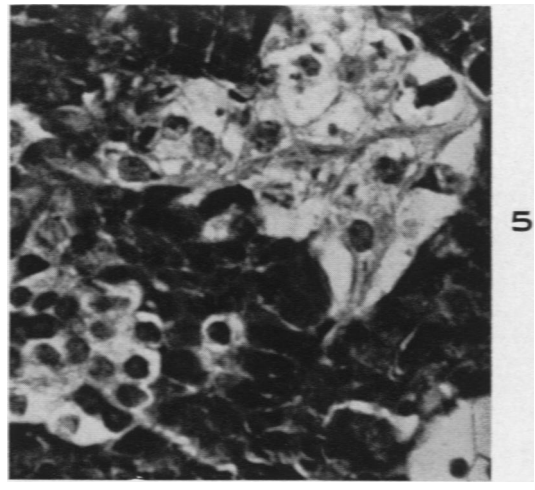
FIG. 5. So-called hydropic degeneration in the pancreatic islet and ductule (lower right) of the rabbit in permanent, severe alloxan diabetes. Helly's fluid; modified Gomori's chromic alum hematoxylin stain. × 530.

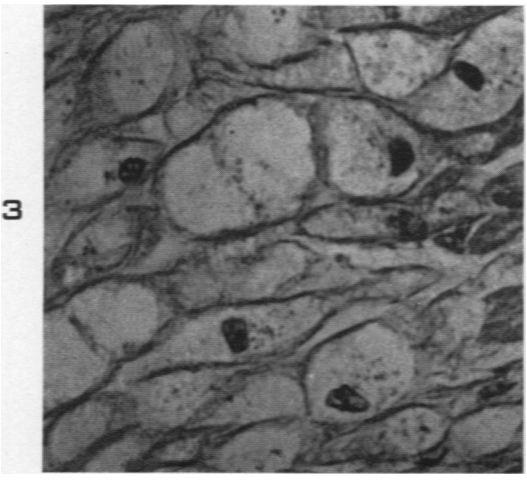
FIG. 6. Human liver in von Gierke's disease. Formalin fixation; hematoxylin and eosin stain. × 530.

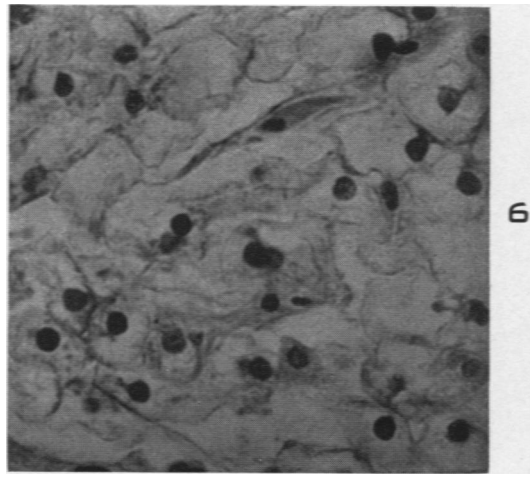












Toreson

Glycogen Infiltration in the Pancreas

PLATE 57

Figs. 7 to 9. Artifactual ipsilateral displacement of histologically demonstrated glycogen.

FIG. 7. Human vaginal epithelium. Formalin fixation; Best's carmine stain. × 530.

FIG. 8. Human "hydropic" heart muscle fibers adjacent to an area of infarction. Formalin fixation; Bauer's chromic acid-Schiff's reagent. × 530.

FIG. 9. Armanni lesion in the renal tubular epithelium of the rabbit in alloxan diabetes. Helly's fluid; Hotchkiss' alcoholic periodic acid-Schiff's reagent. × 530.

Figs. 10 to 12. Pancreas of alloxan diabetic rabbit.

FIG. 10. Glycogen in pancreatic islet cells and centro-acinar cell (lower right). Helly's fluid; Best's carmine stain. × 530.

FIG. 11. Removal of glycogen from pancreatic islet cells and ductule (upper right) by diastase. Helly's fluid; diastase; Hotchkiss' alcoholic periodic acid-Schiff's reagent. × 530.

FIG. 12. Absence of demonstrable mucin from vacuolated pancreatic islet cells. Helly's fluid; Mayer's mucicarmine stain. × 530.

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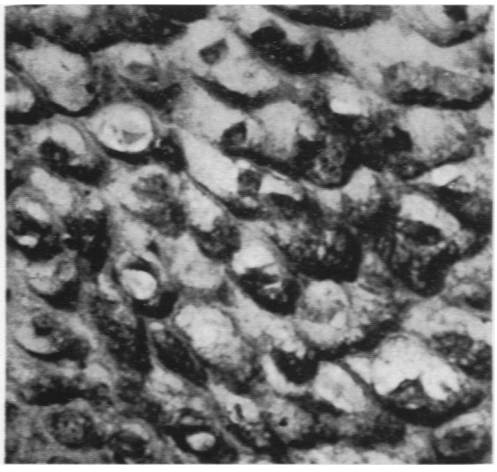

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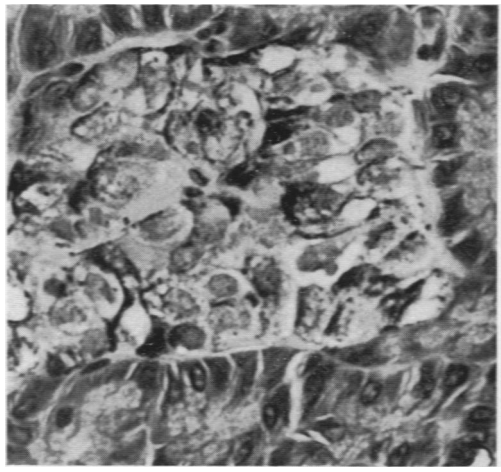

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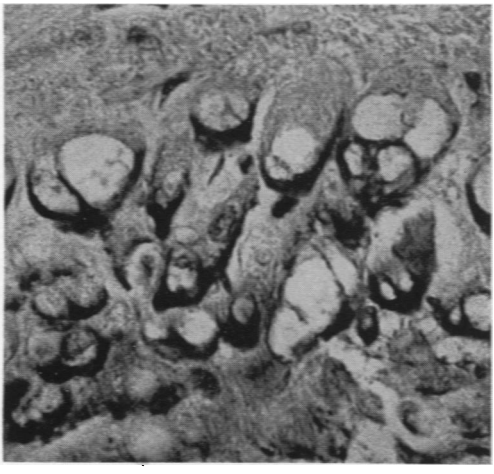

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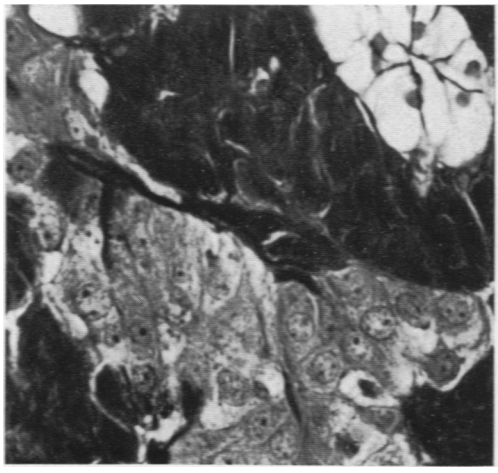

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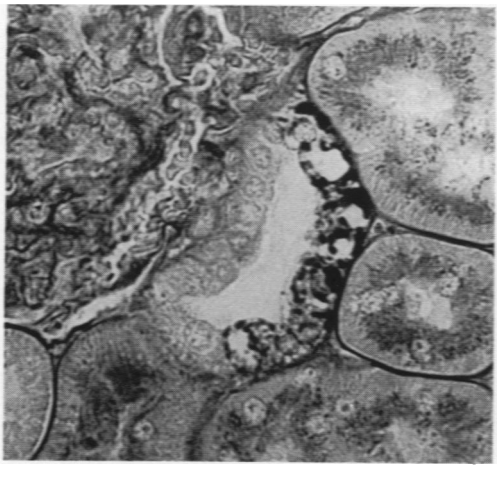

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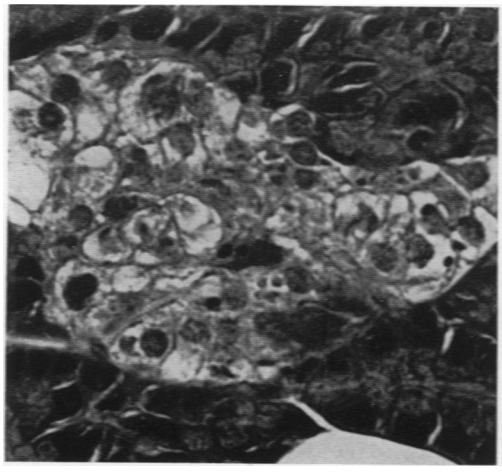


Toreson

Glycogen Infiltration in the Pancreas

PLATE 58

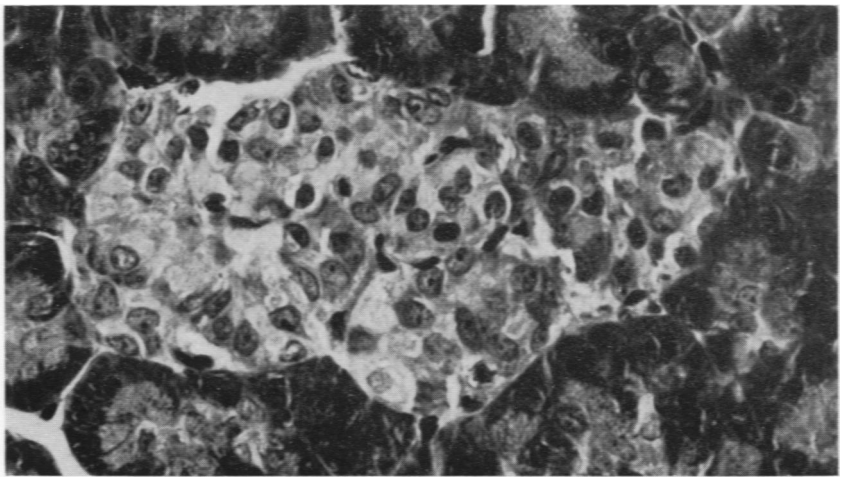
Figs. 13 to 15. Pancreas of a dog treated with anterior pituitary extract.

FIG. 13. Extensive degranulation and slight vacuolation of beta cells of islet. Helly's fluid; modified Gomori's chromic alum hematoxylin stain. × 530.

FIG. 14. Small crescentic bands of glycogen in a few islet cells. Helly's fluid; Best's carmine stain. × 530.

FIG. 15. Removal of glycogen from an islet and ductule by diastase. Helly's fluid; diastase; Best's carmine stain. × 530.

13



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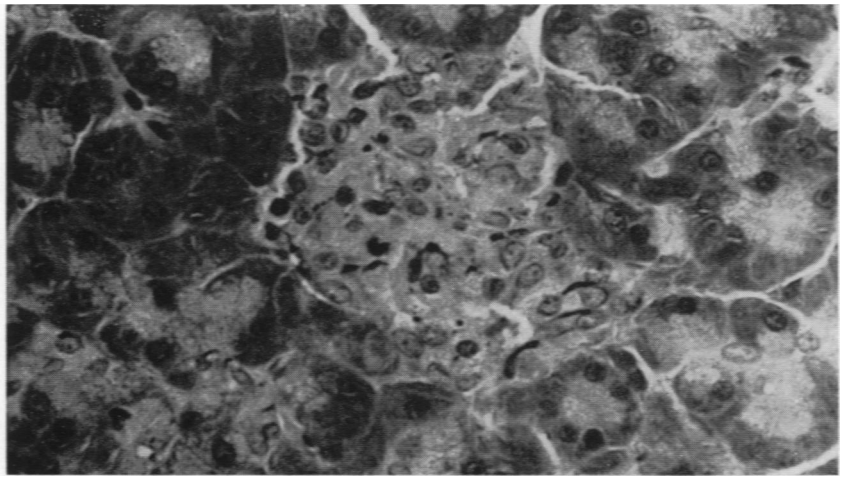

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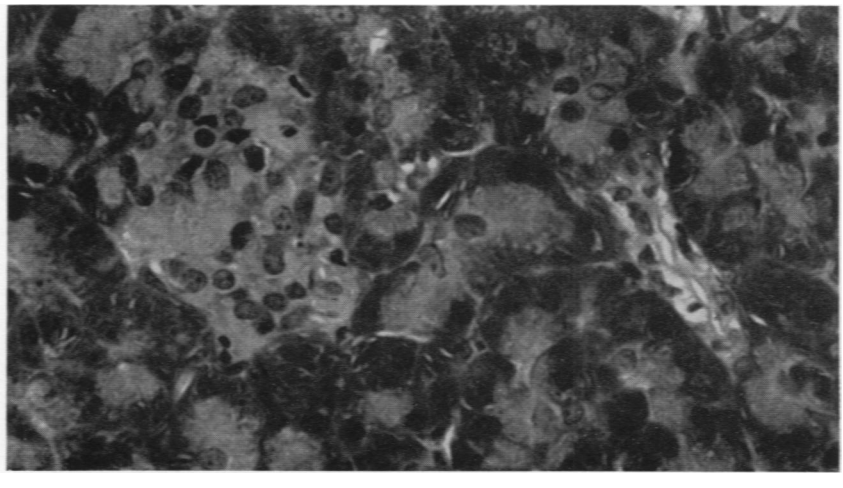


Toreson Glycogen Infiltration in the Pancreas

PLATE 59

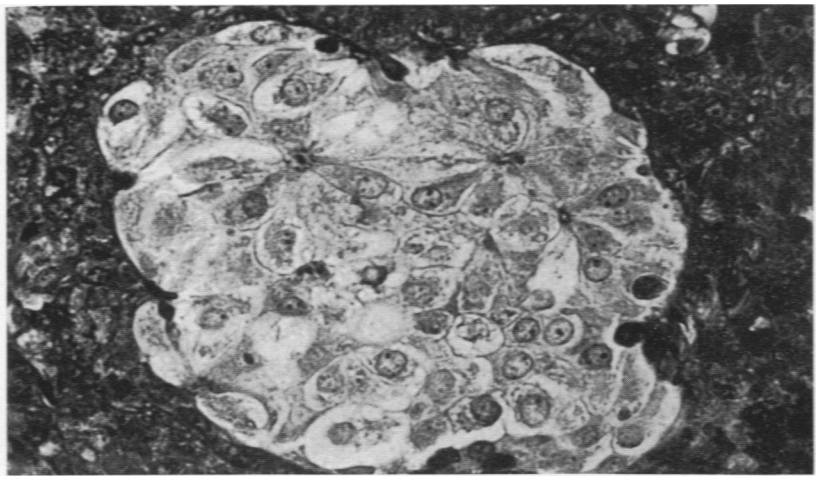
Figs. 16 to 18. Pancreas of cortisone-treated rabbit.

FIG. 16. Marked swelling and vacuolation of beta islet cells without regressive nuclear changes. Vacuolation of centro-acinar cells. Zenker's formal fixation; Masson's trichrome stain. × 530.

FIG. 17. Abundant quantities of glycogen in the cytoplasm of beta islet cells. Several islet and centro-acinar cells incompletely filled by peripherally displaced glycogen. Zenker's formol fixation; Best's carmine stain. × 530.

FIG. 18. Removal of glycogen from beta islet cells by diastase. Zenker's formol fixation; diastase; Best's carmine stain. × 530.

16



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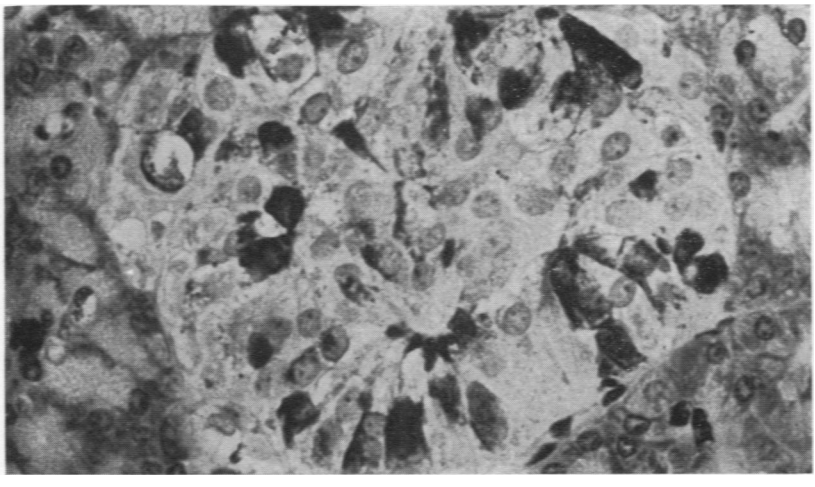

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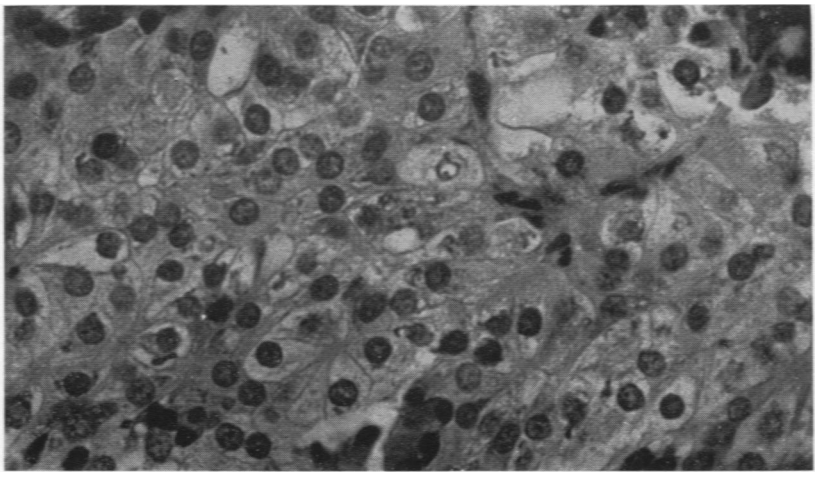


Toreson Glycogen Infiltration in the Pancreas

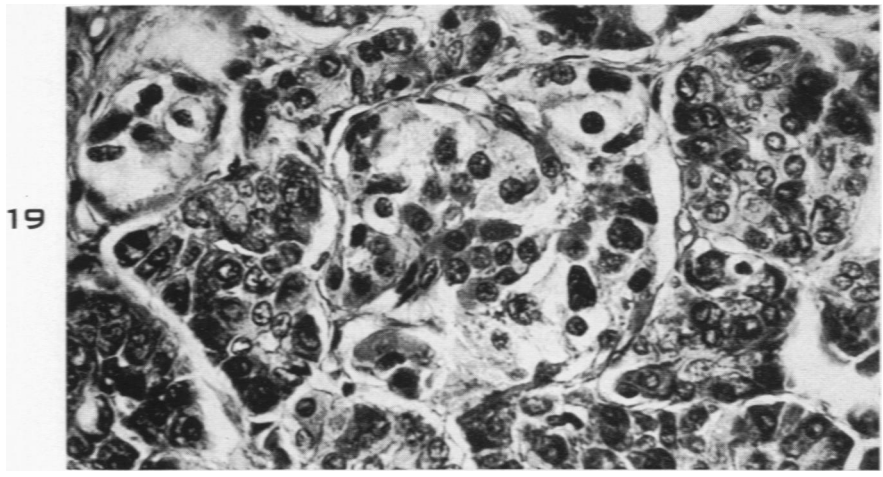
PLATE 60

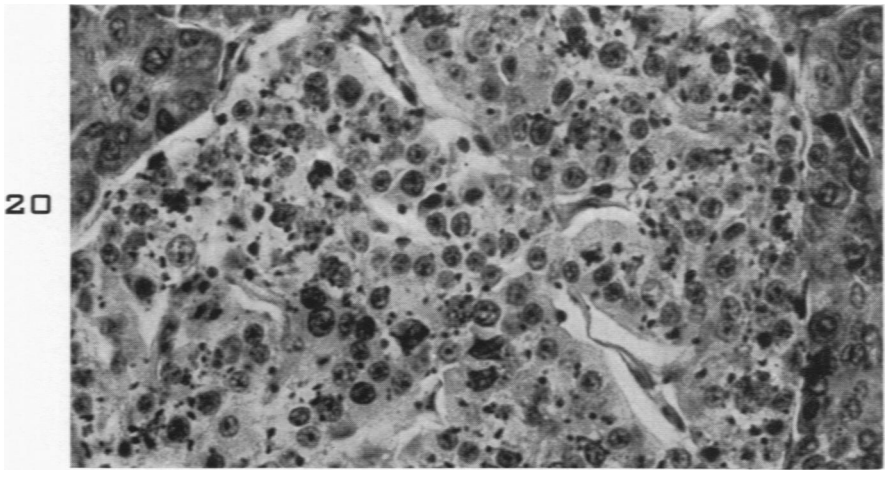
Figs. 19 to 21. Pancreas of a patient who died in diabetic coma.

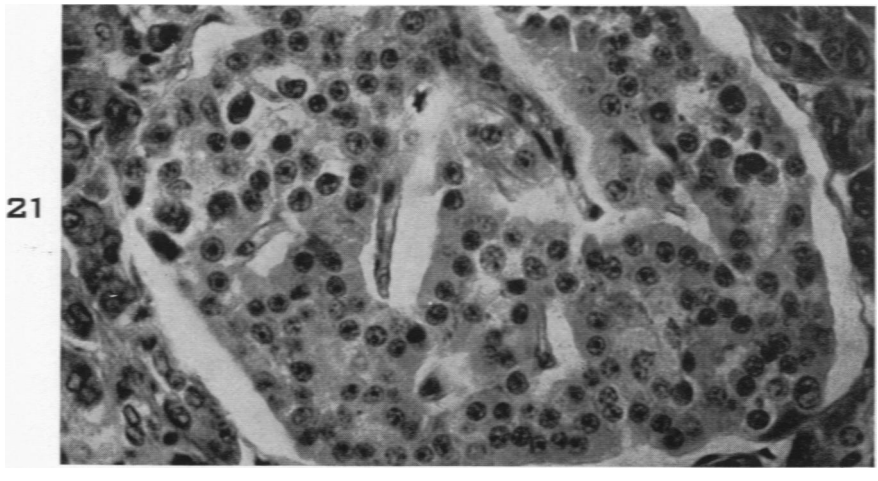
FIG. 19. Swollen and vacuolated beta islet cells. Acetic acid-Bouin's fixation; Masson's trichrome stain. × 530.

FIG. 20. Widespread deposition of granules of glycogen in an islet. Acetic acid-Bouin's fixation; Best's carmine stain. × 530.

FIG. 21. Removal of glycogen from an islet by diastase. Acetic acid-Bouin's fixation; diastase; Best's carmine stain. × 530.







Toreson Glycogen Infiltration in the Pancreas