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Studies on Experimental Diabetes in the Wellesley Hybrid Mouse*

IV. Morphologic Changes in Islet Tissue**

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Summary. Morphologic studies were performed in "Wellesley Hybrid" and commercial laboratory mice fed synthetic chow of two types. The pancreatic islets of diabetic animals of both groups were greatly enlarged, and were composed almost exclusively of beta cells actively synthesizing insulin. The islets of the non-diabetic mice were normal. There was excellent correlation between the presence of diabetes, the level of serum IRI and islet morphology regardless of mouse strain and dietary regimen.

Etudes du syndrome diabétique des souris hybrides de Wellesley. IV. Morphologie des îlots de Langerhans.

Résumé. Nous avons étudié la morphologie des îlots de Langerhans de souris hybrides de Wellesley (C3Hf × I) et de souris albinos ordinaires soumises à deux régimes synthétiques de laboratoire. Nous avons observé une forte augmentation du volume des îlots de Langerhans des animaux diabétiques soumis aux deux types de régime, ces îlots étant composés presque exclusivement de cellules B actives. L'apparence des îlots de Langerhans d'animaux non-diabétiques était normale. Il existe chez ces animaux une excellente corrélation entre la présence de diabète, les taux d'insuline immunoréactive sérique, et l'apparence des îlots, ceci pour les deux régimes.

Der experimentelle Diabetes der hybriden Wellesley-Maus. IV. Morphologische Veränderungen in den Langerhans'schen Inseln.

Zusammenfassung. An "Wellesley Hybrid" und normalen Laboratoriumsmäusen, denen zwei verschiedene Arten von synthetischem Futter gegeben worden war, wurden morphologische Studien durchgeführt. Die Langerhans'schen Inseln diabetischer Tiere beider Gruppen waren stark vergrößert und bestanden beinahe ausschließlich aus aktiv Insulin synthetisierenden β-Zellen. Die Inseln der nicht-diabetischen Mäuse waren normal. Es bestand ein deutlicher Zusammenhang zwischen dem Vorhandensein von Diabetes, dem immunreaktiven Insulin Spiegel im Serum und der Inselmorphologie, unabhängig von Mäusestamm und Diät.

Key-words: Spontaneous Diabetes, Wellesley hybrid mouse, Hybrid mouse diabetes, Genotype: C3Hf × I–Fl, Ultrastructure, Beta cells, Nutrition and diabetes, Diet and diabetes, Insulin in serum, Obesity, Strains of mice.

The "Wellesley Hybrid" mice were produced from matings of male strain I and female strain C3Hf mice, raised in the Department of Zoology and Physiology laboratories of Wellesley College by one of the authors (EEJ).

In earlier studies, the hybrid mice were noted to gain weight more rapidly than the parental strains and to reveal intermittent hyperglycemia and glycosuria most frequently during 4 to 9 months of age. It was noted further that glycosuria was more frequent among the male (53%), in contrast to the female (5%) hybrids, and that the frequency of glycosuria decreased beyond nine to twelve months of age. Previous publications (Jones, 1964; Like et al., 1965) reported the frequent occurrence in the hybrid mice of greatly enlarged islets of Langerhans, consisting almost exclusively of beta cells.

This paper reports the results of morphologic studies of the pancreatic islets in three groups of "Wellesley Hybrid" mice and preliminary observations in a number of commercially available albino mice serving as controls.

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** Paper III of this series is Gleason et. al., 1967.

Materials and Methods

Portions of pancreas from 29 mice were obtained for morphologic study. The mice were separated into the following groups:

Fifteen male "Wellesley Hybrid" mice, six to ten months of age at the time of sacrifice, were maintained on a diet of Purina Laboratory Chow[1]. Thirteen of the fifteen mice were euglycemic (values below 200 mg/100 ml) during the four months of observation, with normal levels of serum immunoreactive insulin (IRI) (below 50 μU/ml). Two mice, eight and ten months of age were intermittently hyperglycemic (blood glucose values between 210 and 280 mg/100 ml) for ten weeks, with significantly elevated serum IRI at sacrifice (505 and 420 μU/ml). The pancreata of this group of fifteen animals were studied by light microscopy.

A second group of fourteen mice, twelve to sixteen months of age, included ten hybrid males and four parental strain mice (2 female C3Hf and 2 male I strain mice). All had been maintained on a diet of Old Guilford Breeding pellets[2]. The four parental strain mice never displayed glycosuria and were normoglycemic

[1] Purina Laboratory Chow: St. Louis, Missouri.

[2] Emory Morse Company: Guilford, Connecticut.

at sacrifice. The hybrid mice revealed intermittent glycosuria and hyperglycemia for variable periods of time. The pancreata of these animals were studied by light and electron microscopy and reported previously (LIKE et al., 1965).

Key for figures' lettering

AC = Acinar cells
AR = Agranular (smooth) reticulum
BM = Basement membrane
CAP = Capillary
cer = Ceroid pigment
D = Duct
F = Fenestrated endothelial cell
G = Golgi complex
GR = Granular endoplasmic reticulum
M = Mitochondrion
N = Nucleus
ne = Unmyelinated nerve
P = Polysomes and "free" ribosomes
RBC = Red blood cell

Fig. 1, 2, 3, 4, 9 and 10. Light micrographs of paraffin embedded tissues fixed in Bouin's solution

Fig. 5–8. Electron micrographs of tissue fixed in collidine-buffered osmium tetroxide, embedded in Epon and stained with aqueous uranyl acetate followed by lead (KARNOVSKY)

A third group of six male hybrid mice, seventeen to twenty-one months of age, manifested either persistent or intermittent hyperglycemia (values in excess of 200 mg/100 ml) while on a diet of Old Guilford Breeding pellets. Blood glucose values, however, returned to normal levels when the diet was changed to either Purina Chow *pellets* or *powdered* Old Guilford Breeding pellets (GLEASON et al., 1967). Reinstitution of Old Guilford Breeding *pellets* did not result in a return of hyperglycemia. Serum IRI levels prior to the change of diet were in excess of 800 μU/ml in all animals. At the time of sacrifice, all but one mouse revealed elevated levels of serum IRI (85 to 915 μU/ml). The pancreata of these animals were studied by light microscopy.

A fourth group of eleven Albino Swiss-Hauschka mice[3] were fed Old Guilford Breeding pellets for six months. Persistent hyperglycemia (340 to 450 mg/100 ml) appeared in one animal after 6 weeks, and in a second after 22 weeks (GLEASON et al., 1967). Serum IRI levels at sacrifice were 2400 μU/ml and 300 μU/ml respectively. The pancreata of these eleven mice were studied by light microscopy.

More than fifty per cent of a larger group of Swiss-Hauschka mice, on an identical diet, have become diabetic and are now under study (GLEASON et al., 1967). One diabetic mouse from this group was killed for light microscopic and ultrastructural studies of the pancreatic islets. Blood glucose at sacrifice was 450 mg/100 ml and serum IRI was in excess of 1500 μU/ml.

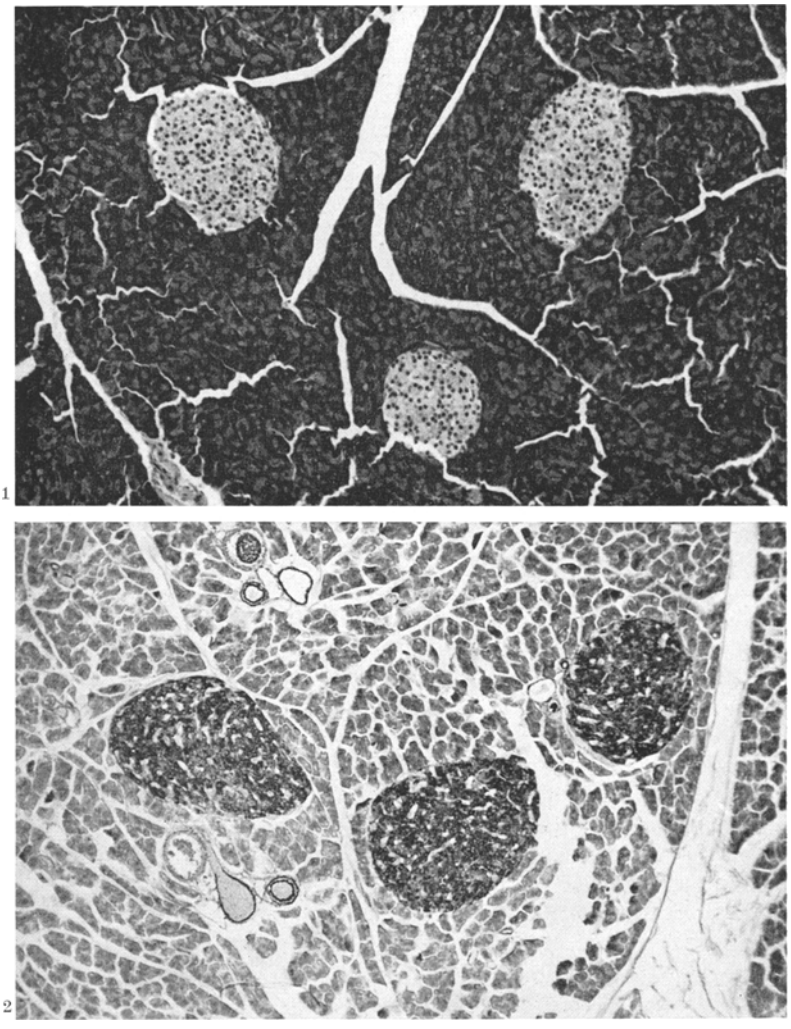


Fig. 1 and 2. Pancreatic islets of normoglycemic mice. Essentially identical in appearance in parental strain (C_3H_f and I), Swiss-Hauschka or Purina Chow-fed Wellesley Hybrid mice. — Fig. 1. Hematoxylin and eosin. 100 × . — Fig. 2. Aldehyde fuchsin stain. The deeply staining, well granulated beta cells are characteristic of normal mice. 70 ×

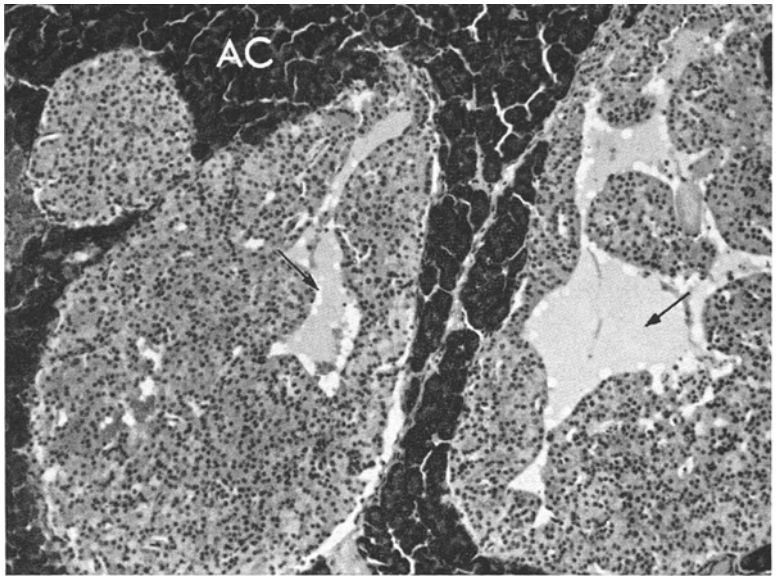
For histological examination, pancreata were processed as previously reported (LIKE and MIKI, 1967). With the exception of the pancreas of the Swiss-Hauschka mouse, which was fixed in glutaraldehyde, all tissues were prepared for electron microscopy in buffered osmium tetroxide. Methods of blood glucose and serum IRI determinations are described in the preceeding reports (GLEASON et al., 1967 and CAHILL et al., 1967).

Results

The pancreatic islets of the Guilford Chow-fed euglycemic parental strains (C3Hf and I) and the

[3] Charles River Breeding Laboratories: Wilmington, Massachusetts.

Swiss-Hauschka mice, and the Purina Chow-fed euglycemic "Wellesley Hybrid" mice were morphologically indistinguishable. Conventional hematoxylin and eosin and aldehyde fuchsin stained preparations revealed the normal size, number, and granularity of the islets in these animals (Fig. 1 and 2).



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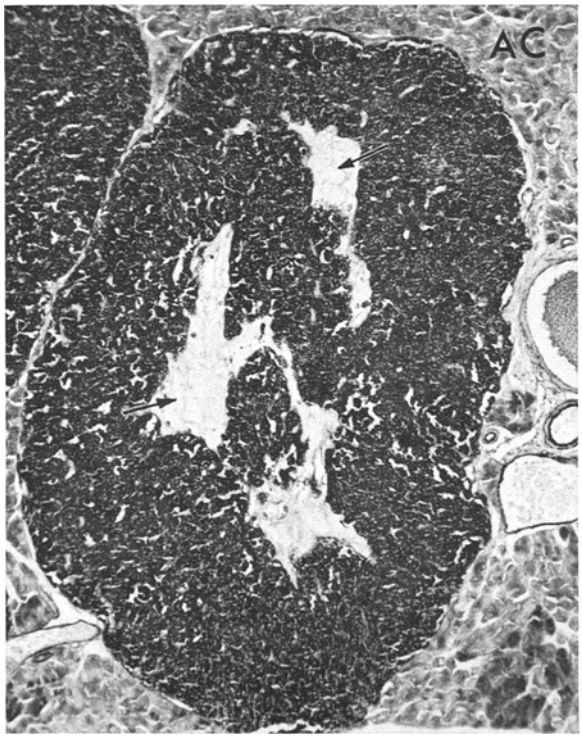

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Fig. 3 and 4. Pancreas of a diabetic Wellesley Hybrid mouse. The greatly enlarged islets reveal central cavitation (arrows). Surrounding exocrine pancreas (AC) is normal. — Fig. 3. Hematoxylin and eosin stain. 100 ×. — Fig. 4. Aldehyde fuchsin stain. 70 ×

The pancreas of one of the two Wellesley Hybrid mice that developed mild intermittent hyperglycemia on a diet of Purina Chow pellets revealed an increase in islet size and both revealed moderate beta cell degranulation. The largest islets, however, were considerably smaller than those previously observed in animals consuming Old Guilford Breeding pellets (JONES, 1964).

The islets of Langerhans of the two groups of diabetic Wellesley Hybrids, ages 12—16 (10 mice) and 17—21 months (6 mice), were comparable and will be described together. The appearance of the greatly enlarged islets observed in all of these animals is illustrated in Fig. 3 and 4. These light micrographs illustrate the tendency to central cyst formation among the larger lesions, and the presence of well granulated (aldehyde—fuchsin positive) beta cells (Fig. 4). Hemorrhagic cysts have also been observed (JONES, 1964). Electron microscopic studies provided further evidence that the greatly enlarged islets consisted almost exclusively of beta cells. The smooth-contoured, non-indented nuclei, as well as the appeareance of the secretory granules, were characteristically those of beta cells (Fig. 6—8). Ultrastructural differences were visible that rendered the hyperplastic cells readily distinguishable from normal beta cells (Fig. 5). The secretory granules of the former were diminished in number and in sharp contrast with normal "resting" beta cells in which cytoplasmic granules were plentiful. The organelles responsible for protein synthesis, i.e., the granular endoplasmic reticulum and free ribosomes, were more abundant in the cells of the enlarged islets. Mitochondria were usually increased in size and number, bizarre in configuration, and contained numerous closely packed cristae mitochondriales. The enlarged Golgi structures were widely dispersed throughout the cells, with many collections of smooth and coated vesicles visible. The small number of remaining beta granules were not aggregated at the cell membranes (Fig. 7), hence evidence of "emiocytosis" was not visible (LACY, 1959). Alpha cells of normal appearance were present, but with greatly decreased frequency (LIKE et al., 1965).

Representative pancreatic islets of the three Swiss-Hauschka mice that became diabetic when fed Old Guilford Chow are illustrated in Figs. 9 and 10. Although the striking increase in islet size was apparent, the magnitude of the hyperplasia fell short of that noted in the Wellesley Hybrids and the aldehyde fuchsin stain revealed relative degranulation of the beta cells. When the electron microscopic appearance of the islet cells in one of these animals was compared with that of the Wellesley Hybrids, striking similarities were apparent. Thus, the beta cells of both diabetic groups revealed evidence of degranulation, increased quan-

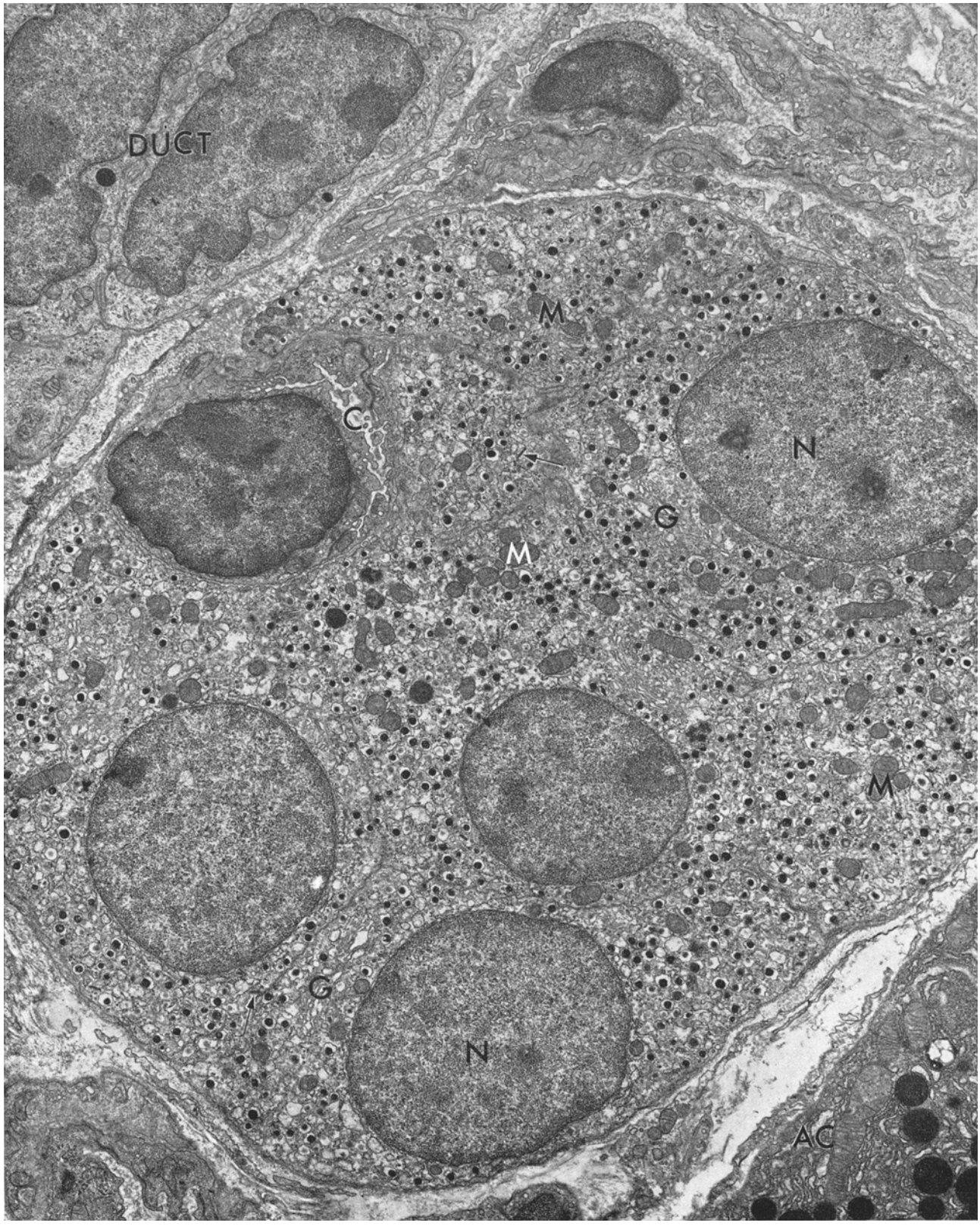


Fig. 5. Islet of a normoglycemic mouse. Lower power electron micrograph of a small islet, composed of normal beta cells and surrounded by elements of the exocrine pancreas. The numerous round cytoplasmic granules, the small Golgi complexes, the inconspicuous granular endoplasmic reticulum and the slender mitochondria are characteristic. Occasional bar-shaped beta granules are visible (arrows). Osmium tetroxide. Approximately 6000 ×

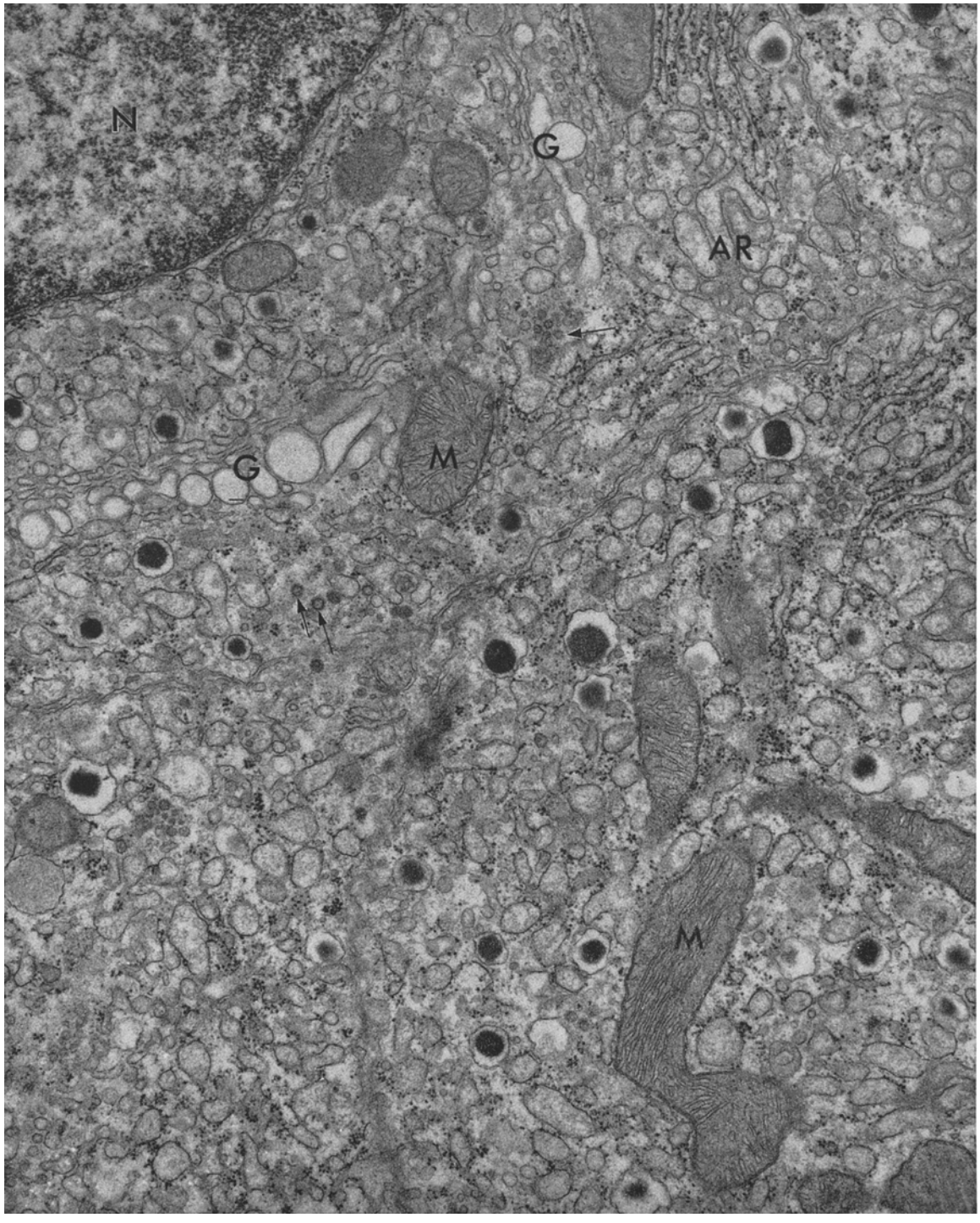


Fig. 6–8. Electron micrographs of beta cells of diabetic Wellesley Hybrids.

Fig. 6. Portions of several cells of a hyperplastic islet. The nucleus is smoothly contoured and the reduced number of cytoplasmic granules are contained in loosely fitting sacs. Note the large mitochondria and Golgi complex. Numerous coated vesicles (arrows) and the smooth tubular structures (? agranular reticulum) are presumably of Golgi origin. Osmium tetroxide. Approximately 21 000 ×.

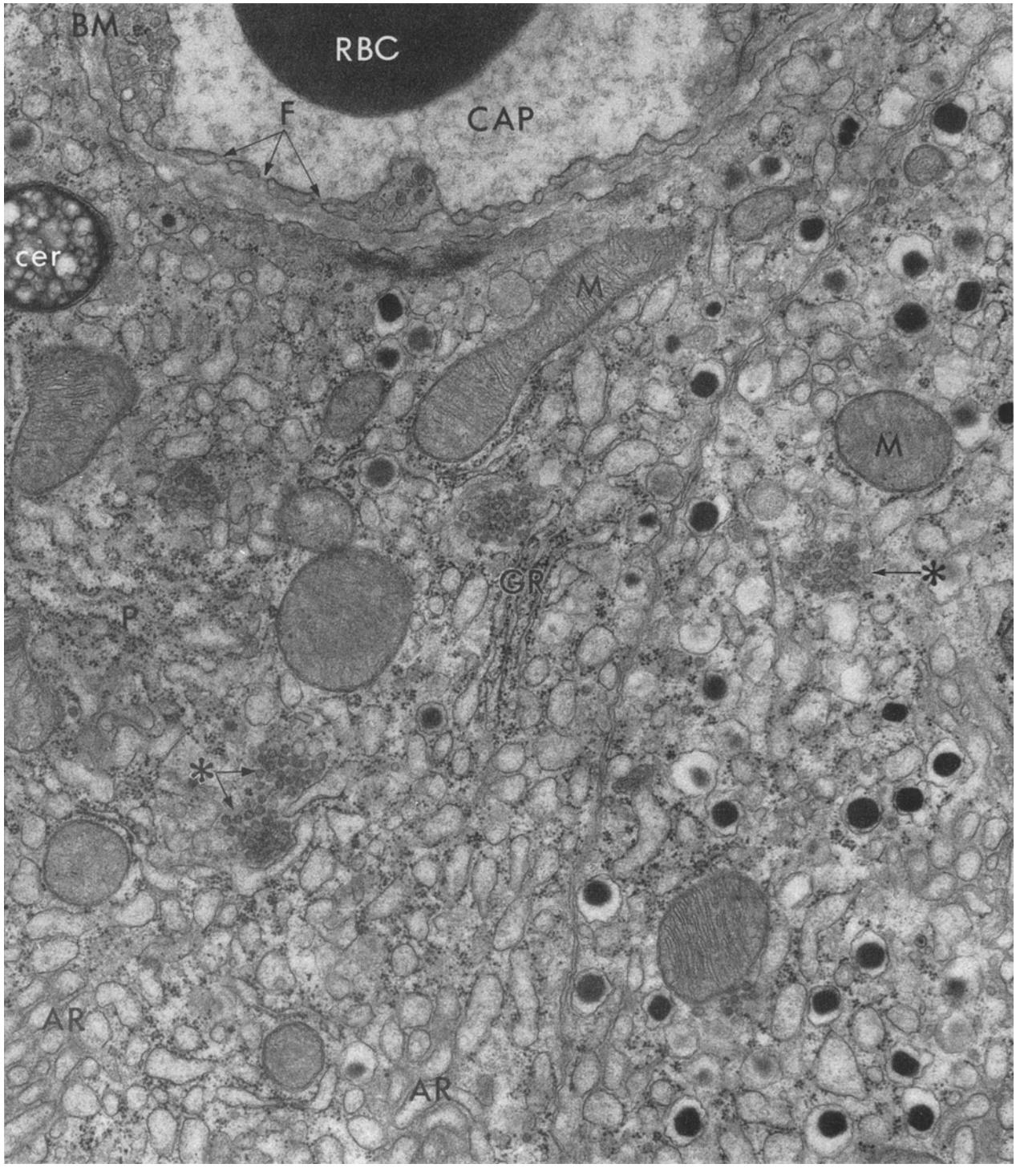


Fig. 7. Beta cells and an adjoining capillary. Beta granules are not localized at the vascular pole of the cells in the hyperplastic islets. Tubules of the hypertrophied granular endoplasmic reticulum are cisternal and often dilated or vesicular. Smooth tubules (AR) are also noted, as are collections of small vesicles (arrows). Osmium tetroxide. Approximately 21000 ×. —

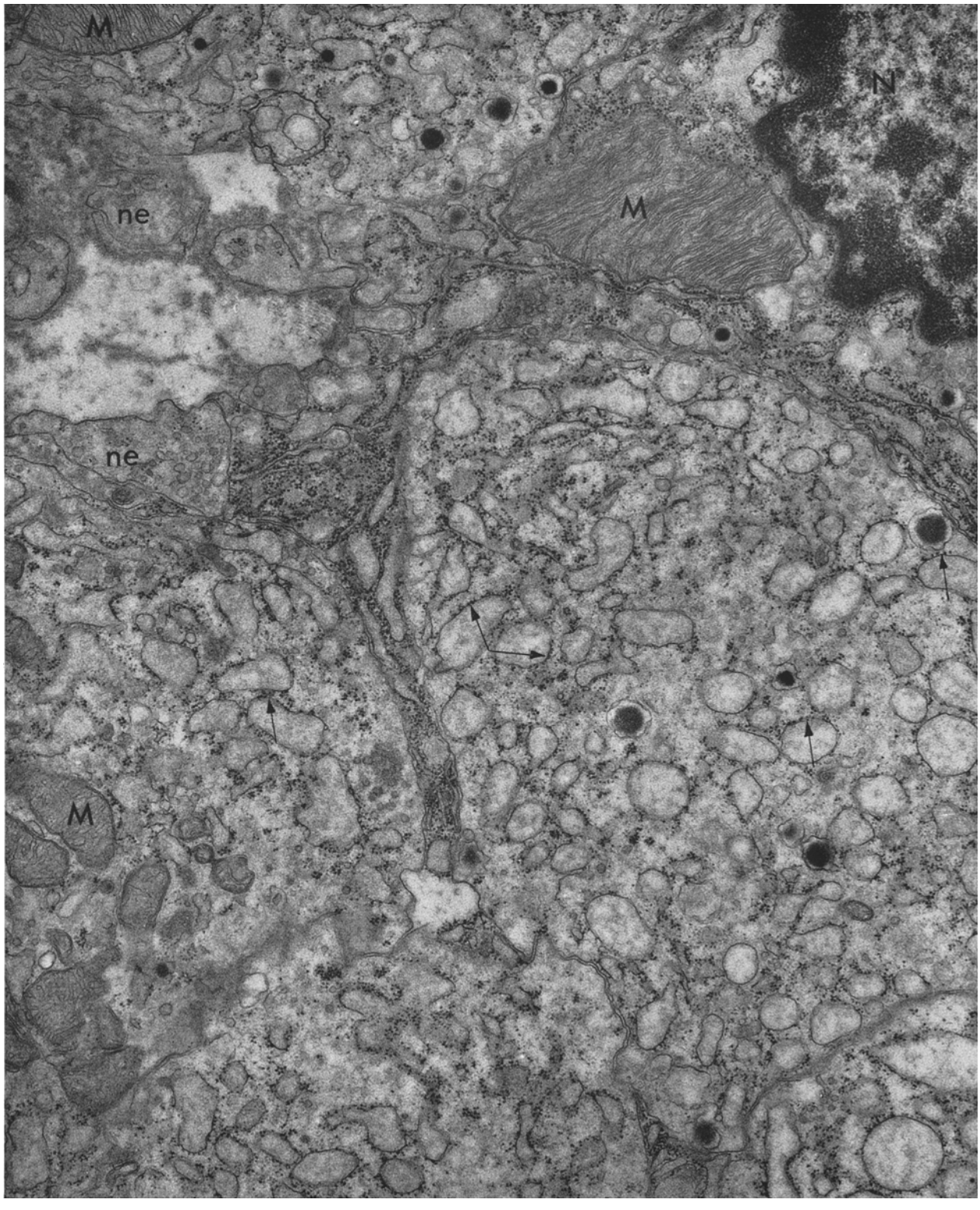
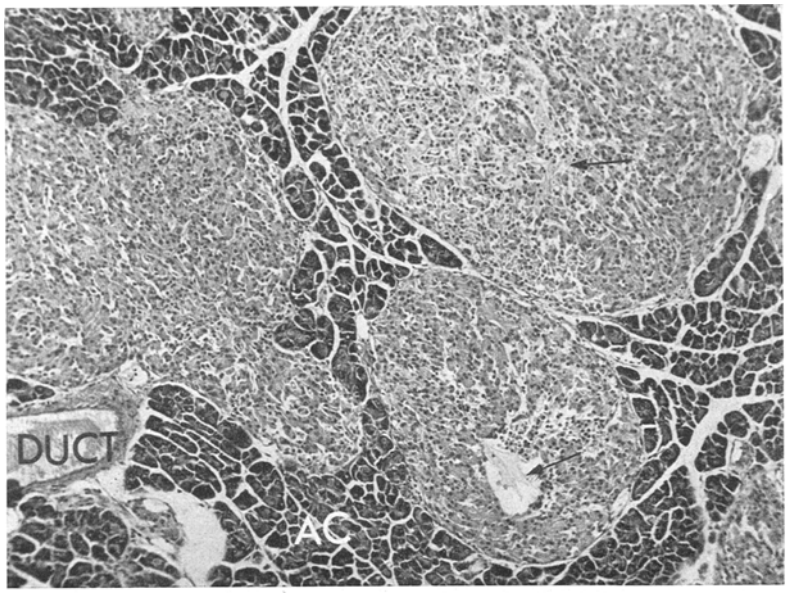


Fig. 8. Another example of a large bizarre mitochondrion. Beta granules are infrequent and the vesicular-appearing, granular endoplasmic reticulum prominent. Small arrows indicate ribosomes attached to vesicular membranes in spotty fashion. Osmium tetroxide. Approximately 21000 ×

tities of granular endoplasmic reticulum, enlarged Golgi structures, and more numerous and often bizarre-appearing mitochondria.



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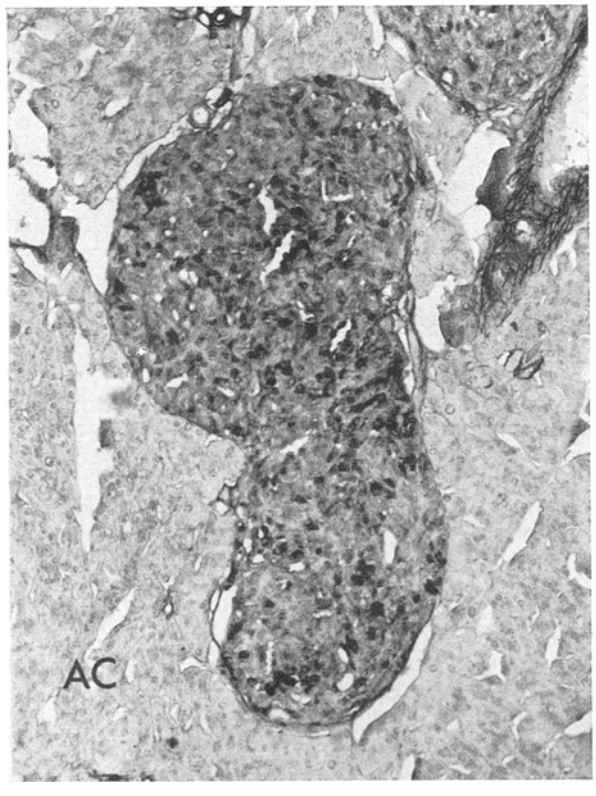

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Fig. 9 and 10. Hyperplastic islets of diabetic Swiss-Hauschka mouse.

Fig. 9. Degeneration (arrow) and eventual cavitation (arrow with asterisk) also a feature of these greatly enlarged islets. Surrounding acinar tissue (AC) normal. Hematoxylin and eosin. 100 ×.

Fig. 10. A significant proportion of the beta cells are degranulated. (compare with Fig. 4). Aldehyde fuchsin. 110 ×

Discussion

Accompanying papers in this issue (Cahill et al., 1967; Gleason et al., 1967) have detailed the critical role of the diet in the evolution of diabetes mellitus in the Wellesley Hybrid mouse and the commercially available Swiss-Hauschka mouse. The morphologic studies reported here demonstrate the close correlation between the presence of diabetes and islet morphology, in both animal groups.

The endocrine pancreas of those mice that failed to develop hyperglycemia at any time during the study were almost always normal regardless of the diet. In contrast, the islets of hyperglycemic animals were significantly altered with their overall size gradually increasing with the duration and severity of the syndrome. Thus, the beta cell degranulation and slight increase in islet size observed in the Purina chow-fed, mildly diabetic hybrids, eventually leads to the greatly enlarged, well-granulated islets of the older animals after prolonged maintenance on Old Guilford Breeding Pellets and long-standing diabetes. The apparent absence of an observable size difference between the pancreatic islets of the two age groups of hybrid mice, might be attributed to an error of sampling. The pancreata of the older group were used only for morphologic study, whereas those of the younger group were processed for insulin extraction as well as histologic procedures.

The maintenance of normal blood glucose levels by the six Wellesley Hybrids, (17—21 months of age) after the reinstitution of Old Guilford Breeding pellets deserves further comment. The continued elevation of serum IRI and the presence of greatly enlarged islets in these mice at a time when blood glucose levels are normal, strongly suggests that more than normal quantities of insulin are required, and are available, to maintain euglycemia. The enlarged islets, with ultrastructural evidence of enhanced protein synthesis, have presumably successfully satisfied the increased demand for insulin. It should be reiterated, that earlier studies had indicated the decreased incidence of glycosuria among the hybrid mice beyond 12 months of age in the face of continued peripheral resistance to insulin (Jones, 1964; Like et al., 1965).

The group of presently diabetic Swiss-Hauschka mice will be followed with interest to determine whether with time, they too can enlarge their mass of pancrea-

tic islet tissue sufficiently to cope with the insulin requirements, and return the blood glucose to normal levels.

Although the obese hyperglycemic mice (BJORKMAN et al., 1963), the KK mice (NAKAMURA, 1967), and the Spiny mouse (PICKET et al. 1967) have been shown to have enlarged islets and increased levels of circulating insulin, there is no reported amelioration of the diabetic syndrome with age in those animal groups.

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