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Diabetic Syndrome in Sand Rats*

IV. Morphologic Changes in Islet Tissue**

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Summary. Ultrastuctural and light microscopic studies of pancreatic islets in normal and diabetic sand rats are reported. Following the institution of a synthetic chow diet, beta cell degranulation and enhanced protein synthesis were observed. With the appearance of diabetes, glycogen infiltration occurred, with displacement of cellular organelles and eventual cytoplasmic degeneration and liquefaction. These alterations were correlated with blood glucose and serum immunoreactive insulin levels. — The sand rats are unique in that they are not able to cope with the increased caloric load of synthetic chow. They respond by marked insulin production; an increase that usually maintains the animal free of ketosis, occasionally returns them to a euglycemic state, and may rarely be terminated by beta cell degeneration and necrosis with fatal ketoacidosis.

Syndrome diabétique chez le rat du sable (Psammomys obesus). IV. Altérations morphologiques du tissu insulaire.

Résumé. Nous avons étudié avec les microscopes optique et électronique les îlots de Langerhans du pancréas de rats des sables normaux et diabétiques. Lorsqu'ils sont maintenus à un régime normal de laboratoire, on observe chez ces animaux une dégranulation des cellules β et les signes d'une synthèse protéique augmentée. Dès qu'apparaît le diabète, on voit apparaître une infiltration glycogénique avec déplacement des organelles cellulaires et, plus tard, une dégénérescence cyptoplasmique avec liquéfaction. L'apparition de ces anomalies morphologiques a été mise en corrélation avec les altérations du glucose sanguin et de l'insuline immunoréactive sérique. Ce qui est remarquable chez le rat des sables, c'est qu'il ne semble pas être à même de s'adapter à l'apport calorique plus concentré de la nourriture de laboratoire. Il réagit par une surproduction insulinique, surproduction qui, en général, évite la cétose. Parfois, cette surproduction suffit à ramener le sucre sanguin à la normale. Dans des cas plus rares, la stimulation de la sécrétion insulinique se termine par une dégénérescence et une nécrose des cellules β avec céto-acidose mortelle.

Das diabetische Syndrom bei der Sandratte (Psammomys obesus): IV. Morphologische Veränderungen des Inselgewebes

Zusammenfassung. Es wird über elektronen- und lichtmikroskopische Untersuchungen an Pankreas-Inseln normaler und diabetischer Sandratten berichtet. Nach Verabreichung einer synthetischen Keks-Diät wurden eine Degranulation der β-Zellen und Zeichen einer vermehrten Proteinsynthese beobachtet. Gleichzeitig mit dem Auftreten von Diabetes erfolgte Glykogeninfiltration, begleitet von einer Verdrängung der Zellorganellen und gelegentlicher Degeneration und Verflüssigung des Cytoplasmas. Diese Veränderungen wurden mit Blutzucker- und immunreaktivem Serum-Insulinspiegel in Zusammenhang gebracht. — Sandratten sind einzigartig in der Hinsicht, daß sie nicht in der Lage sind, das vermehrte Kalorienangebot der synthetischen Diät auf normale Art zu bewältigen. Sie reagieren mit Mehrproduktion von Insulin. Die gesteigerte Insulinausschüttung hält das Tier gewöhnlich frei von Ketose, bringt den Blutzucker gelegentlich auf normale Werte zurück, endet aber in seltenen Fällen mit einer Degeneration und Nekrose der β-Zellen mit anschließender fataler Ketoacidose.

Key-words: Spontaneous Diabetes, Sand rat, Psammomys obesus, Pancreas. Ultrastructure, Beta cells, Alpha cells, Protein synthesis, Insulin in plasma, Insulin in pancreas, Obesity, Nutrition and diabetes, Diet and diabetes.

In the course of a systematic study of the diabetic syndrome in the Egyptian sand rat, *Psammomys obesus*, animals were imported from the United Arab Republic (U.A.R) during the summer of 1964 and 1965. Those received in 1964 were observed to develop a fulminating, acute ketotic diabetic syndrome when Purina laboratory chow was given in place of a diet of mixed fresh vegetables (Miki et al., 1966). The animals imported in 1965, and their American-born offspring, consumed less laboratory chow and developed a milder form of the disease (Miki et al., 1967) resembling more closely the syndrome originally reported by Schmidt-Nielsen (1964) and Hackel (1965).

Material for morphologic examination was obtained from the animals studied physiologically, and additional specimens were obtained in the U.A.R. during July-August, 1965.

This report gives details of the light microscopic and ultrastructural appearance of the pancreatic islets of the normal sand rat, and the changes observed after institution of a synthetic chow diet, with and without the appearance of diabetes mellitus. Since physiologic studies were also performed on these

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** Paper III of this series is De Fronzo et. al. 1967

animals, structural-functional correlations were often possible.

Material and Methods

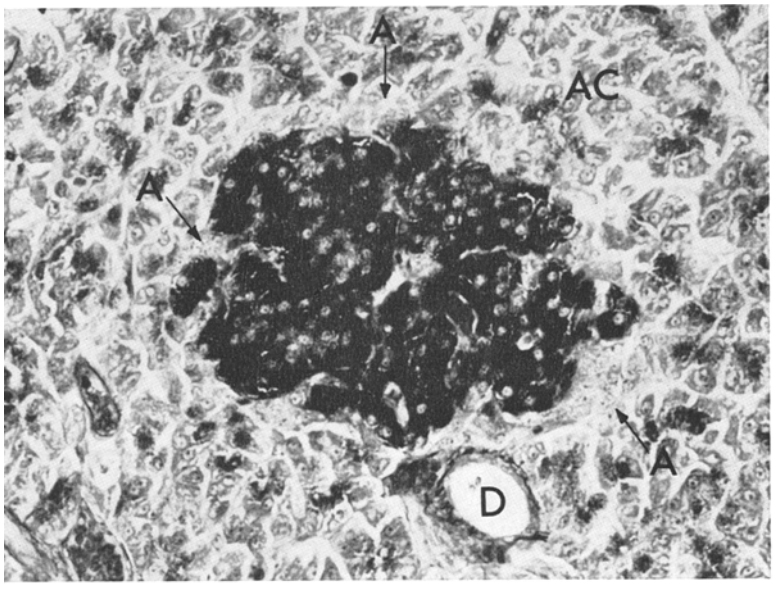
Portions of, or the entire pancreas of 75 sand rats were obtained for morphologic study. The animals were divided into 5 groups.

1. 12 animals killed immediately after or within one day of capture in the U.A.R. with food intake therefore limited exclusively to that found in the vicinity of their burrows (*Salicornia fruticosa* or *Suaeda fruticosa*).

2. 10 members of the group imported to the U.S. in 1964, some of whom developed the acute fulminating diabetic syndrome. This group of rats was maintained exclusively on Purina laboratory chow.[1]

3. 18 animals, including those imported into the U.S. in 1965, and their American-born offspring. These rats consumed Purina laboratory chow or Old Guilford breeding pellets[2] less avidly and developed a milder form of diabetes.

4. 23 rats, all American-born offspring of the group imported in 1965. They were fed a mixed diet of laboratory chow and fresh vegetables and were utilized to evaluate the effect of age on the responsiveness of



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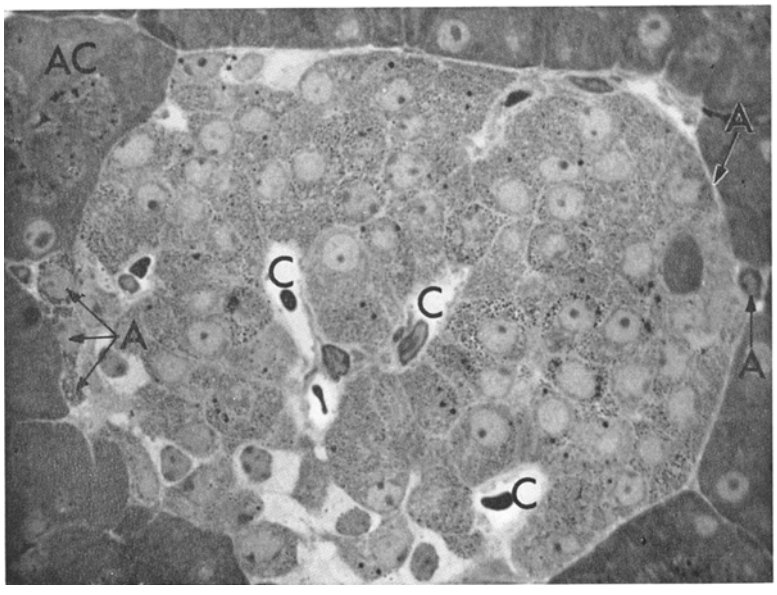

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Key for figures' lettering

A	=	Alpha cell
AC	=	Acinar cells
AR	=	Agranular (smooth) reticulum
B	=	Beta cell
BM	=	Basement membrane
C	=	Capillary lumen
cen	=	Centriole
D	=	Duct
G	=	Golgi complex
GLY	=	Glycogen
GR	=	Granular endoplasmic reticulum
L	=	Presumed Lysosome
M	=	Mitochondrion
mt	=	Micro tubules
N	=	Nucleus
ne	=	Unmyelinated nerve
np	=	Nuclear pore
P	=	Polysomes and "free" ribosomes
RBC	=	Red blood cell
V	=	Vacuole

Figures 1, 7, 12, 14, 15 and 22: Light micrographs of paraffin embedded tissues fixed in Bouin's solution

Figures 2, 8, 13 and 23: Light micrographs of 1 micron sections of Epon embedded tissues fixed for electron microscopy

Fig. 1. Pancreatic islet of normal sand rat, aldehyde fuchsin stain. The deeply staining central beta cells are characteristic. The peripheral alpha cells (arrows) are unstained. 260 ×

Fig. 2. Pancreatic islet of normal sand rat, toluidine blue stain. Beta cells with numerous fine cytoplasmic granules predominate. Alpha cells, (arrows) are difficult to discern. 640 ×

[1] Purina laboratory chow: St. Louis, Missouri.
[2] Emory Morse Company: Guilford, Connecticut.

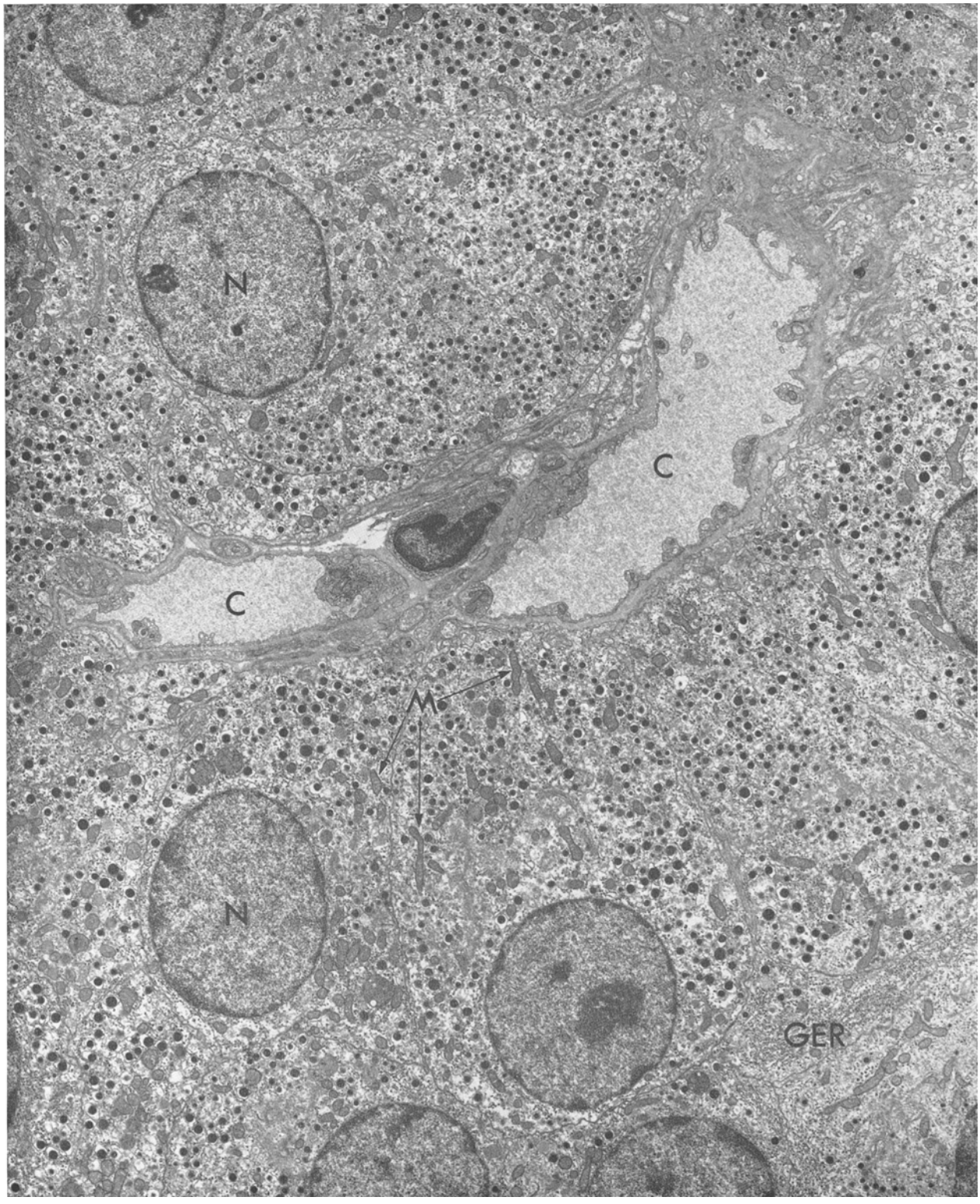

Fig. 3. Beta cells, centre of normal islet. Cytoplasmic granules are numerous and uniform density. Note the slender mitochondria. Osmium tetroxide. Approximately 4300 ×

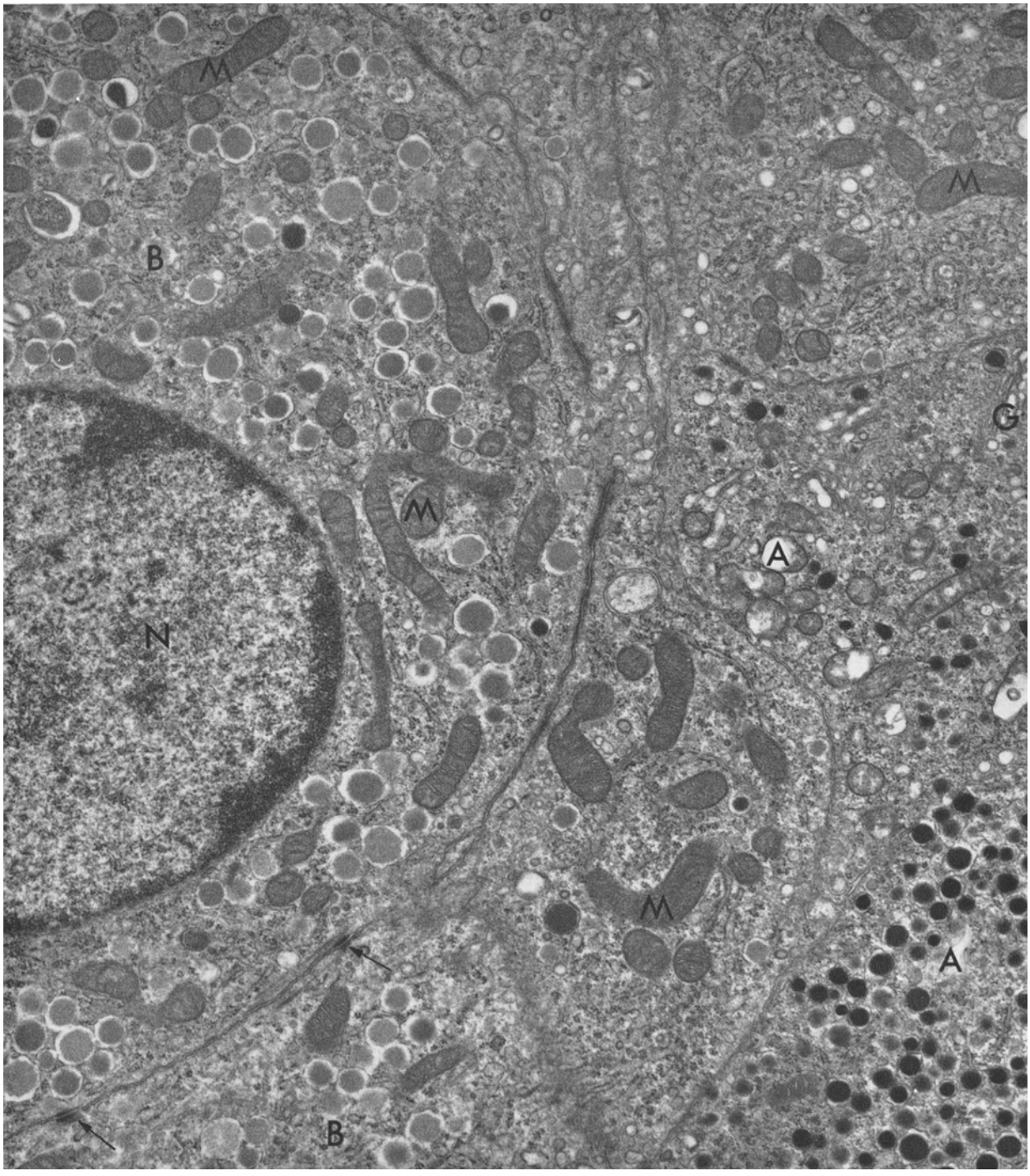

Fig. 4. Peripheral portion of normal islet, after glutaraldehyde fixation. The cytoplasmic granules of the alpha cells are smaller, more electron dense and enclosed in tight sacs. Beta granules are more varied in size and density. Two desmosomes are indicated by arrows. Approximately 13700 ×

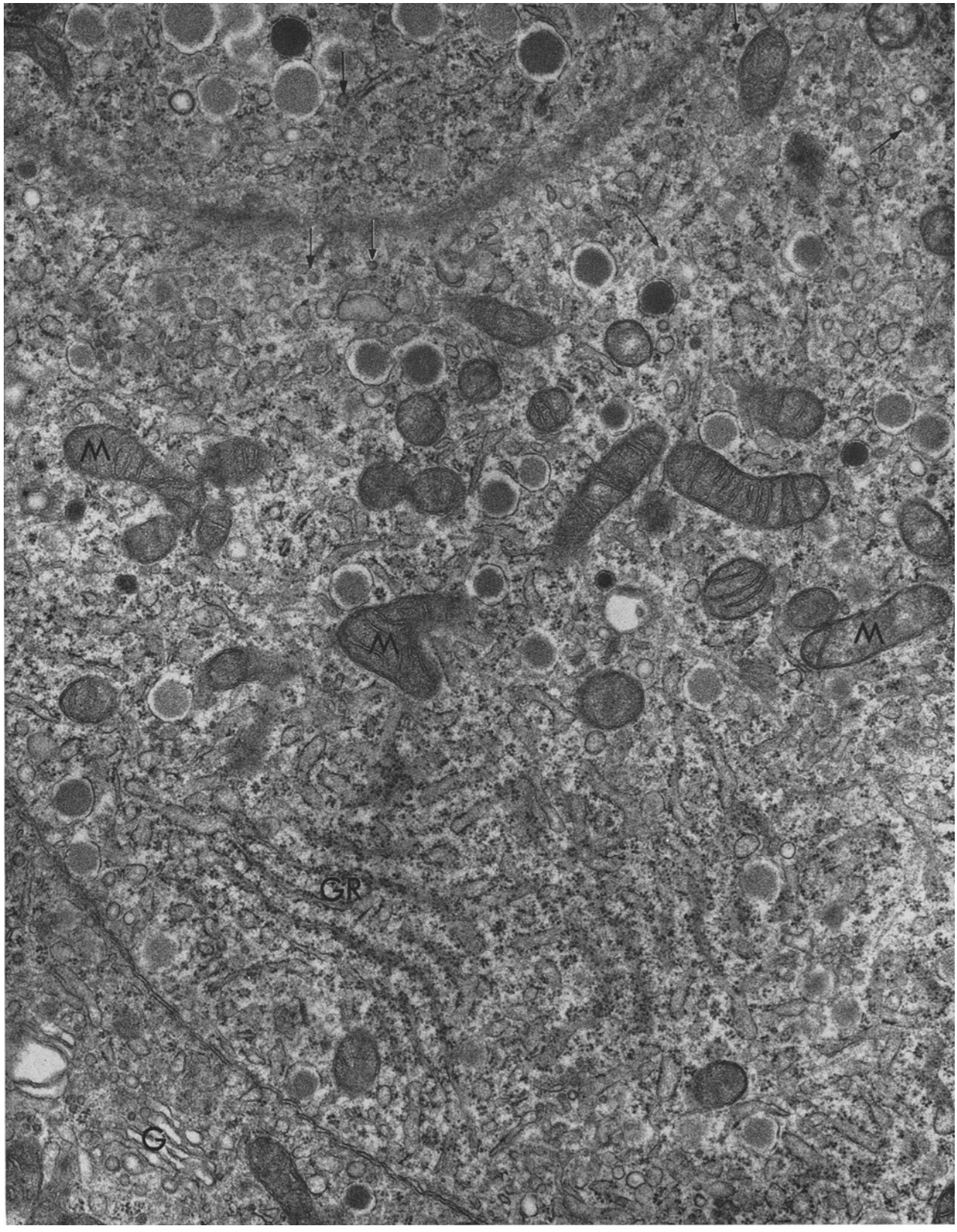

Fig. 5. Normal beta cells with proportionately fewer cytoplasmic granules and more granular endoplasmic reticulum. Note the coated vesicles near the cell membranes (arrows). Glutaraldehyde. Approximately 21 000 ×

the epididymal fat pad to exogenous insulin (De Fronzo et al., 1967).

5. 15 animals, which were imported in 1964 and 1965, maintained exclusively on a diet of mixed fresh vegetables and served as controls for the physiologic studies cited above (Miki et al., 1966, 1967).

For electron microscopy, small fragments of pancreas were fixed at 0—5°C in 1.33% osmium tetroxide, buffered with S-collidine (0.07 M) with added sucrose (0.122 M) and calcium chloride (0.005 M). Final pH was 7.4 to 7.6. Tissue was also fixed at room temperature in 3% glutaraldehyde buffered with sodium

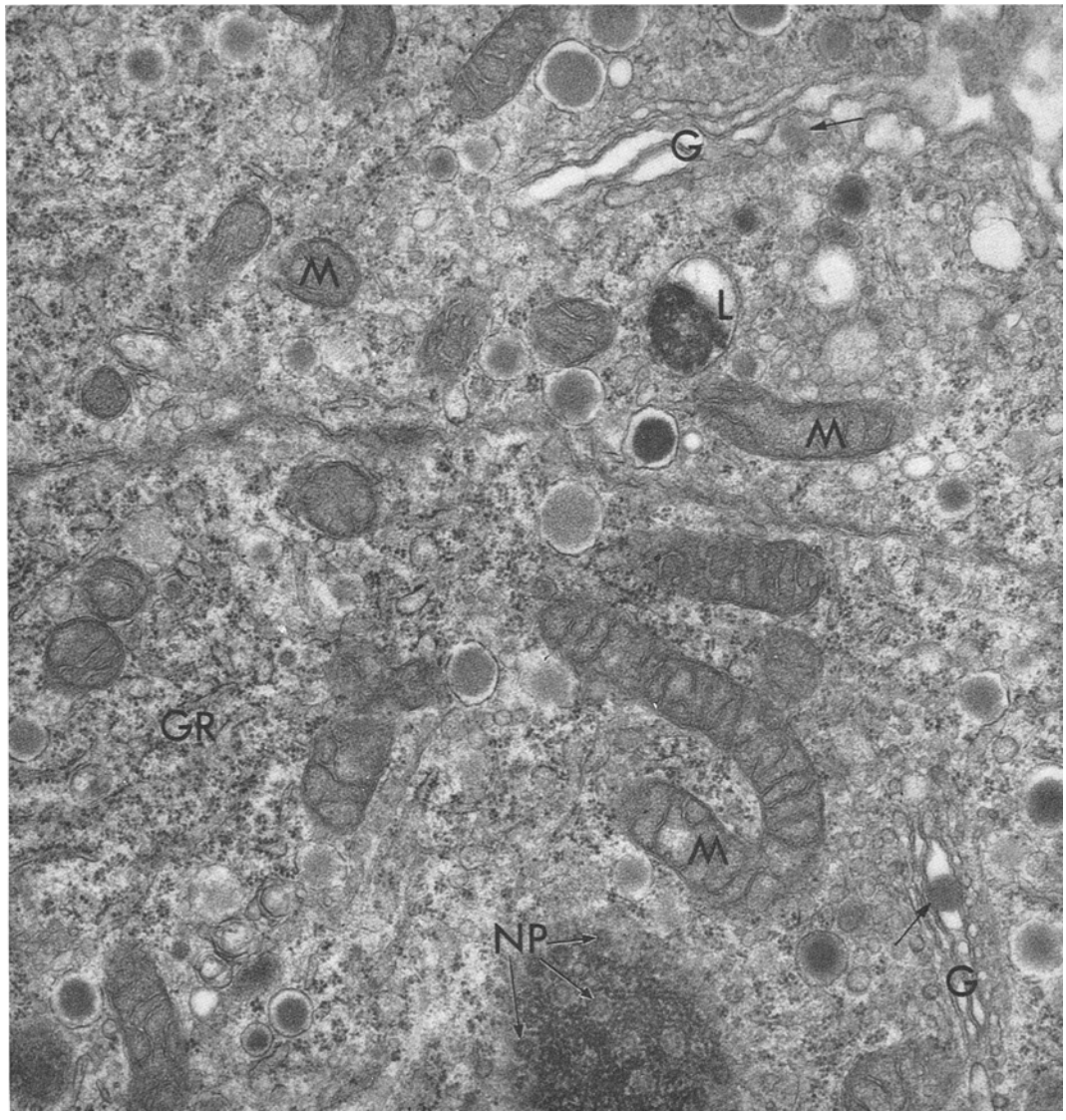

Fig. 6. Portions of two normal beta cells, demonstrating secretory granules within Golgi cisternae (heavy arrows). Glutaraldehyde. Approximately 21 000 ×

For light microscopy, tissue fragments were fixed in Bouin's solution, embedded in paraffin and stained with hematoxylin and eosin and a modified Gomori aldehyde fuchsin method (Warren and LeCompte, 1952). For the demonstration of glycogen, tissue was fixed in alcoholic formalin and paraffin sections were stained with PAS with and without prior digestion with Diastase.

cacodylate (0.1 M) pH 7.4 to 7.6, and a mixture of paraformaldehyde (4%) and glutaraldehyde (5%) buffered with sodium cacodylate (0.07 M) pH 7.1 to 7.2 (Karnovsky, 1965). Calcium chloride (0.005 M) was added to both aldehyde fixatives. After aldehyde fixation, tissues were washed in buffer for variable periods of time and subsequently postfixed at 0—5°C in osmium tetroxide (prepared as detailed above). All

tissues were rapidly dehydrated in a graded series of ethanol solutions and embedded in a mixture of Epon 812 (LUFT, 1961). Sections cut at 1 micron were stained with 1% toluidine-blue-0 in 1% borax for light microscopic identification of the islets. An occasional islet was stained with PAS (CARDNO and STEINER, 1965). Thin sections were cut with a diamond knife, using a Porter-Blum Servall MT-1 ultramicrotome, mounted on 100 or 150 mesh copper grids, without supporting membranes, double stained with aqueous or acetone solutions of uranyl acetate followed by lead (KARNOVSKY, 1961) and examined with an RCA EMU-3G microscope.

Animals, diets, methods of glucose and insulin analysis are all described in the accompanying manuscripts (MIKI et al., 1967; DE FRONZO et al., 1967).

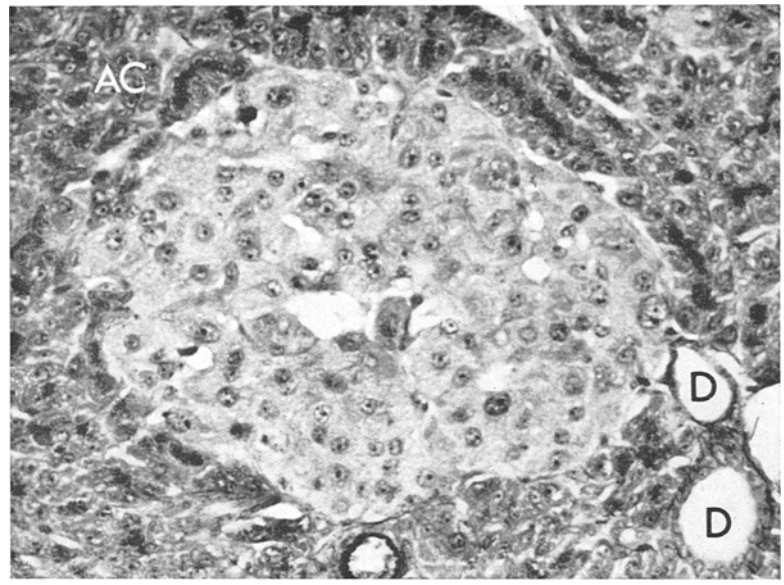

Fig. 7. Pancreatic islet of euglycemic sand rat, fed chow for 4 days. Blood glucose 84 mg/100 ml, serum IRI 240 μU/ml. Beta cell degranulation is striking. Aldehyde fuchsin stain. 260 ×

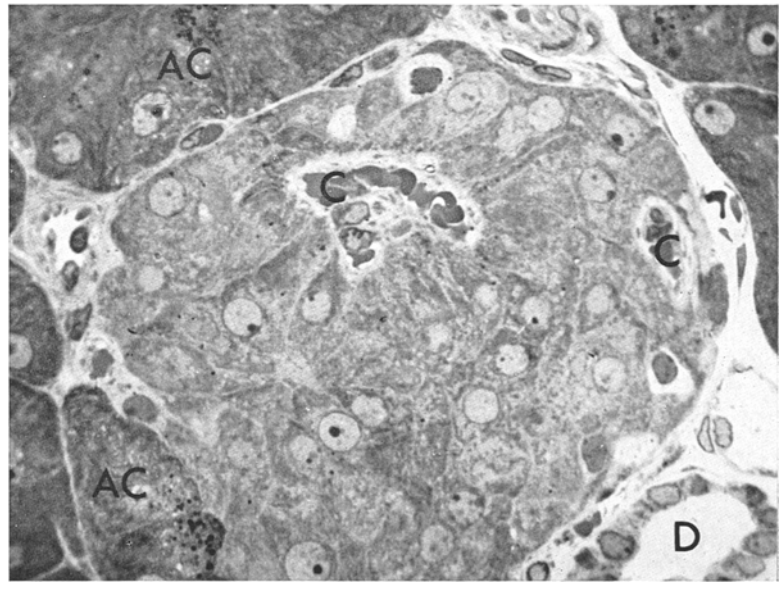

Fig. 8. Islet from same animal illustrated in Fig. 7. Cytoplasmic granules are virtually absent. Enhanced staining intensity is due to increased cellular content of ribosomes. Toluidine blue stain. 640 ×

Results

Normal islets of Langerhans. The pancreatic islets of the sand rats killed immediately or shortly after capture in the U.A.R. and with no change in diet, are identical in appearance to the islets of animals born either in the U.A.R. or the U.S. and fed exclusively a mixture of fresh vegetables. None of the animals killed in the U.A.R. were diabetic. Their blood glucose varied from 35 to 127 mg/100 ml, and the serum IRI from 0 to 45 μU/ml. Only one animal among the vege-

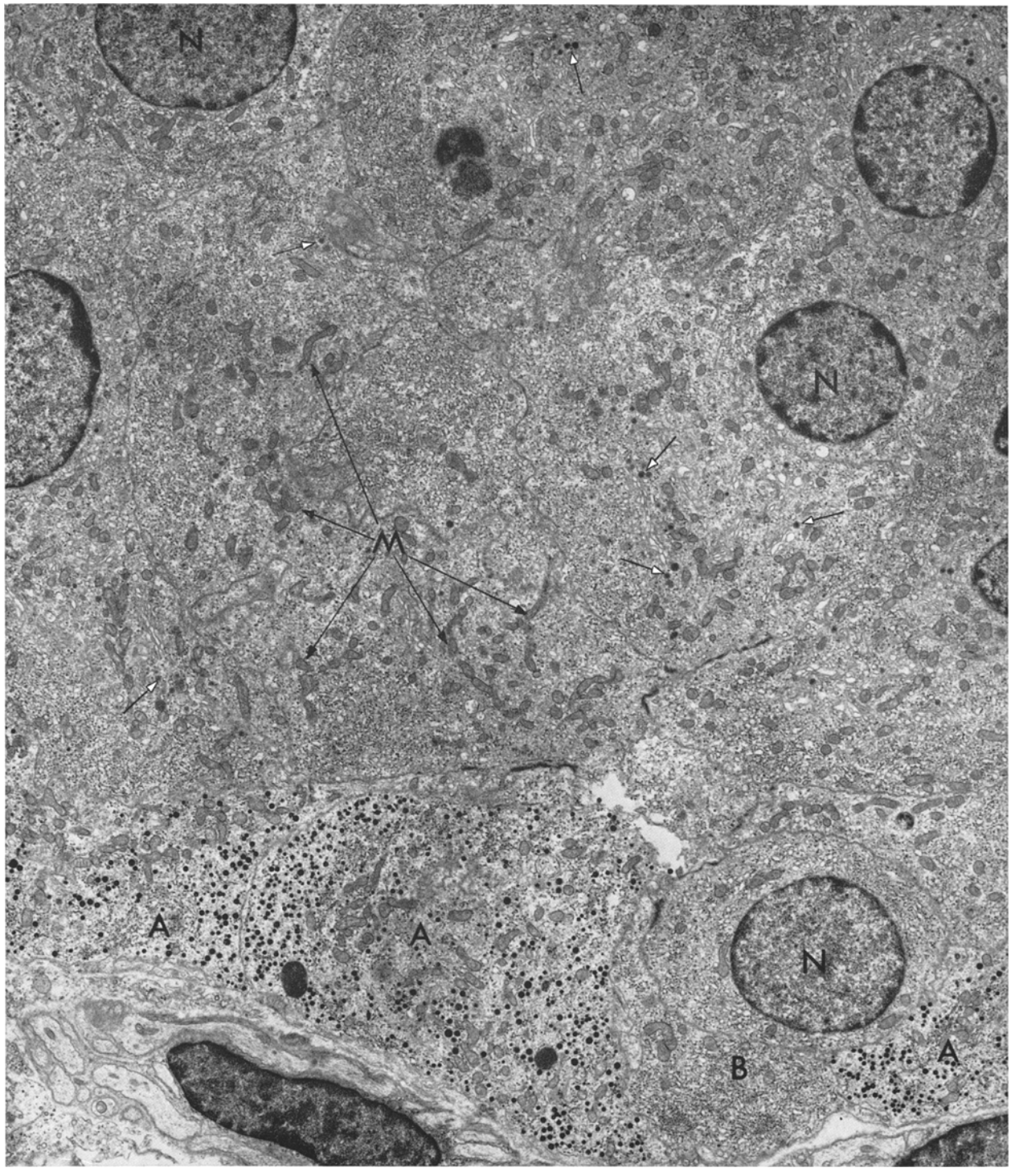

Fig. 9. Degranulated islet of euglycemic sand rat fed laboratory chow for 4 days. Peripheral alpha cells retain their granules. Beta cells occupy the remainder of the micrograph and contain few cytoplasmic granules arrows with white head). Mitochondria are abundant. Osmium tetroxide. Approximately 4300 ×

Fig. 10. (Page 151) Several beta cells from an animal with diabetes of brief duration. Blood glucose 326 mg/100 ml, serum IRI > 1500 μU/ml. Cytoplasmic granules are virtually absent, and granular endoplasmic reticulum is substantially increased. Dilated smooth surfaced tubules and vesicles are prominent (?AR). The latter are sometimes continuous with coated vesicles (*arrow). Small arrows indicate the numerous coated vesicles, perhaps an indication of enhanced protein (insulin) transport from the cell. Glutaraldehyde. Approximately 21000 ×

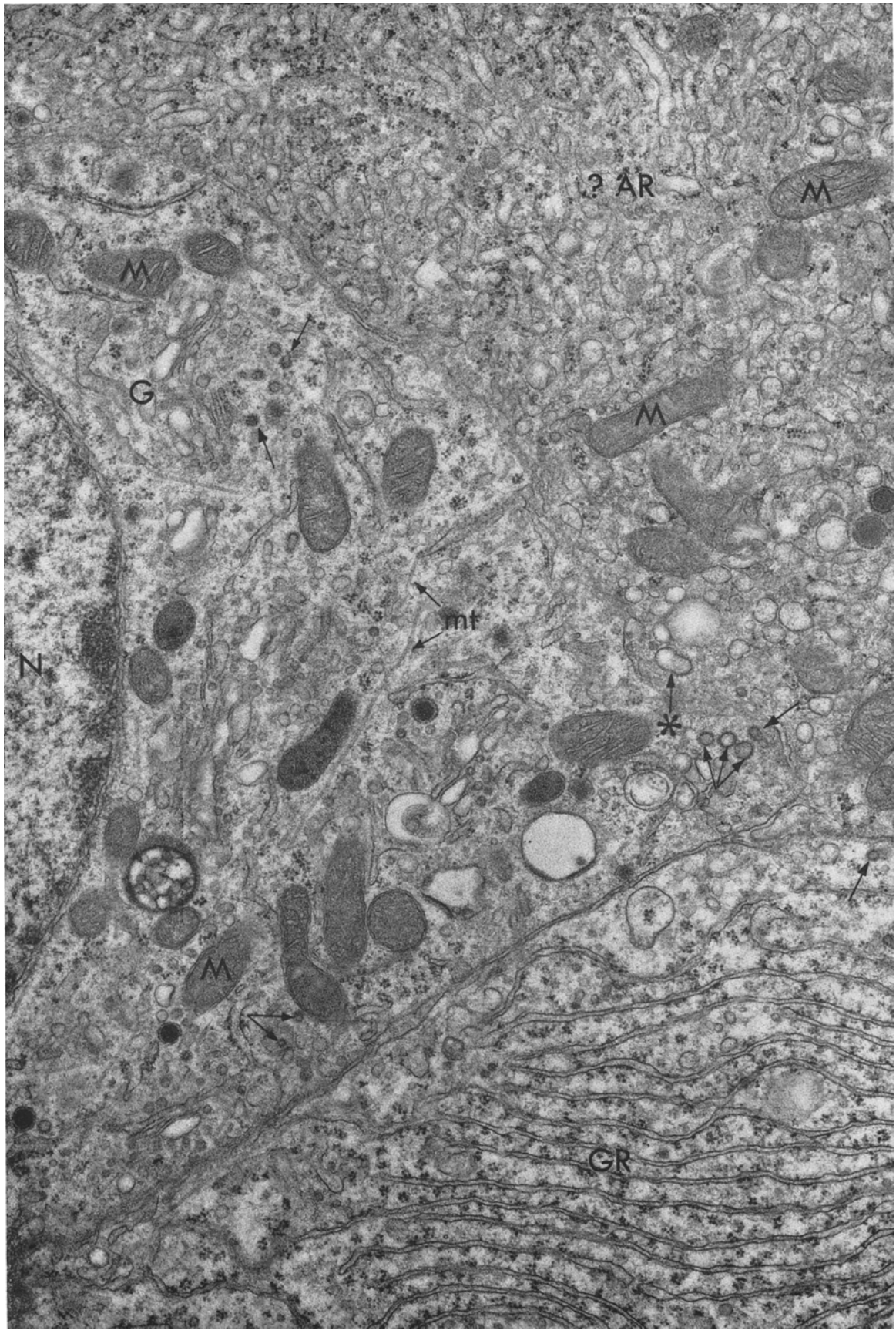
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table-fed group was hyperglycemic, and with this exception the blood glucose and serum immunoreactive insulin levels of both groups were normal (Miki et al., 1966, 1967). Hematoxylin and eosin stained sections showed that the islets of the normal sand rats closely resembled those of the laboratory albino rat in distribution, number and size. The aldehyde-fuchsin stain revealed the numerous well granulated, centrally located beta cells to be surrounded by a rim of alpha cells (Fig. 1). When 1 micron sections of tissue fixed and embedded for electron microscopy were studied after staining with toluidine blue, the cytoplasmic granules of the islet cells were readily visualized without special stains, and it was sometimes possible to distinguish the alpha and beta cells on the basis of the staining intensity of the granules (Fig. 2). With the added resolution and magnification of the electron microscope, the beta and alpha cells were more readily identified and differentiated. No other cell types were observed. The beta cells, which usually occupy the central portion of the islet, were commonly separated from the exocrine tissue by the more peripheral alpha cells. The abundant beta granules of the normal animal were round, and enclosed within loosely fitting smooth-membraned sacs. After primary fixation in osmium tetroxide, the beta granules were of uniform density and size (Fig. 3). After aldehyde fixation, beta granules were more numerous, and more varied in

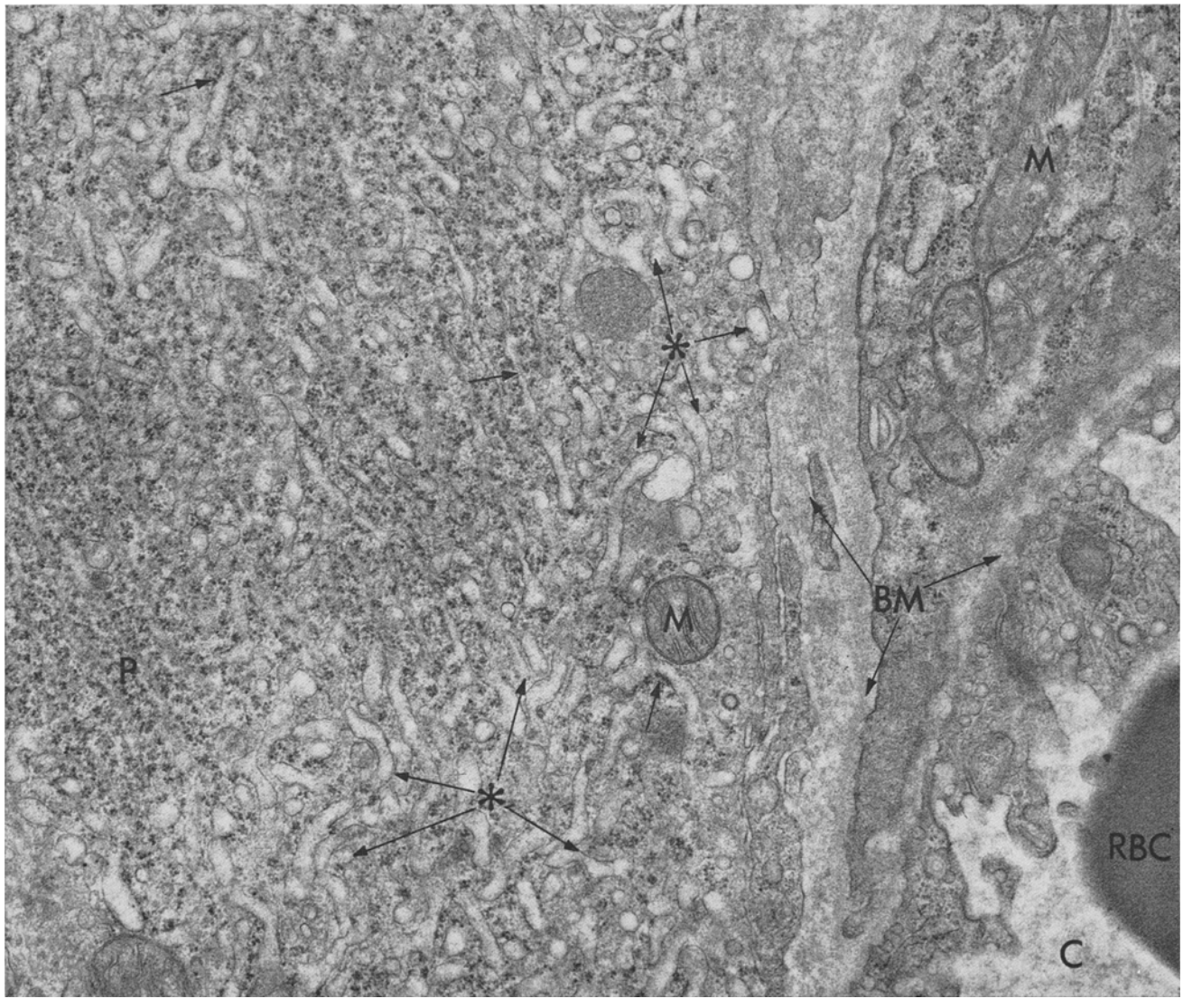


Fig. 11. Another beta cell from the same animal with diabetes of brief duration demonstrating the absence of consistent spatial relationship between the dilated tubules and the numerous ribosomes. In places ribosomes are attached to the membranes (arrows). When dilated, the tubules frequently appear to be smooth (asterisk), and may represent agranular reticulum of Golgi origin. Glutaraldehyde. Approximately 21 000 ×

size and staining intensity (Fig. 4, 5). Mitochondria were relatively numerous, dispersed throughout the cell and appeared as slender structures with transverse cristae mitochondriales. The granular endoplasmic reticulum was not prominent in the well-granulated electron-dense secretory granules, which were contained within more tightly fitting sacs (Fig. 4 and 18).

The uniform size and staining qualities of the alpha granules did not appreciably vary with the fixative utilized.

Fig. 12–15. Pancreatic islets of diabetic sand rats

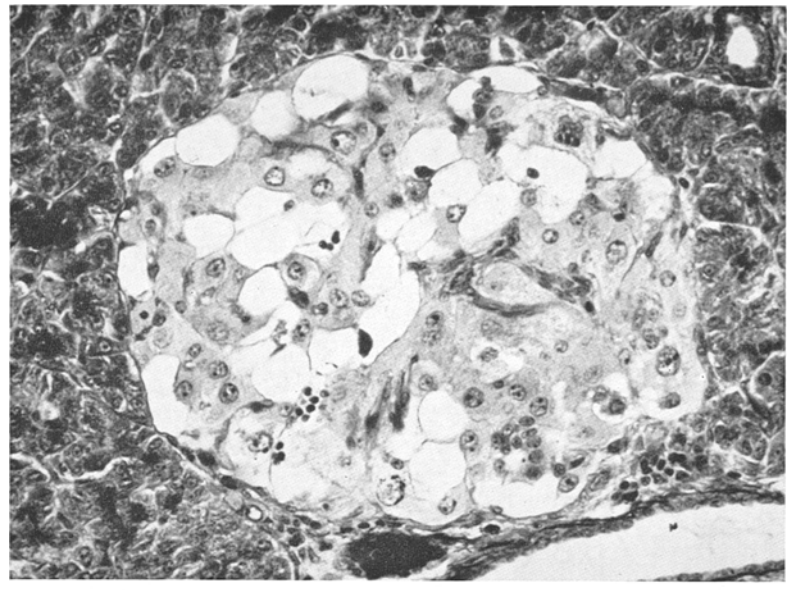


Fig. 12. Beta cell degranulation and vacuolization is pronounced. Vacuoles appear empty in this preparation. Aldehyde fuchsin stain. 260 ×

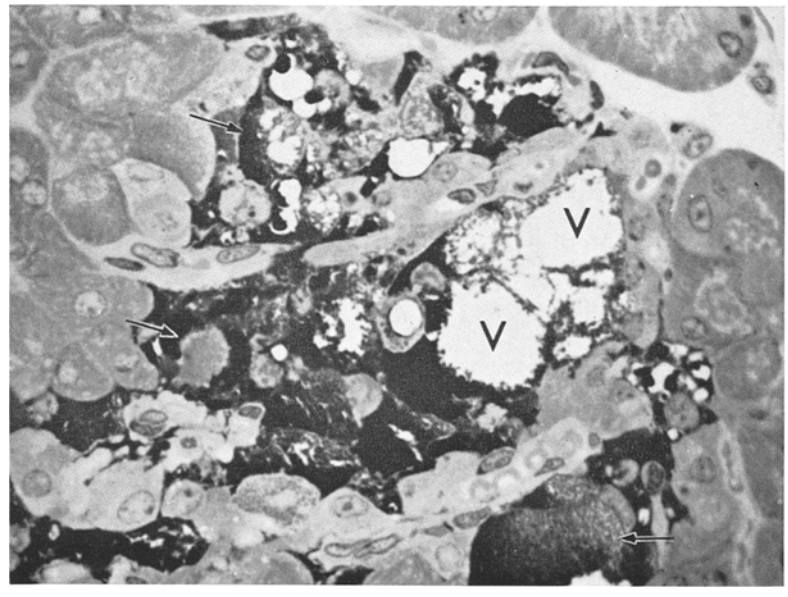


Fig. 13. Most "vacuoles" are filled with PAS stainable material (arrows) in sections of Epon embedded tissue. Several "empty" vacuoles (V) are visible. PAS stain. 640 ×

cell, and varied inversely with the number of cytoplasmic secretory granules (Fig. 5). The inconspicuous Golgi complex rarely contained secretory granules (Fig. 6) but was otherwise typical in appearance with cisternae, vacuoles, smooth vesicles and coated vesicles. Glycogen was not observed in normal islet cells.

The alpha cells were distinguished by their peripheral location and more specifically by their more

Islets of Langerhans after chow feeding. With the institution of a synthetic chow diet (Purina or Old Guilford pellets) the beta cells in susceptible animals eventually became relatively degranulated. This was particularly apparent in those animals offered synthetic chow prior to weaning. Although observed in the presence of euglycemia (Fig. 7 and 8), degranulation was usually accompanied by increased levels of

serum immunoreactive insulin (IRI). In the presence of resting hyperglycemia, beta cell degranulation was more pronounced and the associated serum IRI levels even greater. The degranulated islets of the chow-fed, euglycemic and hyperglycemic rats appeared considerably larger than those of the vegetable-fed animals. Quantitative measurements of islet volume, however, were not performed. The duration of chow feeding required for degranulation of the beta cells was extremely variable and ranged from 4 days to 4 months.

The electron microscopic appearance of beta cell degranulation in euglycemic sand rats is illustrated in Fig. 9, and in a sand rat with diabetes of short duration in Figs. 10 and 11. The peripheral alpha cells were without alterations. The more central beta cells were virtually devoid of secretory granules, contained increased numbers of mitochondria (Fig. 9), a corresponding increase in granular endoplasmic reticulum (Fig. 10, 11) and more prominent Golgi structures. Present in the vicinity of the cell membranes were many more coated vesicles and a significant number of smooth (agranular) membraned tubules (Fig. 10).

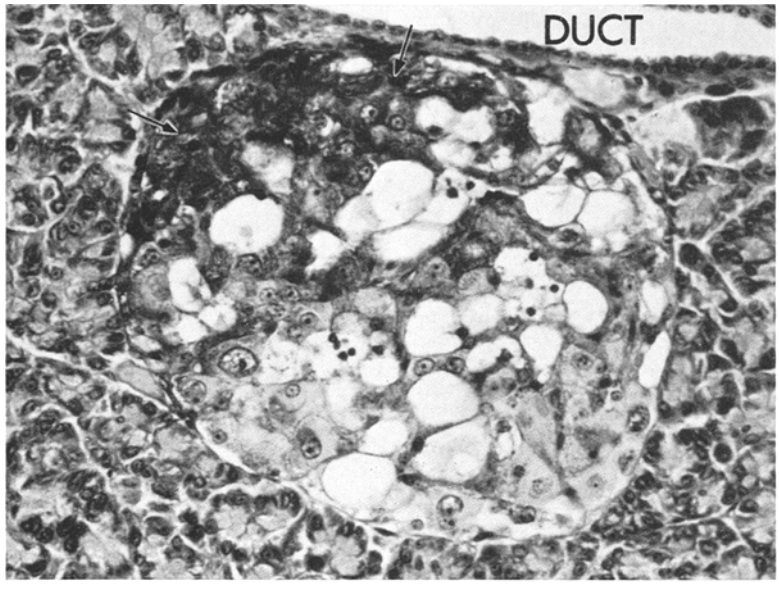


Fig. 14

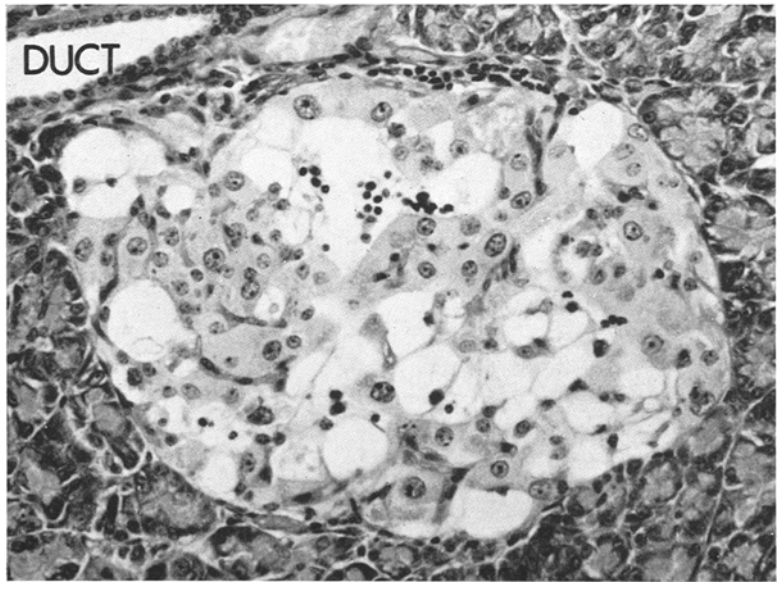


Fig. 15

Fig. 14 and 15. Demonstration of glycogen in islets of tissue fixed in alcoholic formalin. PAS staining of the deposits in Fig. 14 (arrows) was abolished by prior diastase digestion in Fig. 15. 260 ×

Islets of Langerhans in diabetic animals. In the presence of more pronounced resting hyperglycemia, glycogen infiltration was almost always present. By

light microscopic examination, cytoplasmic vacuolization was observed and attributed to PAS-stainable, diastase-digestible material within greatly enlarged beta cells. The "empty" vacuoles observed in paraffin sections (Fig. 12) were usually filled in Epon sections with a non-structured material that stained strongly with PAS (Fig. 13). Alcoholic formalin fixation and paraffin embedding permitted diastase digestion prior to PAS staining, and therefore proved the presence of glycogen in the beta cell vacuoles (Fig. 14 and 15).

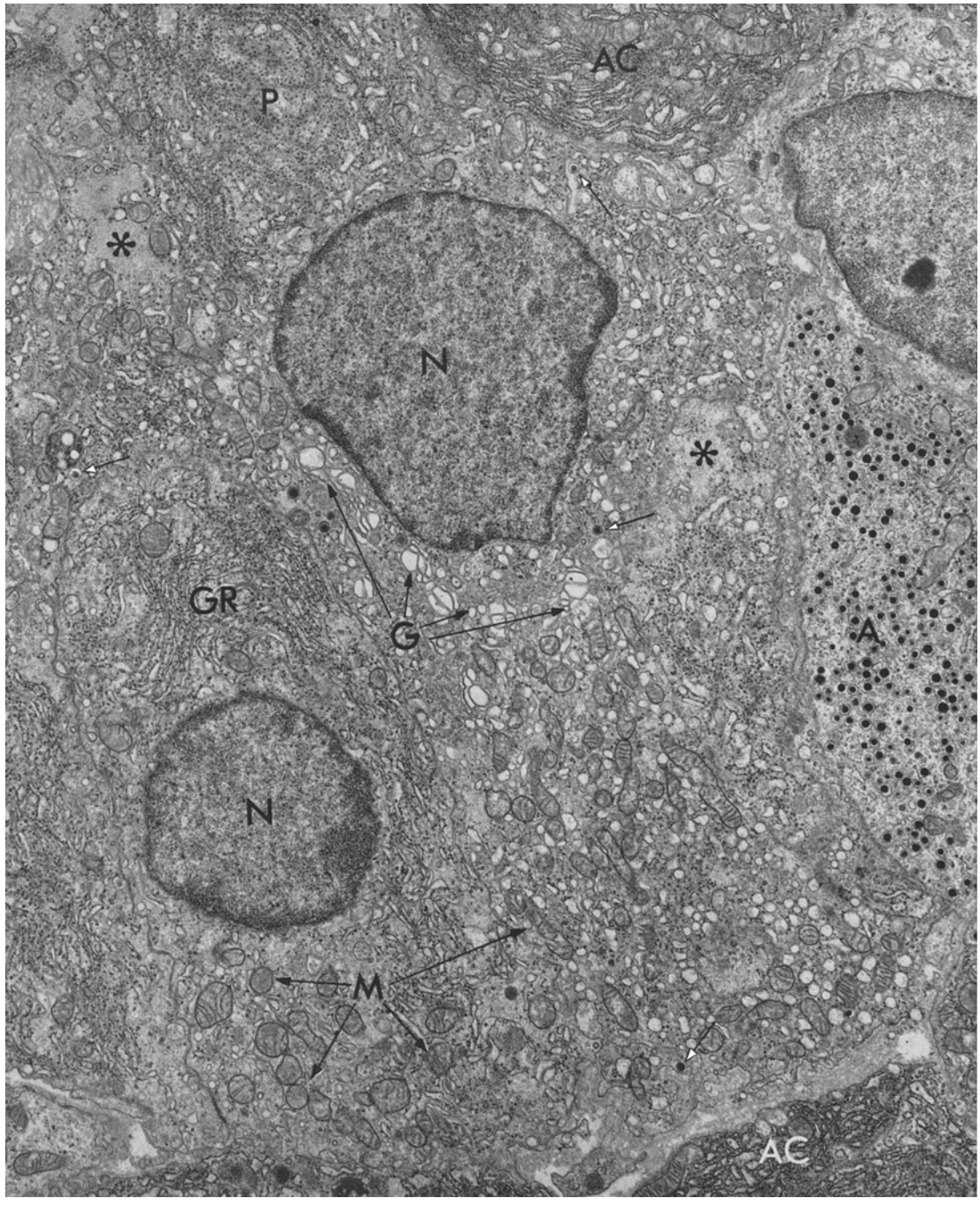


Fig. 16—21. Illustrate electron micrographs of pancreatic islets of diabetic sand rats with glycogen infiltration of beta cells

Fig. 16. Low power micrograph of periphery of an islet with adjacent pancreatic acinar cells. Beta granules (with white head) arrows are few in number. Granular endoplasmic reticulum and free ribosomes are plentiful. The Golgi complex is enlarged and mitochondria are more numerous. Glycogen deposits are indicated by asterisks. Alpha cells are normal. Blood glucose 252 mg/100 ml. Serum IRI > 160 μU/ml. Osmium tetroxide. Approximately 6000 ×

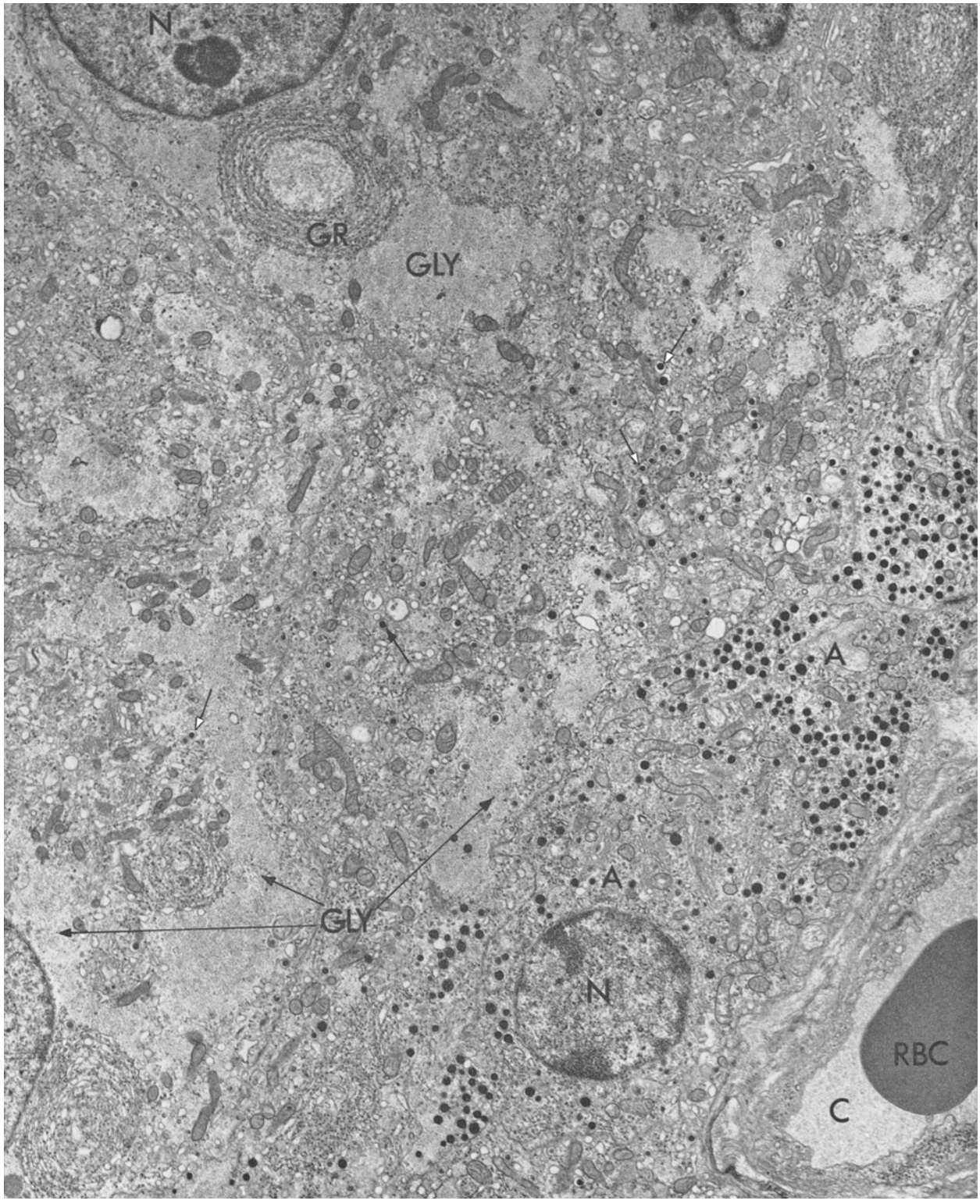


Fig. 17. Low power micrograph of an islet with prominent cytoplasmic glycogen deposits. Similar deposits have not been observed in nuclei. Blood glucose 605 mg/100 ml. Serum IRI > 160 μU/ml. Osmium tetroxide. Approximately 6000 ×

The electron microscopic appearance of degranulated, glycogen-filled beta cells is illustrated in Figs. 16—21. The peripheral alpha cells were unaltered and retained their usual complement of secretory granules (Fig. 16—18). The degranulated beta cells were distorted by the intracellular accumulation of glycogen, which displaced and compressed cytoplasmic organelles (Fig. 17—21). The cytoplasmic remnant of these cells contained increased quantities of granular endoplasmic reticulum, enlarged Golgi structures, and numerous enlarged mitochondria. The morphologic evidence of enhanced protein synthesis was most obvious in those beta cells with smaller accumulations of glycogen (Fig. 18). Nuclei were never observed with glycogen accumulations.

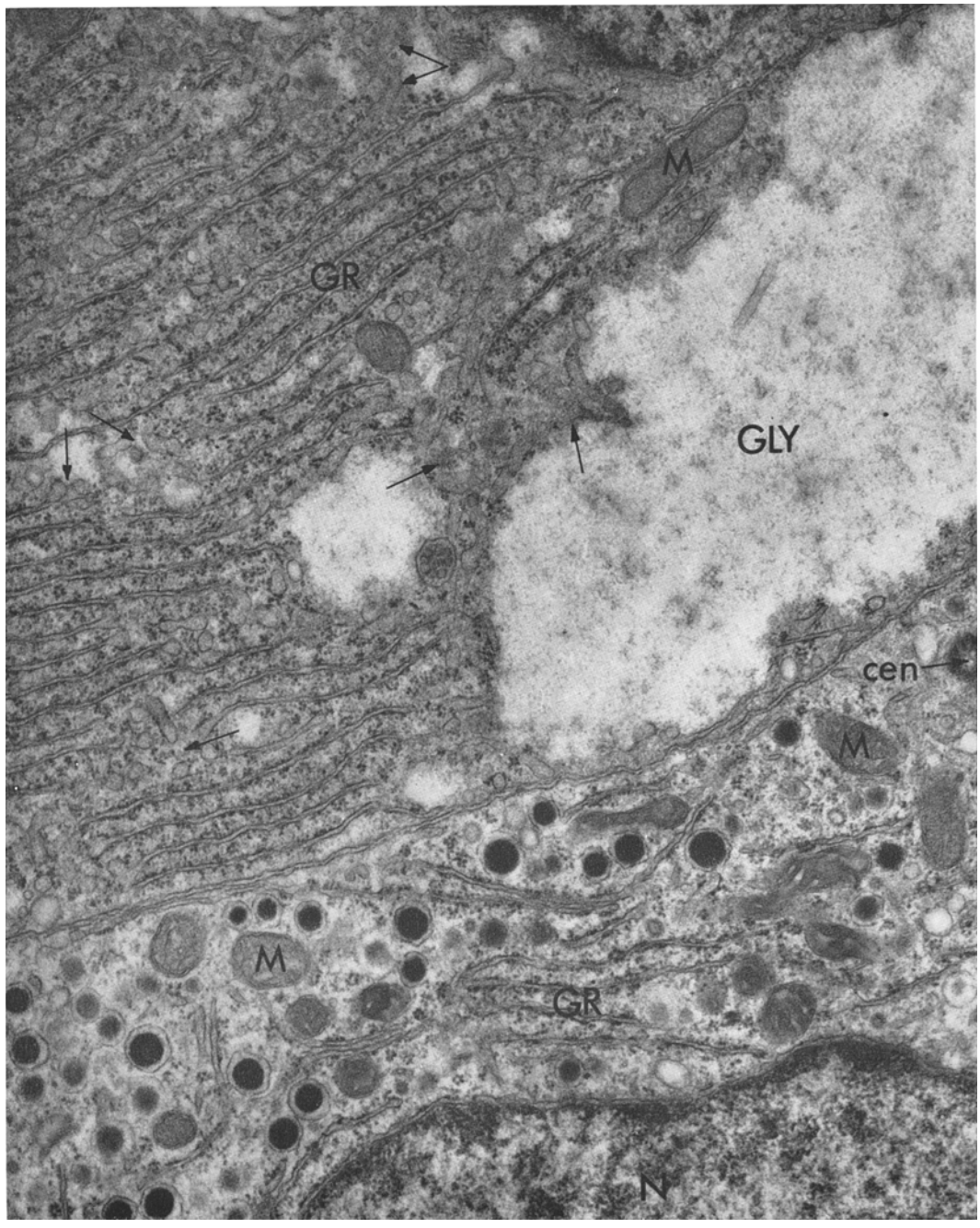


Fig. 18. Portions of normal alpha cell and glycogen-infiltrated beta cell. Beta granules are absent and the granular endoplasmic reticulum is hypertrophied. Foci of transition between the GR and smooth surfaced tubules and vesicles are indicated (arrows). Approximately 21 000 ×

In one sand rat with sustained hyperglycemia

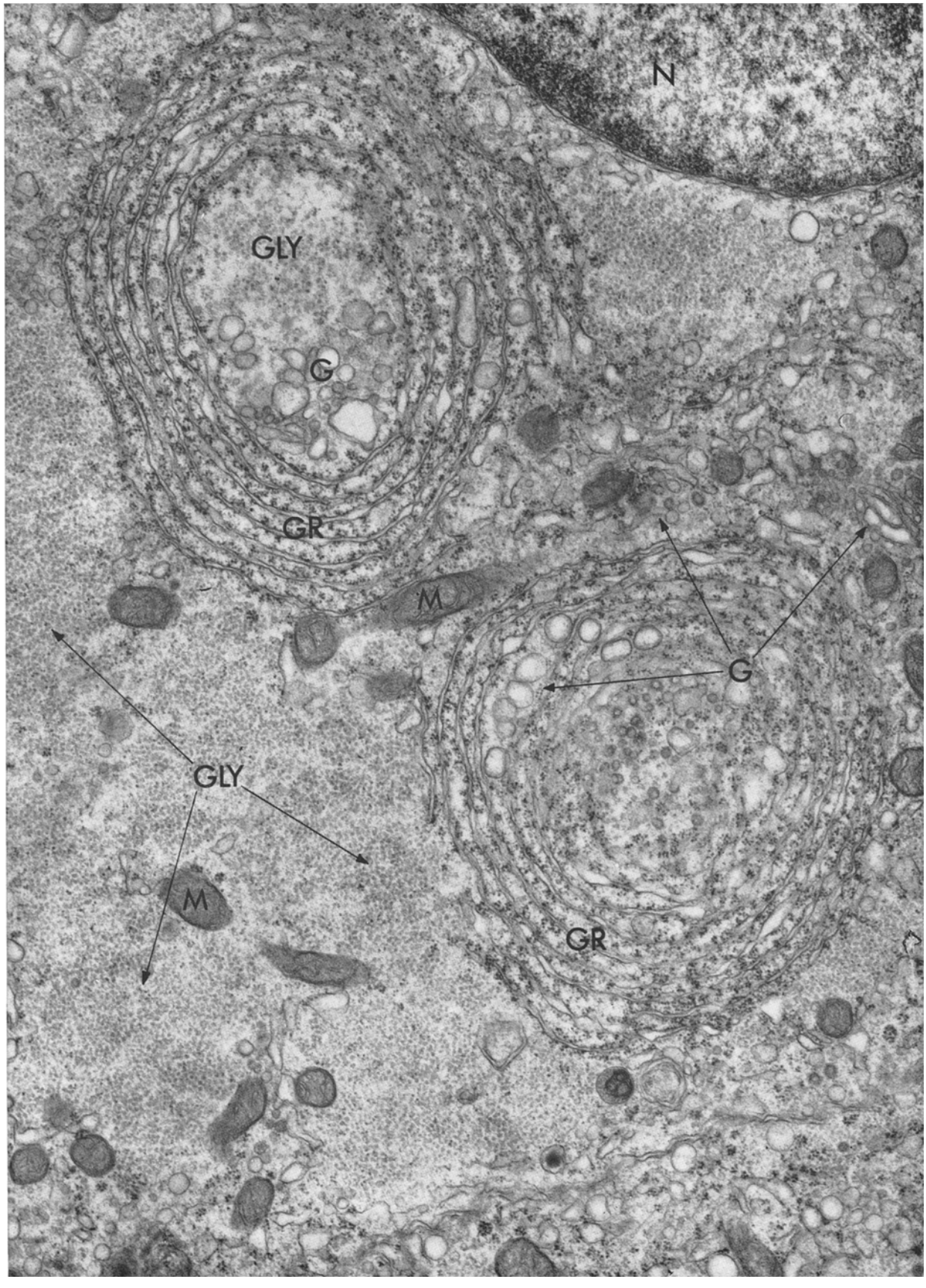


Fig. 19. Beta cell with glycogen infiltration and an enlarged Golgi complex. Whorled ("finger print") pattern of the granular endoplasmic reticulum is indicative of enhanced protein synthesis. Same animal as in Fig. 17. Osmium tetroxide. Approximately 21 000 ×

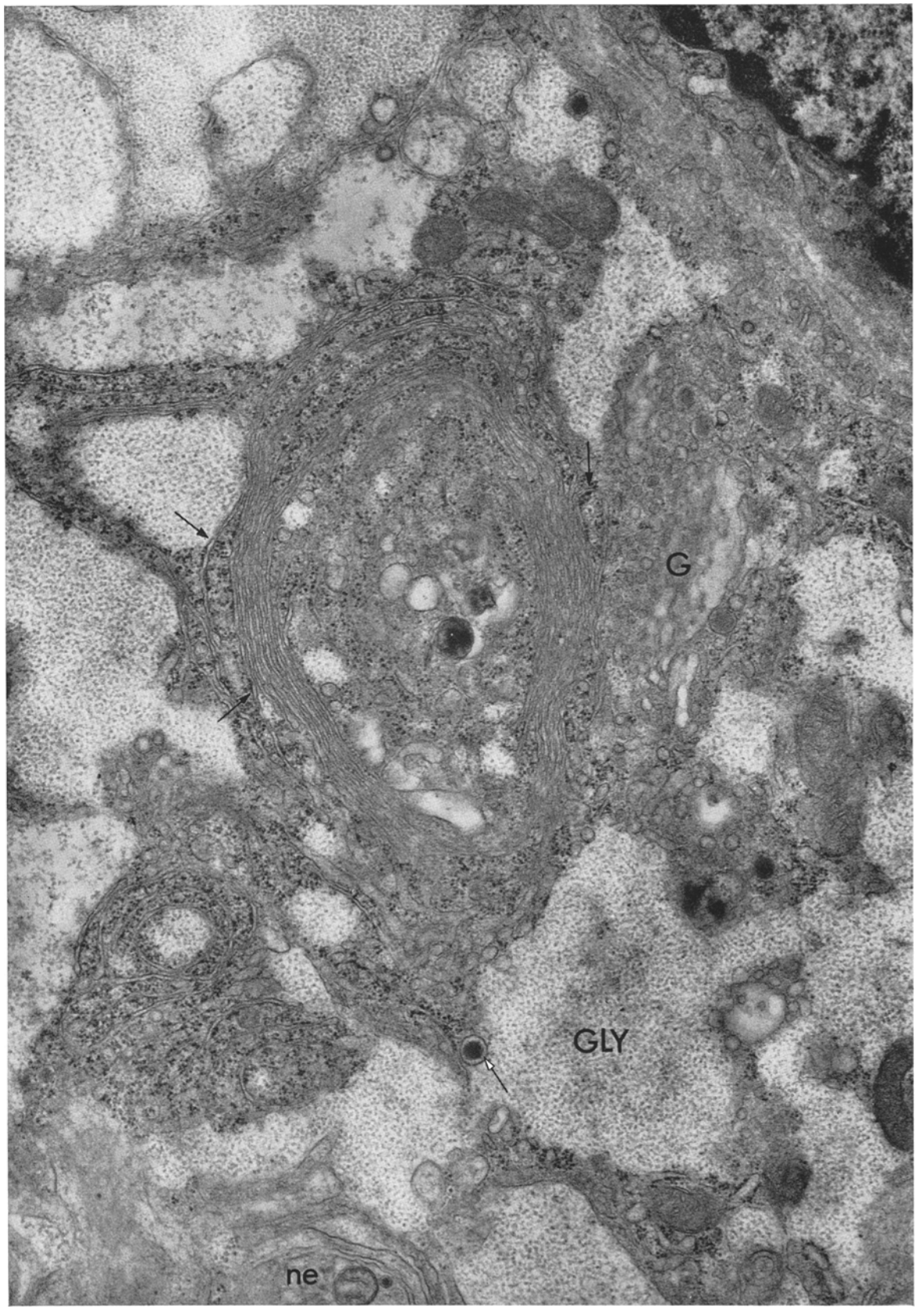


Fig. 20. Beta cell with marked glycogen infiltration and distortion of cytoplasmic organelles. The central whorled pattern of smooth membranes is continuous, at arrows, with obvious granular endoplasmic reticulum from which it originated. The Golgi complex and related coated vesicles are prominent. Paraformaldehyde-glutaraldehyde. Approximately 21 000 ×

(600 mg/100 ml) and a disproportionally low serum IRI (51 μU/ml), evidence of beta cell degeneration was noted by light (Fig. 22 and 23) and electron microscopy (Fig. 24—27). True vacuoles first appeared within the foci of glycogen accumulation (Fig. 24). Together with the increasing number and size of these "true vacuoles" (i.e., not glycogen filled) there appeared a number of degenerating beta cells (Fig. 25, 26) and small numbers of inflammatory cells (Fig. 27). The evidence of degeneration and possible necrosis was limited to the single sand rat obtained for electron microscopic study in which elevated levels of blood glucose were not associated with the expected markedly elevated levels of serum IRI (Miki et al., 1967).

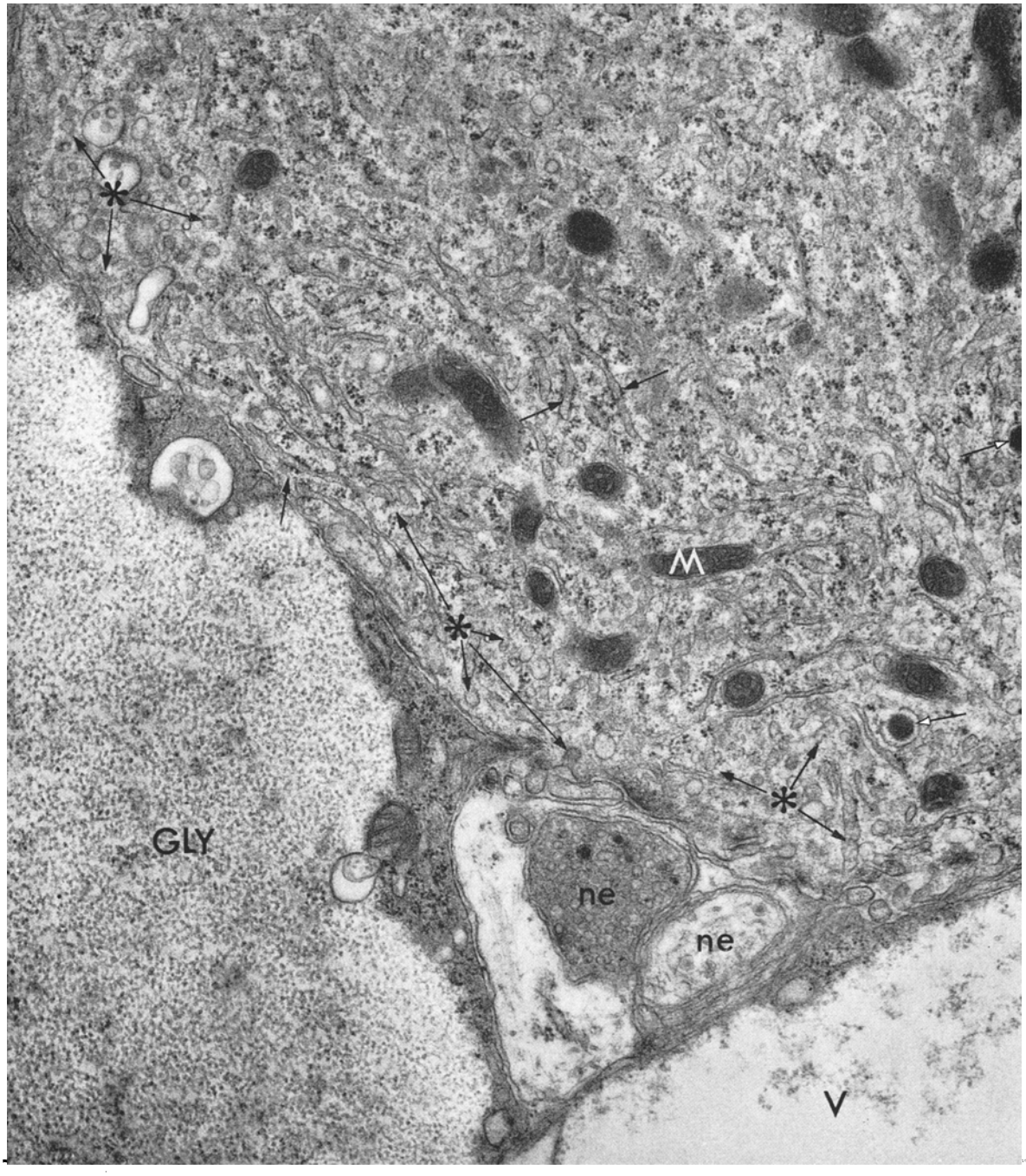
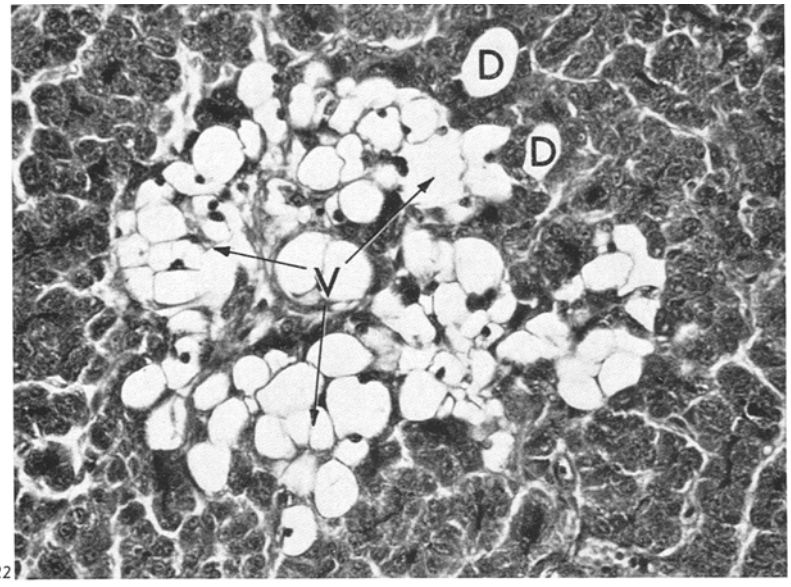


Fig. 21. Several beta cells demonstrating glycogen infiltration, with compression of organelles against cell membrane (lower left), true vacuolization (lower right), and degranulation with proliferation of granular endoplasmic reticulum (arrows), and numerous agranular tubules and coated vesicles (asterisks). Non-myelinated nerves are visible (ne). Paraformaldehyde-glutaraldehyde. Approximately 21 000 ×

Discussion

The light microscopic and ultrastructural appearance of the pancreatic islets in the normal sand rat cannot be distinguished from that of the albino laboratory rat (Lacy, 1961). A third granular cell was not observed in the sand rat (Caramia, 1963); and although alpha granules varied somewhat in diameter, these differences occurred within the same cell and hence no basis for subdividing the alpha cells into secondary groups was present (Caramia et al., 1965). Beta cells were more adequately preserved after aldehyde fixation with retention of a greater number of beta granules and the occasional presence of secretory granules within the Golgi apparatus. In general, the amount of granular endoplasmic reticulum was inversely proportional to the number of secretory granules.



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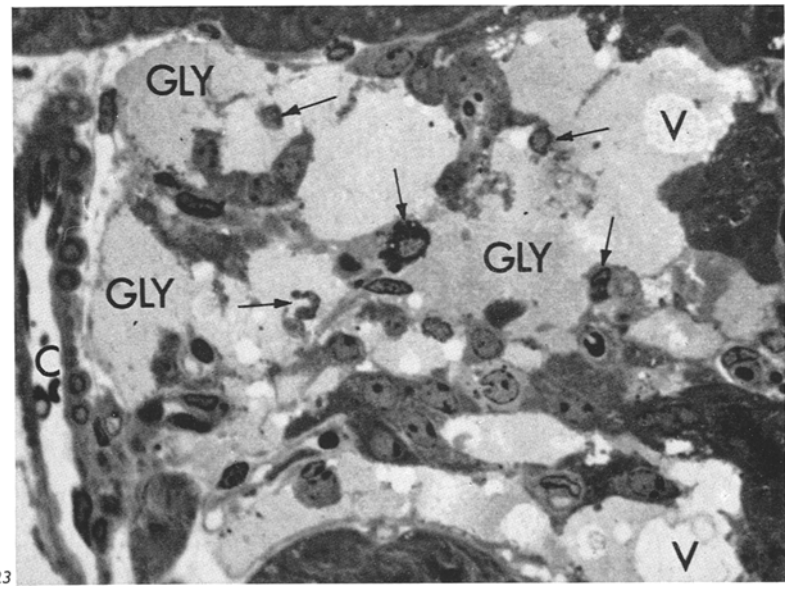

23

Fig. 22 and 23. Pancreatic islets of sand rat with sustained hyperglycemia and a disproportionally low IRI. Blood glucose 600 mg/100 ml. Serum IRI 51 μU/ml

Fig. 22. Almost ubiquitous vacuolization of beta cells. Aldehyde fuchsin stain. 260 ×

Fig. 23. The transformation of many beta cells into sacs of glycogen with isolated, deeply staining nuclei and cytoplasmic remnants is well demonstrated. The irregularly shaped nuclei are presumably undergoing degenerative changes (arrows). Toluidine blue stain. 640 ×

When susceptible sand rats were fed a diet of synthetic chow, instead of mixed fresh vegetables or their native plants, increased levels of serum IRI and beta cell degranulation became manifest, at times in the absence of hyperglycemia. Ultrastructural evidence of enhanced protein synthesis was invariably present. Evidence suggestive of increased release of protein from the cell included the prominently enlarged Golgi complex, and the increased number of coated vesicles and agranular tubules, possibly of Golgi origin, in the vicinity of the cell membranes. Evidence of "emiocytosis" (Lacy, 1961) was not present. With the appearance of hyperglycemia, protein synthesis was further increased and the beta cells revealed the presence of variable quantities of glycogen limited to the cytoplasm. The glycogen was not related to any particular cell organelle, but appeared to displace and compress all of the cytoplasmic structures. The extent of the glycogen infiltration varied considerably from cell to cell, perhaps accounting for the maintenance of elevated levels of serum IRI in these animals. The ultrastructural appearance of the glycogen in the beta cells of the sand rat differs from the classical (Revel, 1960) in that the granules are less electron dense, regardless of the fixative utilized. That the granular infiltrate is indeed glycogen was proven by the diastase digestibility of comparable infiltrates, as visualized by light microscopy. The explanation for the different ultrastructural appearance is not presently available.

The appearance of liquefaction and true vacuolization was apparently limited to the areas of most concentrated glycogen infiltration. Concomitant alterations include cytoplasmic degeneration, nuclear irregularity with decreased size and increased electron density (?pyknosis), and presumably cell death. The presence of these alterations, in the only diabetic animal without evidence of increased IRI in whom tissue was obtained for electron microscopic study, provides the basis, albeit slim, for suggesting that these changes may also have been present in those animals that developed the fulminating ketotic diabetic syndrome.

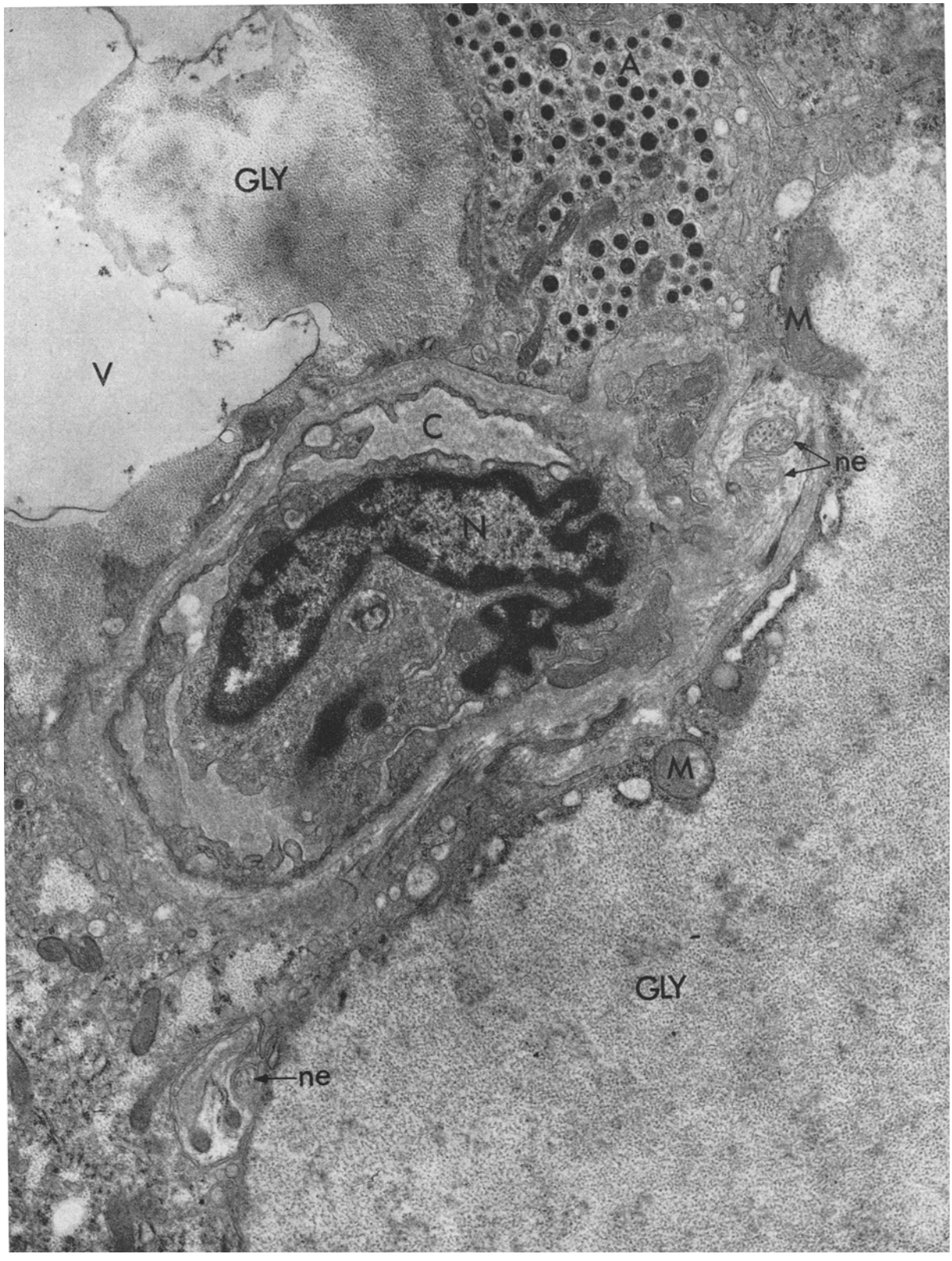

Fig. 24–27. Electron micrographs of degenerating beta cells. Same animal illustrated in Fig. 22 and 23

Fig. 24. Cytoplasmic remnants of beta cells at upper left and lower right are flattened against cell membranes by accumulated glycogen. Vacuole formation within glycogen "stuffed" cells parallels other degenerative changes. Alpha cell and capillary are normal. Paraformaldehyde-glutaraldehyde. Approximately 13700 ×

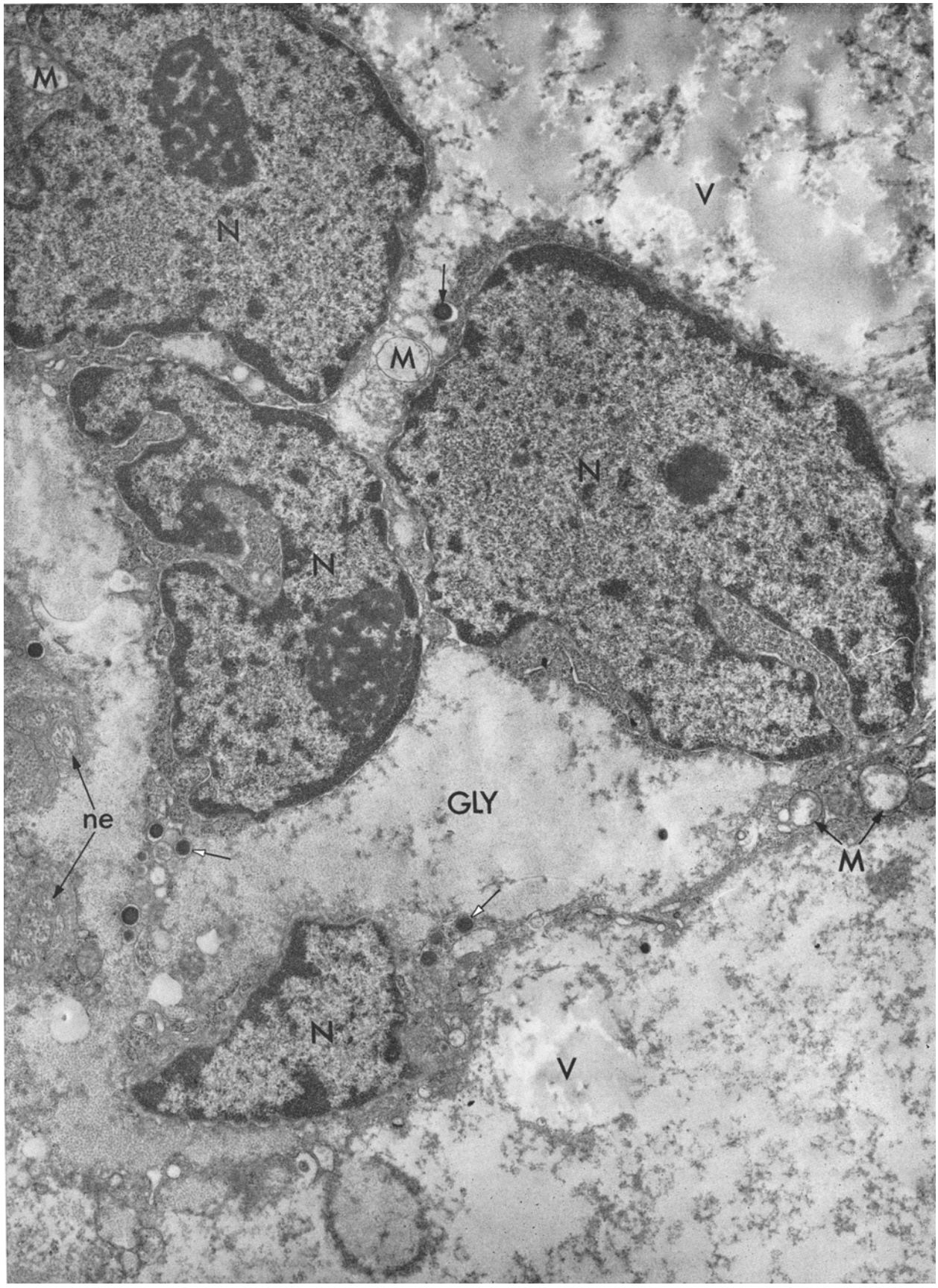

Fig. 25. Examples of degenerating (perhaps necrotic) beta cells. Irregularly shaped and electron-dense nuclei are in close apposition suggesting loss of intervening cytoplasm. Cytoplasmic remnant adheres to periphery of nuclei and cell membranes. Vacuoles with clumping of glycogen and mitochondrial ballooning suggest imminent cell breakdown

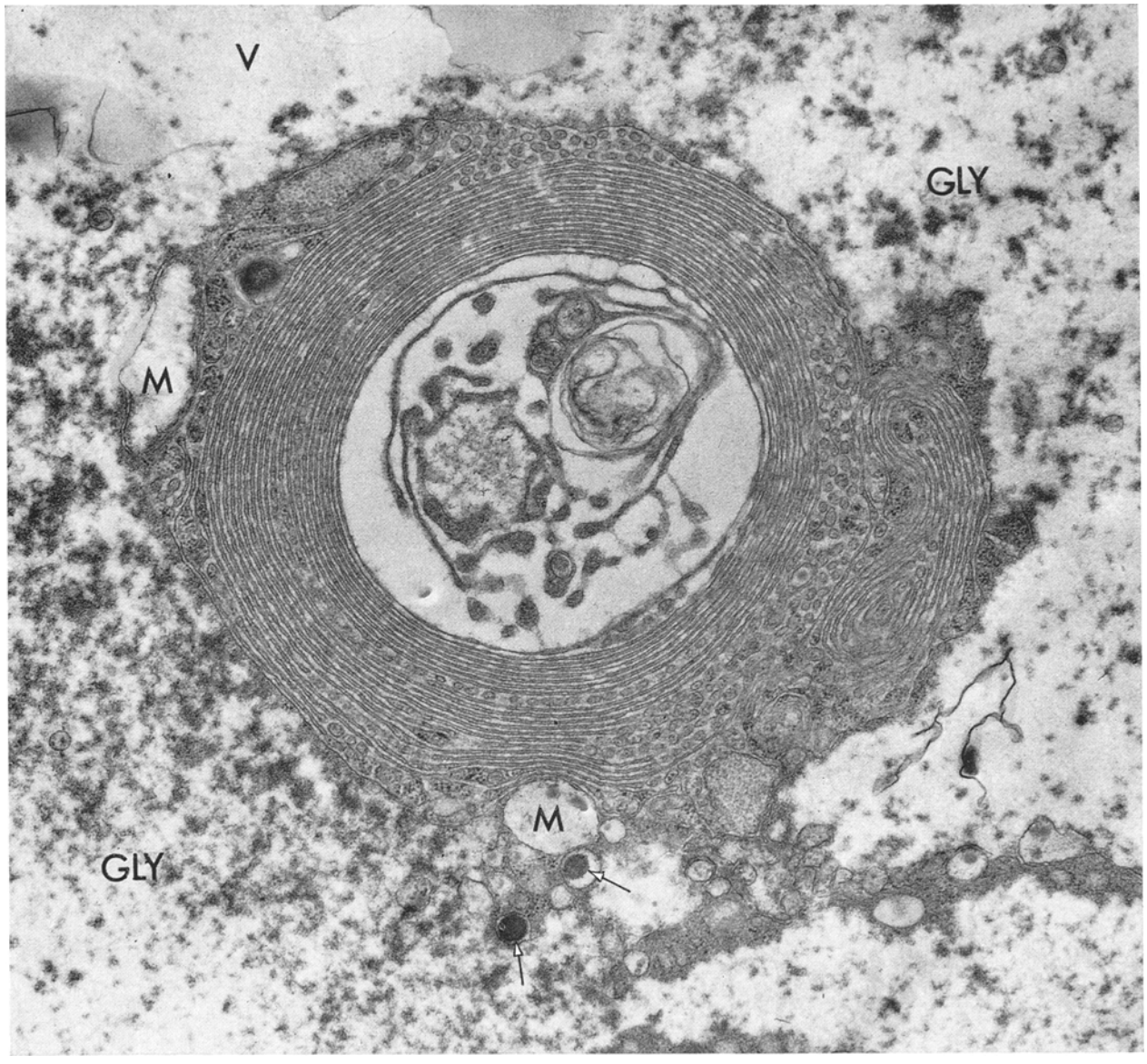

Fig. 26. Degenerating or necrotic beta cell, with a whorled collection of continuous agranular cisternae, presumably a stage of development beyond that illustrated in Fig. 20. The centrally situated membranous debris closely resembles a rearranged collection of lipoprotein (myelin figure). Glutaraldehyde. Approximately 21 000 ×

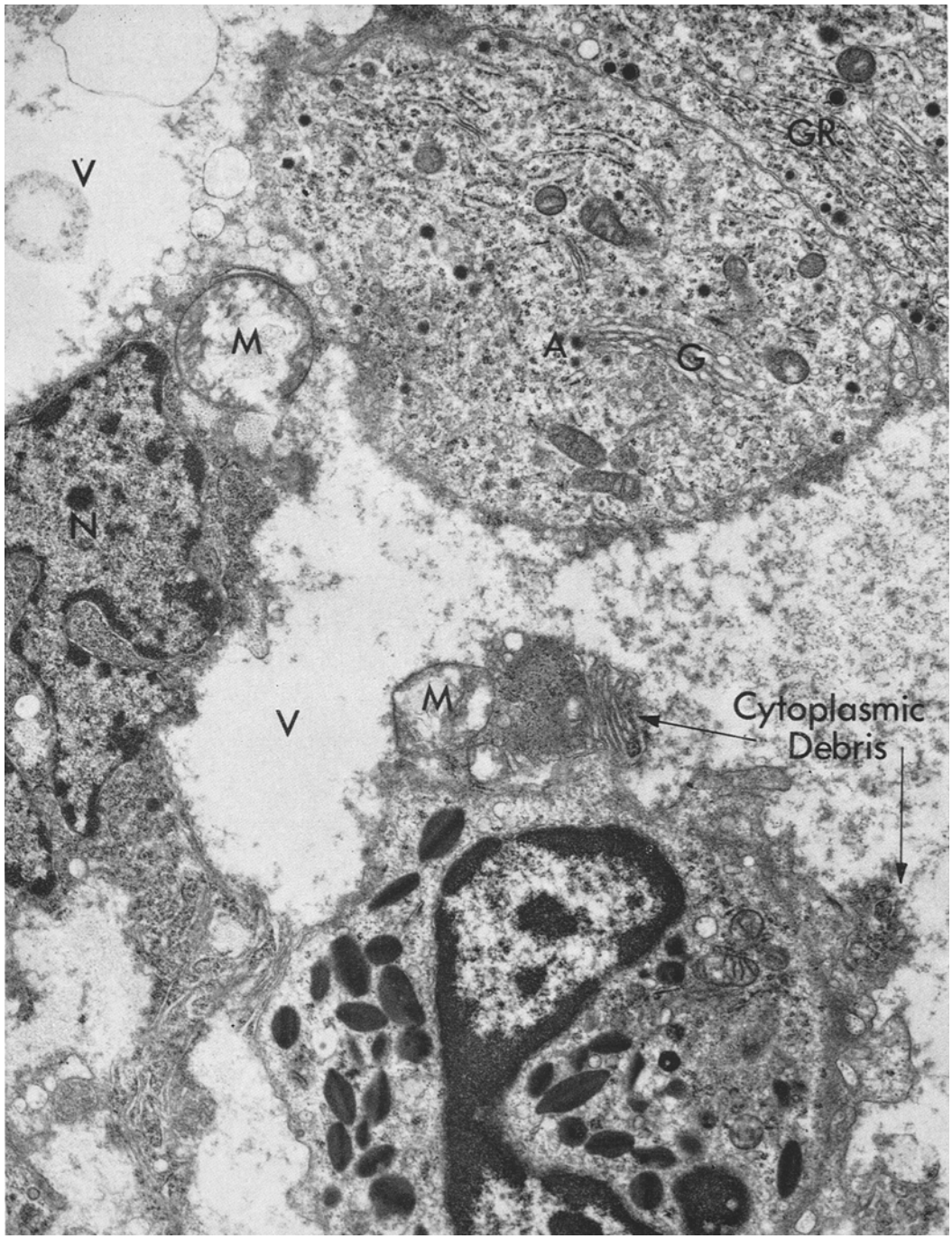

Fig. 27. Evidence of an inflammatory cell (eosinophilic leucocyte) in the vicinity of cell degeneration. Distorted beta cell nucleus, ballooned mitochondria and cytoplasmic debris are visible. The alpha cells are reasonably normal. Glutaraldehyde. Approximately 13700 ×

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