Pancreatic Beta Cell Replication Induced by Glucocorticoids in Subhuman Primates

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Pancreatic islets were studied by means of light microscopy, autoradiography and electron microscopy in untreated *Macaca cyclopis* monkeys and after the administration of large quantities of adrenal glucocorticoids. Mild hyperglycemia and profound elevations of serum immunoreactive insulin were induced by glucocorticoid injections of 1 to 3 weeks duration, with a gradual return to pretreatment levels within 2 months after cessation of treatment. Morphologic alterations included degranulation and hyperplasia of pancreatic beta cells. These were noted in association with increased numbers of labeled islet cells after the administration of ^{3}H-thymidine and beta cells undergoing mitotic division, and could be correlated directly with the magnitude of serum insulin elevation. Evidence of acinar–islet or duct–islet cell transformation was absent. Beta cell regranulation and the twofold increase in extractable pancreatic insulin which followed the cessation of injections demonstrated the survival and functional integrity of the newly formed beta cells (Am J Pathol 75:329–348, 1974).

For the past several years, attention has again been directed to the beta cell as a primary site of pathology in genetic human diabetes mellitus. The fact that there is a decrease in beta cell numbers and pancreatic extractable insulin in human diabetes, most notably the juvenile form,[1–3] suggests that alterations in the size of the beta cell population may play a primary role in the etiology of the disease in man. Support for this hypothesis can be found among laboratory models of spontaneously occurring diabetes mellitus. It is clearly apparent from several of these models that the course and outcome of the disease may be a function of the ability of the animal to produce new beta cells during periods of hyperglycemia. Hence the disease is most lethal in the Chinese hamster[4–6] and the C57BL/Ks–*db/db* mouse,[7–10] models in which beta cell replication is severely limited and a progressive diminution of the beta cell mass eventually leads to insulin insufficiency. On the

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contrary, those laboratory models which exhibit a more mild diabetic syndrome, successfully increase their beta cell mass and, therefore, their insulin synthetic capacity. Hence the C57BL/6J–*ob/ob* mice,[11,12] the C3Hf $\times$ I F_1 (Wellesley hybrid) mice,[13,14] the Swiss Hauschka mice [15] and the great majority of Egyptian sand rats [16,17] have greatly enlarged pancreatic islets, and all either co-exist with their disease or with time may actually return to the normoglycemic state.

Little is known of the factors regulating the proliferation of pancreatic beta cells in the adult human. Since all of the experimental studies on beta cell replication cited above have been collected in rodents and because the broad species gap between man and rodent may preclude meaningful correlative interpretation of data collected in rodents, we chose the subhuman primate as a hopefully more suitable model to answer the following questions: a) Are adult rodent beta cells unique in their ability to undergo mitotic division, or is this phenomenon also observed in adult members of species more closely related to man (*eg*, the subhuman primate)? b) Do subhuman primates possess the ability, observed in some rodents, to respond to diabetogenic stress or hyperglycemic stimulation by increasing beta cell mitotic activity, as a means of expanding their beta cell mass and insulin synthetic capacity?

To answer these questions, large quantities of a potent adrenal glucocorticoid were administered to fully mature *Macaca cyclopis* monkeys. The decision to utilize this classic diabetogenic agent was based upon a large number of early studies which reported the induction of hyperglycemia and islet hyperplasia in guinea pigs,[18,19] rabbits,[20,21] rats [22–24] and mice,[25] with adrenal corticosteroids, ACTH and ACTH-secreting tumors. The need for studying this higher species was underlined by the virtual absence of data collected in the subhuman primate. Indeed, only one laboratory has reported the induction of islet hypertrophy and hyperplasia in cortisone and ACTH-treated monkeys.[26] When published more than 20 years ago, the authors concluded that the alterations of islet morphology were the result of hyperplasia of centroacinar cells with neoformation of islets. There was no mention of beta cells undergoing mitotic division, and physiologic data such as blood glucose, serum insulin and pancreatic insulin levels were not reported.

In this communication we shall present the results of light microscopic, autoradiographic and ultrastructural studies of the pancreatic islets of untreated monkeys and the changes observed after adrenal glucocorticoid administration. Since physiologic studies were also performed, structural–functional correlations will be presented when pos-

sible. A detailed report of the physiologic investigations will be published separately.[27]

Materials and Methods

All animals utilized in these experiments were maintained at the New England Regional Primate Center where they had free access to Purina monkey chow and water. Daily supplements of fresh fruit were provided. Eight mature male and female *Macaca cyclopis* monkeys received daily intramuscular injections (8 mg/kg body weight) of a suspension of methyl-prednisolone-acetate (Depo-Medrol®, donated by the Upjohn Co), a long acting adrenal glucocorticoid. Body weights, blood glucose [28] and serum immunoreactive insulin (IRI) levels [29,30] were determined at weekly intervals. Groups of animals were sacrificed after receiving the glucocorticoid continuously for 1, 2 or 3 weeks. Eight control animals either were untreated or received daily intramuscular injections of the vehicle (an aqueous solution of polyethyleneglycol (30 parts), sodium chloride (9 parts) and myristyl-gamma-picolinium chloride (0.2 parts) for periods of 3 weeks. On the day of sacrifice, samples were obtained for blood glucose and serum IRI determinations. Tritiated thymidine, (5 μCi/g body wt, specific activity approximately 15 Ci/mM), was injected intravenously 2 hours prior to the induction of Nembutal® anesthesia, at which time the pancreases were removed, and the animals were sacrificed by exsaguination.

To insure the identification of labeled beta cells which may have been degranulated after 3 weeks of glucocorticoid or vehicle injections, a separate group of 9 animals received Depo-Medrol or vehicle injections for 3 weeks; following the intravenous administration of tritiated thymidine, they received no further injections and were allowed to recover for an additional 7 to 8 weeks prior to sacrifice with the expectation that beta cell regranulation would occur. It became apparent as the study proceeded, that this experimental group would also permit one to ascertain whether the newly formed beta cells would persist and function [27] for this period of time.

For routine light microscopy, pancreatic samples were fixed in Bouin's solution and/or 10% formalin and paraffin sections stained with hematoxylin and eosin, periodic acid–Schiff and a modified aldehyde fuchsin stain.[31] For autoradiography, paraffin sections mounted on gelatin-coated slides were exposed to 0.5% periodic acid solution, washed, dehydrated, air-dried and coated with Kodak nuclear track emulsion NTB-2. After exposure for periods of 1 to 4 weeks in Drierite®-containing air-tight boxes at 4 C, the emulsion was developed in Kodak Dektol developer at 18 C, fixed and the slides placed in Schiff's reagent for the completion of the PAS stain. To identify the labeled islet cells, pancreatic sections from several animals sacrificed 7 to 8 weeks after cessation of steroid administration were stained according to the aldehyde fuchsin or aldehyde thionin [32] method, prior to coating with Kodak NTB-2 or Ilford L-4 emulsion. After suitable periods of exposure (up to 10 weeks) the emulsion was developed as indicated above, and the sections lightly counterstained with nuclear fast red.

For electron microscopy, the pancreases were fixed at room temperature by immersion in either 3% glutaraldehyde buffered with sodium cacodylate (0.1 M), pH 7.4, or a paraformaldehyde-glutaraldehyde solution, diluted 1:1 with sodium cacodylate (0.1 M), final pH 7.2 to 7.5.[33] Calcium chloride (0.005 M) was added to both aldehyde fixatives. After fixation for approximately 2 hours, tissues were washed in 0.1 M sodium cacodylate and postfixed at 0 to 4 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 7.4. Rapid dehydration in a graded series of ethanol solutions was followed by embedding in an Epon-Araldite mixture. Sections cut at approximately 1 μ thickness were stained with 1% toluidine blue-O in 1% borax for light microscopic identification of islets. Thin sections were then prepared with an LKB Ultratome using DuPont diamond knives, stained sequentially with aqueous uranyl acetate and lead citrate [34] and examined with an RCA EMU-3G electron microscope.

For the determination of extractable pancreatic IRI content, tissue samples obtained from each pancreas were rapidly frozen with dry ice, stored at −20 C and subsequently extracted with cold acid ethanol [35] for immunoassay.[29,30]

Results

Physiologic Studies

Only a brief resume of these studies is presented here. A full report of the physiologic data will be published separately.[27] Table 1 shows blood glucose (BG), serum immunoreactive insulin (IRI) and extractable pancreatic IRI values in untreated control monkeys, in animals after 3 weeks of methylprednisolone-acetate injections, in animals treated with the glucocorticoids for 3 weeks and allowed to recover for a period of 7 to 8 weeks and in monkeys after 3 weeks of vehicle injections. In spite of the large quantities of steroid administered, most animals failed to develop severe hyperglycemia. In comparison with the pretreatment mean BG of 54 mg%, the mean glycemic level after 3 weeks of glucocorticoid injection was 75 mg%. Depo-Medrol injections did, however, induce markedly increased serum IRI levels with elevation from a pretreatment mean of 120 μ units/ml to 1217 μ units/ml after 3 weeks of injections. With the cessation of glucocorticoid administration, BG and serum IRI values gradually returned towards and were not significantly greater than pretreatment levels after 7 to 8 weeks. Extract-

Table 1 — Effects of Glucocorticoid on Blood Glucose, Serum and Pancreatic IRI Content in Subhuman Primates (mean ± SE)

Group	Blood glucose (mg/100 ml)	N	P	Serum IRI (μunits/ml)	N	P	Pancreatic IRI (units/g)	N	P
Control	54 ± 2	16	—	120 ± 45	16	—	6.81 ± 0.96	9	—
Steroid (3 weeks)	75 ± 7	11	0.005	1217 ± 388	11	0.005	2.92 ± 0.38	3	0.05
Recovery*	59 ± 4	6	NS	212 ± 103	6	NS	13.14 ± 1.58	6	0.005
Vehicle (3 weeks)	67 ± 10	6	NS	208 ± 80	6	NS	6.73 ± 2.18	3	NS

P is the significance of the difference compared to the control group, as determined by the Student t test.

Blood glucose and serum IRI values are nonfasting. A human insulin standard (Eli Lilly Co) was used in measuring IRI. Standard curves were essentially identical to those obtained from purified monkey insulin (kindly supplied by Dr. Donald F. Steiner).

* Values obtained on the day of sacrifice, 7 to 8 weeks after steroid was discontinued.

able pancreatic IRI content fell from control values of 6.8 units/g of wet tissue to 2.92 units/g after 3 weeks of steroid injections. However, 7 to 8 weeks after the cessation of steroid administration, pancreatic insulin content was strikingly increased at 13.14 units/g.

Blood glucose levels in control animals injected only with the vehicle were essentially unchanged. Although serum IRI levels in these animals were moderately elevated, extractable pancreatic IRI content did not differ significantly from the untreated controls either during therapy or following the recovery period.

Light Microscopic Studies

Untreated Control Monkeys

The light microscopic appearance of the pancreatic islets of untreated *Macaca cyclopis* monkeys (Figure 1) could not be readily distinguished from those of the human primate previously studied in this laboratory.[36] Although rounded and oval-shaped structures (when visualized in two dimensions) were most numerous, occasional islets were quite irregular and stellate in configuration. The intraislet capillary network separated the endocrine cells into cords or trabeculae, collagen was not abundant within or around the islets, and the peripherally situated endocrine cells were in close contact with but clearly demarcated from the surrounding acinar tissue. Aldehyde-fuchsin-stained preparations (Figure 1) revealed a random distribution and orientation of alpha cells and well-granulated beta cells. Most islets possessed the random admixture of endocrine cell types characteristic of the human. Others displayed central or eccentric clusters of alpha cells with a symmetrical or asymmetrical mantle of beta cells. Examples of islets with a central grouping of beta cells surrounded by peripheral alpha cells, as observed in rats and mice, were not encountered. Although beta cells were in the majority in virtually every islet examined, there were rare examples in which the alpha cells were predominant. The endocrine cell nuclei showed very little variation in size and shape, and evidence of mitotic division was absent in untreated animals.

Monkeys Receiving Glucocorticoids

The most important and prevalent histologic feature of animals receiving glucocorticoids was the presence of beta cell degranulation as observed in aldehyde fuchsin stained preparations (Figure 2). Although noted in all monkeys receiving steroids, the relative number of degranulated beta cells and the degree of degranulation was variable and could

be correlated directly with increased levels of seurm IRI. The degranulated beta cells were increased in size, and their enlarged nuclei revealed a variability in size, shape and staining intensity that suggested an increased chromatin content. Furthermore, beta cells undergoing mitotic division were identified with relative ease (Figure 2). Although quantitative data were not collected, the frequency of mitosis appeared to be directly related to the decreased beta cell granularity. The islets of animals receiving steroids were also increased in size, and the orderly trabecular pattern of the normal islets was often replaced by irregular sometimes nodular-appearing collections of cells. In general, the severity of all of the above changes was best correlated with the magnitude of serum IRI elevation rather than the glycemic elevation or the duration of steroid administration.

The pancreatic islets of animals sacrificed 7 to 8 weeks after cessation of glucocorticoid injections were still enlarged, but were characterized once again by the presence of well-granulated beta cells and the apparent absence of cells undergoing mitotic division (Figure 4).

Vehicle-Injected Control Monkeys

The islets of these animals were variable in appearance. Although most could not be distinguished from those of untreated animals, there were examples of mild degranulation of individual beta cells and the subtle suggestion of increased islet size. Degranulation, however, was never prominent and islet cell mitoses were observed only infrequently.

Autoradiographic Studies

General Comments

The terms *labeled cell* and *labeling* are used interchangeably and denote the presence of a cluster of black silver grains in the photographic emulsion overlying the cell nucleus. The silver grains identify the paths of beta particles, originating in DNA synthesized at the time of ^{3}H-thymidine injection, which pass into and expose the overlying photographic emulsion. For control purposes, the presence of heavily labeled crypt cells of the duodenal mucosa (in acute experiments) and of labeled lymphocytes in pancreatic lymph nodes (in animals sacrified 7 to 8 weeks after ^{3}H-thymidine injection) was required to verify the introduction of ^{3}H-thymidine into the thymidine pool of the monkey.

Control Monkeys (Untreated and Vehicle Recipients)

Labeled islet cells were identified only with great difficulty in untreated control animals. In most instances a random section of pancreas

in which 100 to 150 islets were identified contained at most two to five labeled islet cells. Labeled exocrine and connective tissue cells (vascular endothelial cells, fibroblasts, etc) were observed with greater frequency but were also quite uncommon. The incidence of labeled endocrine, exocrine and connective tissue cells was increased in vehicle-treated monkeys. The increase however, was patchy and always considerably less than that observed among glucocorticoid injected animals.

Monkeys Receiving Glucocorticoids

The number of labeled islet cells was increased in all animals treated with steroids (Figure 3), and was especially pronounced in monkeys with the greatest degree of beta cell degranulation, the most numerous islet cell mitoses and the highest levels of serum IRI. A 20- to 30-fold increase in islet cell labeling was estimated in the pancreata of glucocorticoid injected animals. The presence of nuclear labeling appeared to be unrelated to individual islet size and the location (periphery vs central) of labeled cells within individual islets was apparently random. Exocrine and connective tissue cell labeling was also greater than that observed in control monkeys, but the magnitude of the increase was less than among endocrine cells and was apparently unrelated to alterations in blood glucose and serum IRI levels. Labeled acinar, centroacinar and ductal cells were randomly situated within the exocrine pancreas and were not necessarily in proximity to the islets.

The pattern of islet labeling in animals allowed to recover for 7 to 8 weeks prior to sacrifice was unchanged, although the number of labeled cells and the intensity of labeling may have decreased. Aldehyde-fuchsin and aldehyde-thionin stained autoradiographs clearly demonstrated only labeled beta cells (presumably regranulated during the recovery period). Although labeled alpha cells were *not* identified, several labeled islet nuclei could not be attributed accurately to either cell type. Lastly, although many labeled lymphocytes persisted within pancreatic lymph nodes, and labeled connective tissue cells were identified, only a rare labeled exocrine cell was observed after the preiod of recovery.

Ultrastructural Studies

Normal Untreated Monkeys

The pancreatic islets of normal monkeys resembled those of other mammals in that they were composed of beta, alpha and delta cells, organized into cords or trabeculae by the fenestrated capillaries. Delicate collagen fibrils were frequently enmeshed within the capillary basal lamina, and small numbers of unmyelinated nerve endings were

noted, most commonly at the islet periphery, where they came into close proximity with adjacent endocrine cells. The numerous *beta cells* were relatively uniform in appearance and could be identified on the basis of their characteristic secretory granules (Figure 5). Although variable in size and electron opacity, the abundant beta granules were almost always rounded and enclosed within tightly fitting granule-limiting membranes. The majority were also larger and more electron lucent than alpha and delta granules; however, smaller and more electron-dense granules were also identified, as were rare crystalloid forms which resembled typical human beta granules. Elements of the inconspicuous rough endoplasmic reticulum (RER) and Golgi complex were characteristically dispersed throughout the cell and among the secretory granules which filled most of the cytoplasmic volume. Mitochondria were elongated, slender or circular depending upon the plane of section and contained relatively few loosely packed cristae mitochondriales. Infrequently, focal cell-to-cell junctional contacts (macula adherens) were visualized but were always very small and often incomplete. In the occasional beta cell which contained a smaller number of secretory granules, the RER and Golgi components were relatively more prominent. The *alpha cells* occupied no characteristic location within the monkey islet and were identified therefore on the basis of secretory granule morphology, since other cellular structures did not differ in any consistent manner. Granules were smaller and more electron opaque than beta granules and were contained in more loosely fitting sacs (Figure 5). There was less variation in granule size and density than among the beta granules and elements of the RER and Golgi complex were more abundant and compactly organized. The *delta cells* were encountered least frequently and were also identified on the basis of their characteristic secretory granules, which were the smallest of the three cell types. The electron opacity of the delta granules was similar to that of alpha granules, but the granule limiting membranes were very closely applied to the periphery of the granules making it possible for one to distinguish the two cell types. Other endocrine cell types, or undifferentiated (stem) cells were not observed.

Monkeys Receiving Glucocorticoids

Ultrastructural alterations in animals receiving methyl-prednisolone-acetate were essentially restricted to the beta cells, wherein the magnitude of the structural modifications was a reflection of the elevated serum IRI levels. In the presence of serum IRI levels between 250 and 500 μ units/ml, beta cell alterations included; partial secretory degranu-

lation, a larger more compact Golgi and an expanded RER (Figure 6). When serum IRI levels exceeded 750 μ units/ml, evidence of beta cell degranulation was striking, with few small secretory granules remaining in cells occupied by a greatly expanded RER, a large and complex Golgi structure, larger and more numerous mitochondria and an assortment of Golgi related vesicles and tubules (Figure 7). Beta cells undergoing mitotic division (Figure 8) were observed in those steroid-injected monkeys evidencing beta cell degranulation and in every instance were identified by the presence of typical secretory granules. Alpha and delta cells undergoing mitosis were not observed. The beta cells of the glucocorticoid injected monkeys allowed to recover for 7 to 8 weeks prior to sacrifice were crowded with characteristic secretory granules. The Golgi structures and the RER were correspondingly diminished in magnitude and again dispersed among the other cytoplasmic components. In contrast, however, with the untreated control monkeys, the islets after recovery contained greater numbers of beta cells evidencing mild or partial degranulation.

Monkeys Receiving Vehicle

Most pancreatic islets in this experimental group were unaltered and could not be distinguished from the untreated controls. The islets of animals with moderately elevated levels of serum IRI also revealed ultrastructural evidence of mild beta cell degranulation as described above.

Exocrine Tissue

The acinar and ductal cells of treated animals did not reveal any significant ultrastructural alterations. Specifically, examples of acinar-islet or ductal-islet mixed cells or transformation were not observed. In a number of injected animals, foci of inflammation and/or fibrosis were observed within the exocrine tissue. These alterations were observed in steroid-treated as well as vehicle-treated monkeys, could not be related to any of the functional or structural observations reported above and will therefore not be further described or discussed.

Discussion

The findings reported above indicate that the pancreatic beta cells of rodents are not unique in their ability to undergo mitotic division. The evidence of low level uptake of ^{3}H-thymidine by pancreatic islet cells of untreated control monkeys is in agreement with previously published data for the mouse [9,10,37,38] and guinea pig.[19] It clearly indicates that the

basal turnover of islet cells is a normal phenomenon in adult primates and presumably necessary for maintaining an optimum population of pancreatic islet cells under normal conditions of "wear and tear." Furthermore, our data which indicate an increase in nuclear labeling and an increased frequency of mitotic division among differentiated beta cells following glucocorticoid injections confirm and extend previous studies in rodents [9,10,19] to the primate. Although many earlier investigators [18-26] reported the phenomenon of pancreatic islet hyperplasia following adrenal glucocorticoid administration and most described the presence of beta cell mitoses, the importance and relevance of mitotic activity among fully differentiated beta cells to the genesis of islet (specifically beta cell) hyperplasia was not appreciated in the rodent prior to the work of Kern and Logothetopoulos [19] and was not previously [26] recognized as significant in the primate. The findings reported above of a twofold increase in extractable pancreatic insulin, 7 to 8 weeks after the cessation of steroid injections, indicate in addition, that the population of newly formed beta cells are not only capable of synthesizing and storing insulin, but appear to survive as well for at least 2 months. The ability of these beta cells to respond to a physiologic stimulus, such as the intravenous administration of glucose has also been established.[27]

The findings reported above unfortunately shed no light on the mechanism by which glucocorticoid administration stimulates beta cell replication. Although all animals received equal quantities (per kilogram body weight) of Depol-Medrol, the magnitude of the beta cell response was variable. Hence, it is unlikely that the adrenal glucocorticoid per se was the signal responsible for increased beta cell replication. Furthermore, the degree of blood glucose and serum insulin elevations were neither uniform nor predictable among the treated animals. Although quantitative measurements of beta cell labeling and mitoses were not made, it appeared clear that beta cell replication was most marked in those animals with the most profound elevations in serum IRI during the course of steroid injections. This suggested association of mitotic and functional activities of pancreatic beta cells is supported by a previous report from this laboratory [38] in which increased beta cell replication appeared to be correlated best with enhanced beta cell functional activity, as reflected in elevated levels of serum IRI.

With regard to the possibility that the increase in islet size, and specifically beta cell hyperplasia, was the result of islet cell neogenesis from primitive ductal, centroacinar and/or acinar cells, the extensive and controversial literature concerning the morphogenesis of experi-

mentally induced islet hyperplasia has been thoroughly reviewed by Lazarus and Volk.[39] In the present experiments, neither undifferentiated precursor cells nor mixed cell types were observed within the islets of control or treated animals. Furthermore, the observation that nuclear labeling was most frequent among islet cells and that labeled islet cells were not preferentially localized at the islet periphery, together with the observation of nuclear labeling and mitotic division among differentiated beta cells, all strongly support the belief that in adult subhuman primates, as in adult mice[9,10,37,40] and guinea pigs,[19] beta cells serve as their own progenitors.

The mechanism of action of the vehicle-induced sporadic hyperglycemia with associated increases in serum IRI and pancreatic labeling is unknown. Although it would appear to be desirable from a pharmacologic point of view to determine the component(s) of the vehicle responsible for the induction of hyperglycemia, the physiologic and morphologic alterations induced appeared to be unimportant in so far as the present experiments were concerned. In future experiments however, it would certainly be preferable to use an agent without this particular vehicle.

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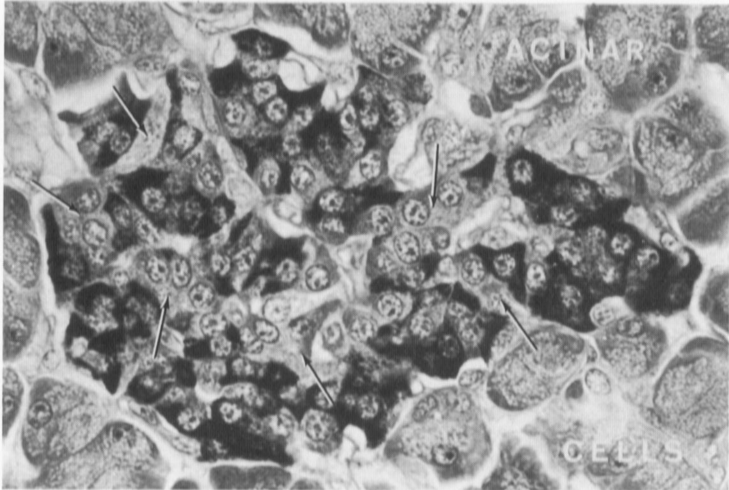
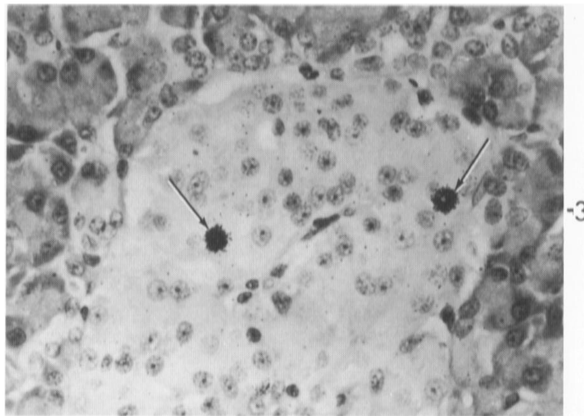
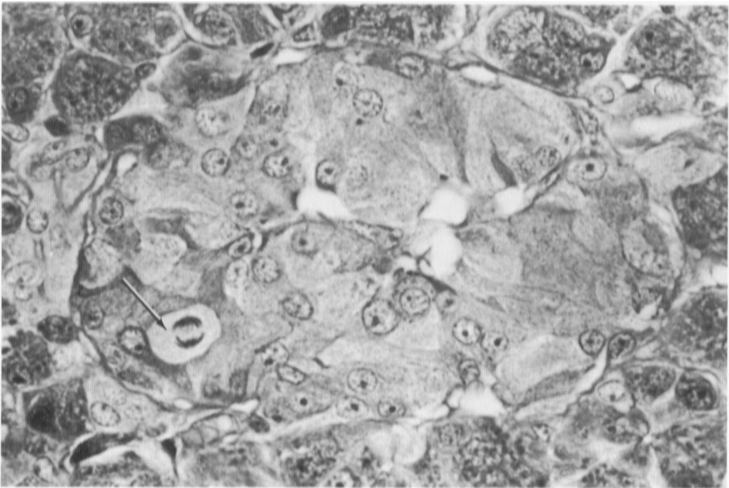
[*Illustrations follow*]







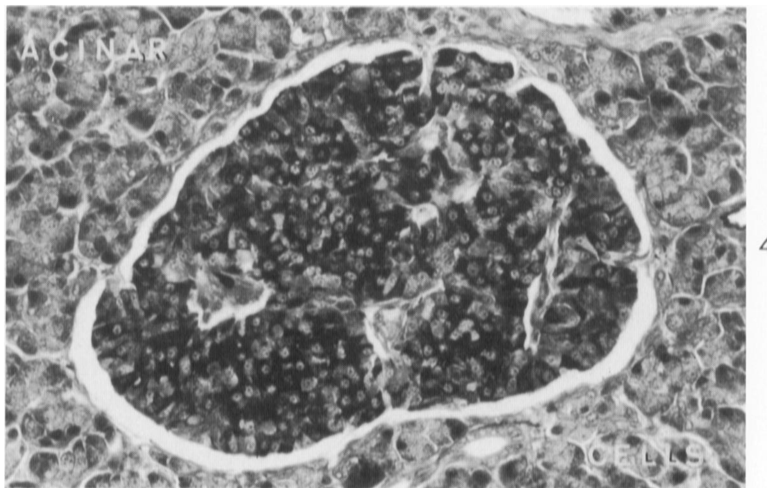
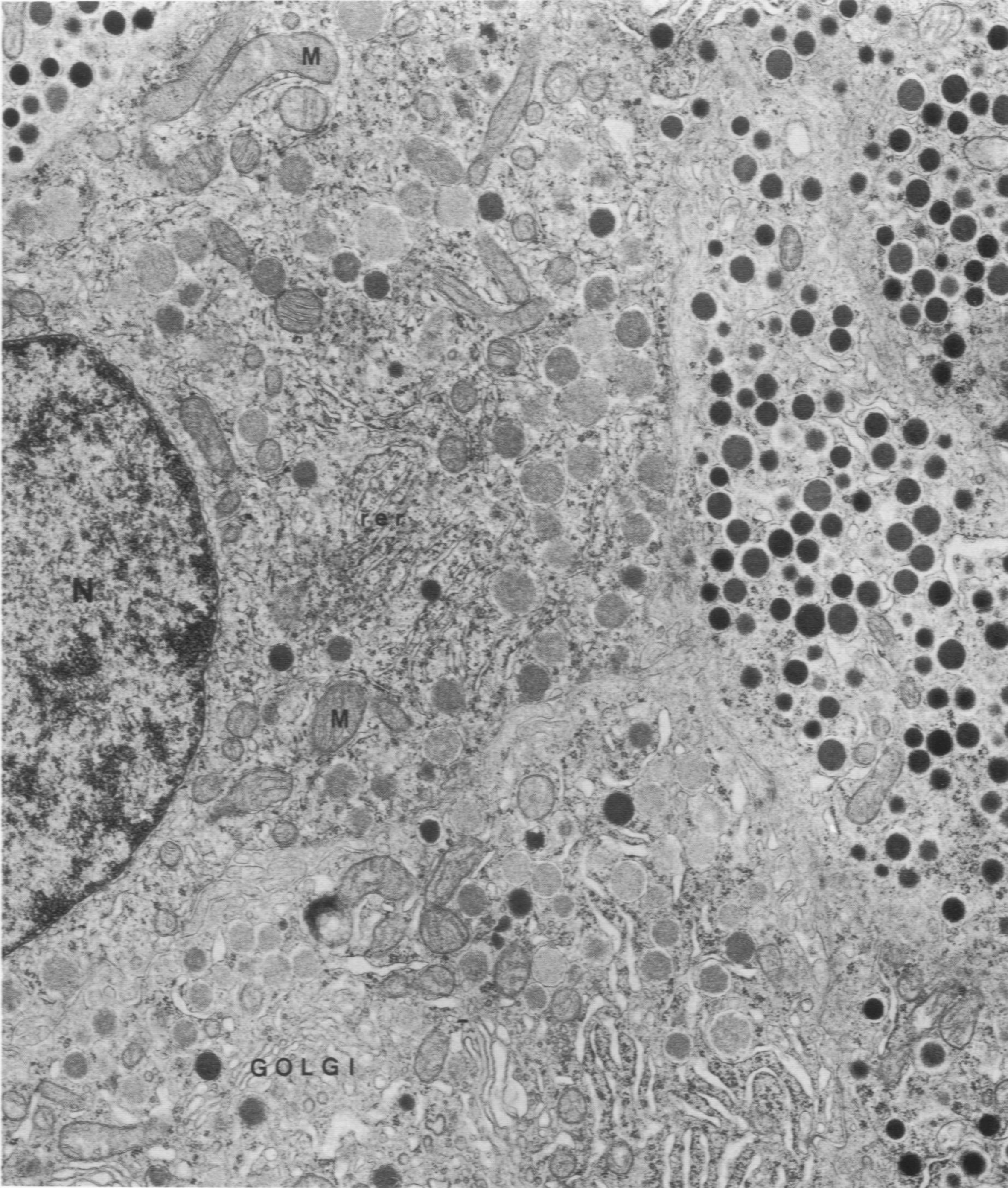


Fig 1—Pancreatic islet of untreated *Macaca cyclopis* monkey. The deeply staining well-granulated beta cells (black in photograph) are characteristic of normal animals. Randomly situated alpha cells are indicated by *arrows* (Aldehyde fuchsin, × 560). **Fig 2**—Pancreatic islet of monkey injected with glucocorticoids for 3 weeks. Beta cell degranulation is pronounced. Beta cell undergoing mitotic division is indicated by *arrow*. Cell type was established by the presence of cytoplasmic aldehyde fuchsin positive granules, which are not discernible in the black and white micrograph. Blood glucose, 94 mg/100 ml; serum IRI, 2240 μunits/ml (Aldehyde fuchsin, × 560). **Fig 3**—Pancreatic islet of monkey treated with glucocorticoids for 2 weeks. Autoradiograph after ^{3}H-thymidine administration. The nuclei of two islet cells are labeled (*arrow*). Blood glucose, 224 mg/100 ml; serum IRI 860 μunits/ml (PAS, × 560). **Fig 4**—Pancreatic islet of monkey treated with glucocorticoids for 3 weeks and allowed to recover for 7 weeks. The greatly enlarged islet is composed predominately of well-granulated beta cells. Blood glucose, 66 mg/100 ml; serum IRI, 370 μunits/ml (Aldehyde fuchsin, × 225).

Fig 5—Portions of beta and alpha cells from an untreated control monkey. The micrograph is largely occupied by a well-granulated beta cell with smaller portions of an alpha cell to the right and upper left. The numerous beta secretory granules are contained within closely fitting granule limiting membranes and are of varying electron densities. The rough endoplasmic reticulum (*rer*) and Golgi components occupy small portions of the cell cytoplasm, and mitochondria (***M***) are elongated and slender. The alpha secretory granules are somewhat smaller and more electron opaque (Approximate magnification, × 14,200).


M
rer
N
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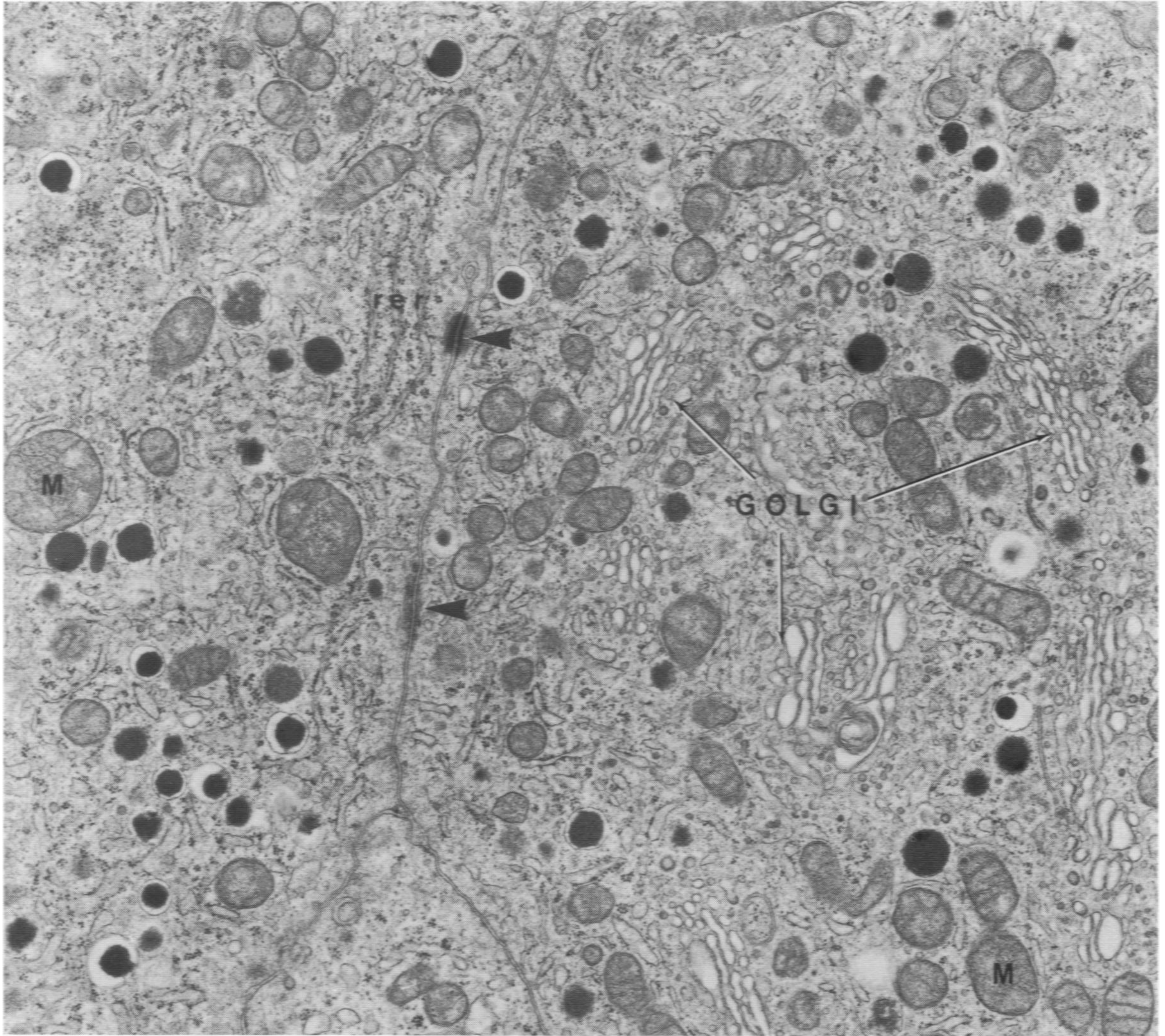

Fig 6—Portions of partially degranulated beta cells from a glucocorticoid-injected animal. Secretory granules are diminished in number, the rough endoplasmic reticulum (*rer*) and Golgi components are augmented and mitochondria (*m*) sometimes enlarged. *Arrowheads* indicate junctional complexes joining adjacent beta cells. Blood glucose, 74 mg/100 ml; serum IRI 285 μunits/ml (Approximate magnification × 14,200).

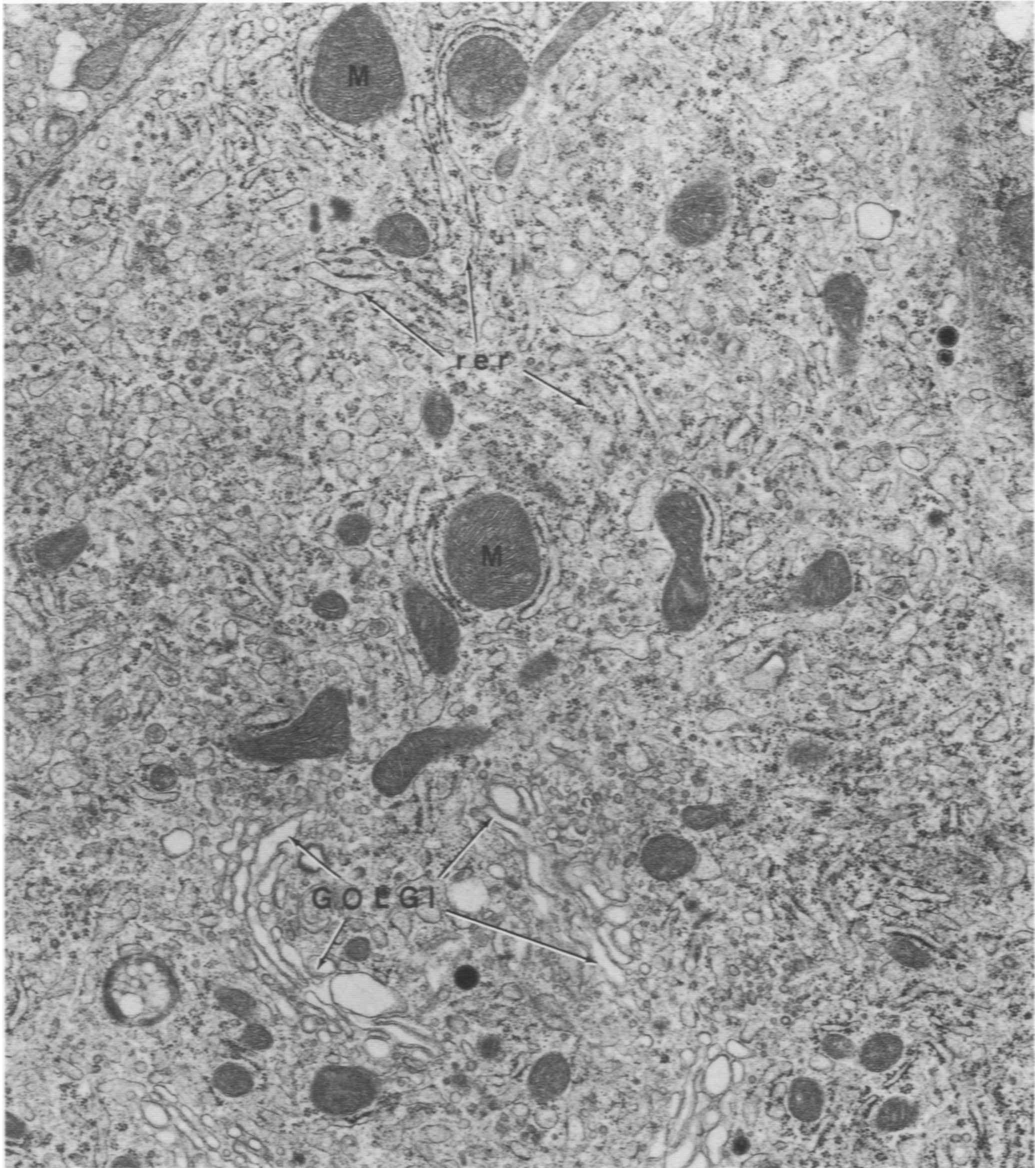

Fig 7—Beta cell of monkey after treatment with glucocorticoids for 2 weeks. Secretory degranulation is virtually complete with pronounced expansion of the rough endoplasmic reticulum (*rer*) and an enlarged Golgi. Individual mitochondria (*M*) are enlarged. Blood glucose, 224 mg/100 ml; serum IRI, 860μ units/ml (Approximate magnification × 14,200).

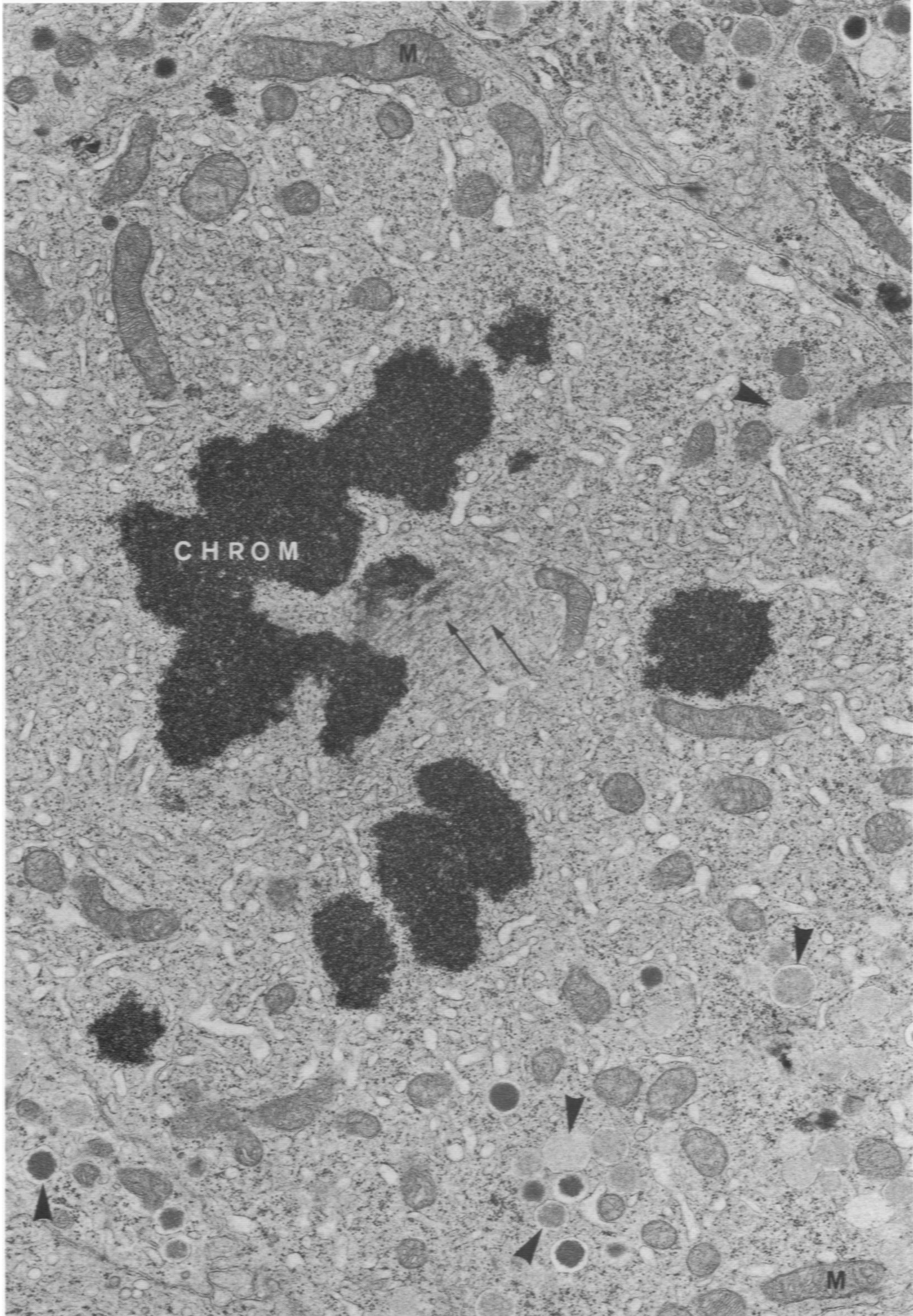

Fig 8—Beta cell undergoing mitotic division. This animal received glucocorticoids for 3 weeks. The nuclear membrane is absent and the nuclear chromatin is divided among the obliquely sectioned chromasomes (*CHROM*). Profiles of mitotic spindle fibers (*arrows*) are recognized as are numbers of beta secretory granules (*arrowheads*). Blood glucose, 60 mg/100 ml; serum IRI 385 μunits/ml (Approximate magnification × 14,200).