FINE STRUCTURE OF THE A AND D CELLS OF THE RABBIT ENDOCRINE PANCREAS IN VIVO AND INCUBATED IN VITRO

I. Mechanism of Secretion of the A Cells

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ABSTRACT

The cytological changes observed in the A and D cells of rabbit endocrine pancreas incubated in a medium containing 0.6 mg/ml or 3 mg/ml of glucose are described. These cells showed no changes in their fine structure nor any signs of degranulation. When the A cells were incubated in a medium without glucose, they released A granules and synthesized new hormone. The way in which A granules are eliminated is compared to that following insulinic hypoglycemia in the animal in vivo. In both cases, the mechanism of secretion involves margination, emiocytosis of the entire granule, and formation of microvilli, in contrast to previously reported observations (9). The D cells showed no alteration of their fine structure after incubation with different concentrations of glucose in the medium. Only very rarely could we observe morphological changes which were suggestive of emiocytosis of the entire D granule.

INTRODUCTION

The behavior of the B cells of the rabbit endocrine pancreas has been investigated, by electron microscopy, in pieces of pancreas maintained in a shaking incubator for experimental periods up to 1 hr (2). These investigations have established that the changes observed in B cells following a 1-hr incubation with 0.6 mg/ml glucose consist of (1) degranulation, (2) hyperplasia and vesiculation of ergastoplasm, and (3) partial regranulation. The B cell preparation in vitro has been used to great advantage for the study of the mechanisms of secretion of insulin (13). Up to the present time, no morphological study has been undertaken with A or D cells in vitro, with the aim of finding a system in vitro in which the mechanisms of secretion of these cells could be investigated under the same conditions as the B cell. We undertook the present study to determine the cytological changes observed in A and D cells maintained in an incubation medium with different concentrations of glucose. Since it was found that the release of the A granule in vitro was different from that described in a previous report (9), we also studied the way in which the A granules are released after insulinic hypoglycemia in the animal in vivo.

MATERIALS AND METHODS

The pancreases of six rabbits (1.5–2 kg body weight) which had been fasted for 18–20 hr were removed after the animals were killed by a blow on the head

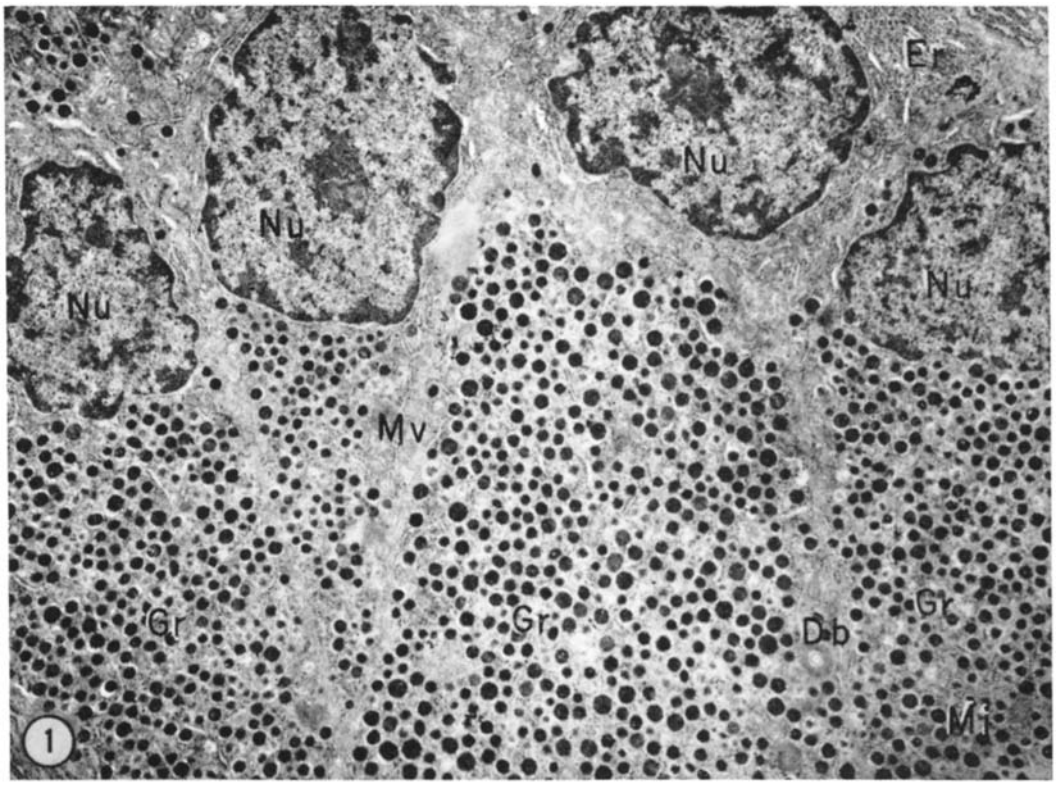

FIGURE 1 Normal A cells of rabbit endocrine pancreas showing nuclei (*Nu*), A secretory granules (*Gr*), ergastoplasmic sacs (*Er*), microvilli (*Mv*), dense bodies (*Db*), and mitochondria (*Mi*). × 7800.

and decapitation. A piece of the pancreatic tail was incubated in 10 ml of medium (Ringer solution, supplemented with glucose 0.6 mg/ml, pyruvate, fumarate, and glutamate, 5 mM each) at 38°C in a gas phase 95% O_2 + 5% CO_2. Each piece was kept in this incubation medium for 1 hr. For morphological studies, tissue samples were taken at 0, 15, 30, and 60 min.

In a second group of eight experiments, the pancreatic tissue was maintained for 1 hr in the medium described above, and then transferred to a medium in which the amount of glucose was 3 mg/ml and kept in this medium for another ½ hr. In this second set of experiments, tissue samples of morphological studies were taken at 0 and 60 min of the 1st hr of incubation time and at 5, 15, and 30 min of the last ½ hr of incubation time. In a third group of four experiments, pancreatic tissue was maintained in the same medium as described above, except that no glucose was present in the medium. For morphological studies, portions of tissues were taken at 0, 15, and 60 min of incubation time.

In a fourth group of eight experiments, rabbits which had been fasted for 18–20 hr were injected intravenously with ten units of insulin per kg body weight. All the animals were killed, by decapitation after a blow to the head, at different intervals after the injection time: two after ½ hr, three after 1 hr, and three after 2 hr. Blood glucose was measured in all of these animals.

For the electron microscope, small pieces of pancreas were fixed in distilled glutaraldehyde (1) with Millonig buffer (total osmolarity of the fixation vehicle, 480 milliosmols) and postfixed for 2 hr in 2% osmium tetroxide in the same buffer. The fixed tissue was embedded in Epon 812 (7). Sections were prepared in the routine manner and stained with a combination of uranyl acetate and lead citrate (12). Sections mounted on uncoated grids were examined with a Siemens Elmiskop I.

For light microscopy, pieces of pancreas were fixed in Zenker's solution. Sections were stained with aldehyde-thionin (11) and then counterstained with Gomori's one-step trichrome stain (3).

RESULTS

Normal Rabbit Pancreatic Islets

FINE STRUCTURE OF THE A CELLS: The A cell of the rabbit pancreas has been described

previously (9, 10). The A cells tend to be located near the periphery of the islet. In an electron micrograph the A cells can easily be distinguished from the B cells on the basis of the structure of the cytoplasmic granules, as described by Lacy (4, 5, 10). The A granules possess a dense internal portion separated by a less dense space from the distinct, closely applied limiting membrane (Fig. 1). Most normal A cells contain large numbers of such secretory granules filling the cytoplasm. Bundles of delicate filaments are dispersed throughout the cytoplasm of the A cells. Other typical cytoplasmic organelles present are flattened ergastoplasmic sacs and ribosomes. The mitochondria are rod shaped. Dense bodies are noticed quite often. In our material, we have often observed a few microvilli in the plasma membranes of some A cells (Fig. 1).

FINE STRUCTURE OF THE D CELLS: The fine structure of the D cell of the rabbit pancreas has been described recently (10, 8). It has been demonstrated that this cell corresponds to the argyrophilic cell of the islet. These cells in glutaraldehyde-fixed tissue have secretory granules which are less dense than the A-cell granules (Fig. 2). The limiting membrane of the secretory granule is very closely applied to the granule core which has a uniform opacity. Meyer and Bencosme (8) have shown that epithelial filaments are present in large amounts in D cells and that they have the same characteristics as those described in A and B cells of the rabbit pancreas (Fig. 3). Other organelles of these cells are rod-shaped mitochondria, free ribosomes, and ergastoplasmic sacs. The Golgi apparatus is usually perinuclear in location, and

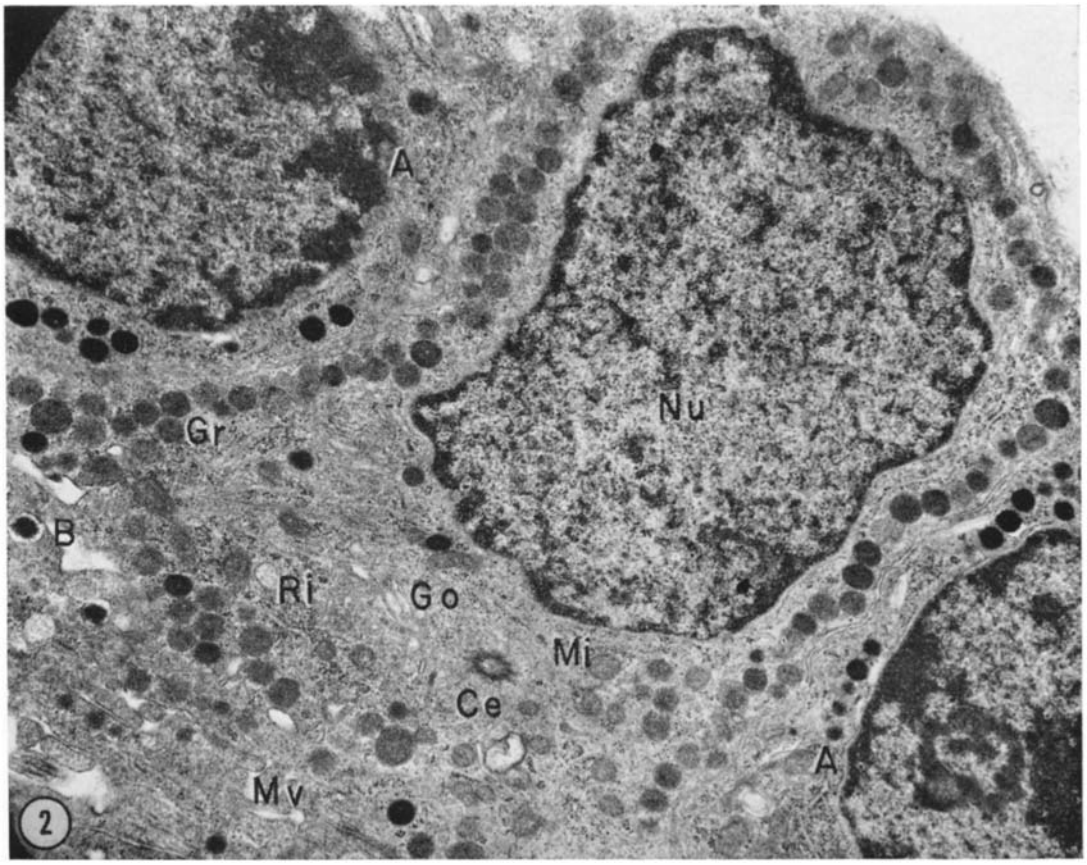

FIGURE 2 Normal D cell of rabbit endocrine pancreas showing nucleus (*Nu*), D secretory granules (*Gr*), ribosomes (*Ri*), Golgi apparatus (*Go*), mitochondria (*Mi*), centriole (*Ce*), and microvilli (*Mv*). A cells (*A*) and B cells (*B*) are also shown. × 16,000.

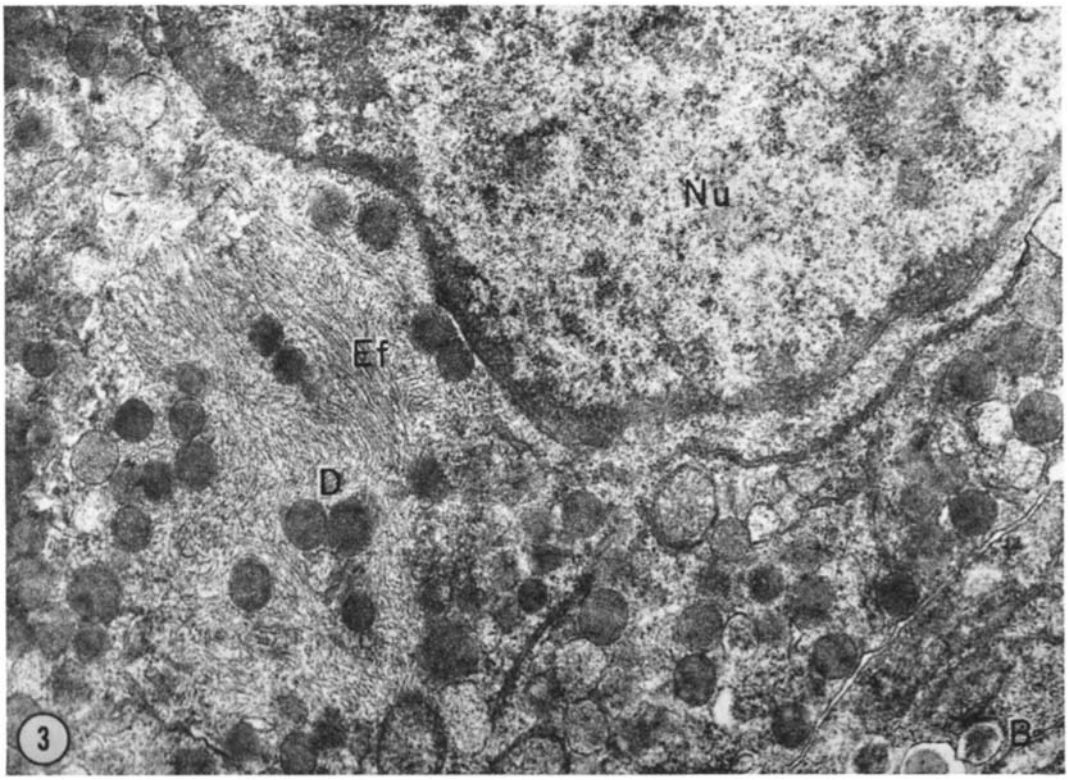

FIGURE 3 Epithelial filaments (*Ef*) in normal D cell of rabbit endocrine pancreas. Also shown are D secretory granules (*D*) and nucleus (*Nu*). × 27,500.

the secretory granules are very often close to the plasma membrane of the cell.

Rabbit Pancreatic Islets Incubated In Vitro

MORPHOLOGY OF A CELLS INCUBATED IN VITRO WITH GLUCOSE: There were no visible morphological changes in the A cells after the 1st hr of incubation in medium containing 0.6 mg/ml of glucose. The majority of the A cells were filled with granules, and only very rarely could we observe a partly degranulated cell. We observed no fragmentation and elimination of granules as described by Munger (9). The mitochondria and other cytoplasmic organelles were well preserved, and in only a few cells was an increase in the number of ergastoplasmic sacs or prominent Golgi complexes noted. Some intercellular spaces were widened, and a few microvilli were seen in the plasma membranes. Transferral of the cells, after this 1st hr of incubation, to an incubation medium containing 3 mg/ml glucose produced no changes in the morphology of these cells apart from that described above (Fig. 4).

MORPHOLOGY OF A CELLS INCUBATED IN VITRO WITHOUT GLUCOSE: At 15 min of incubation time, most of the A cells contained numerous secretory granules. Nevertheless, in some of the A cells the A granules showed apparent margination, with their surrounding sacs adjacent to the surface of the cell, and, in many instances, the plasma membrane had fused with the enveloping sacs of the granules and had ruptured. At this point, some of the granules were lying entirely free in the extracellular space (Fig. 5). In some places, these concave spaces were empty, suggesting that the secretory granule had been released there and then dissolved. This phenomenon could be seen on most of the islets and in many of the cells examined, and no more than one or two granules were seen in the process of emiocytosis per cross-section of a cell. A few of the cells showed an increase in the number of microvilli projecting into

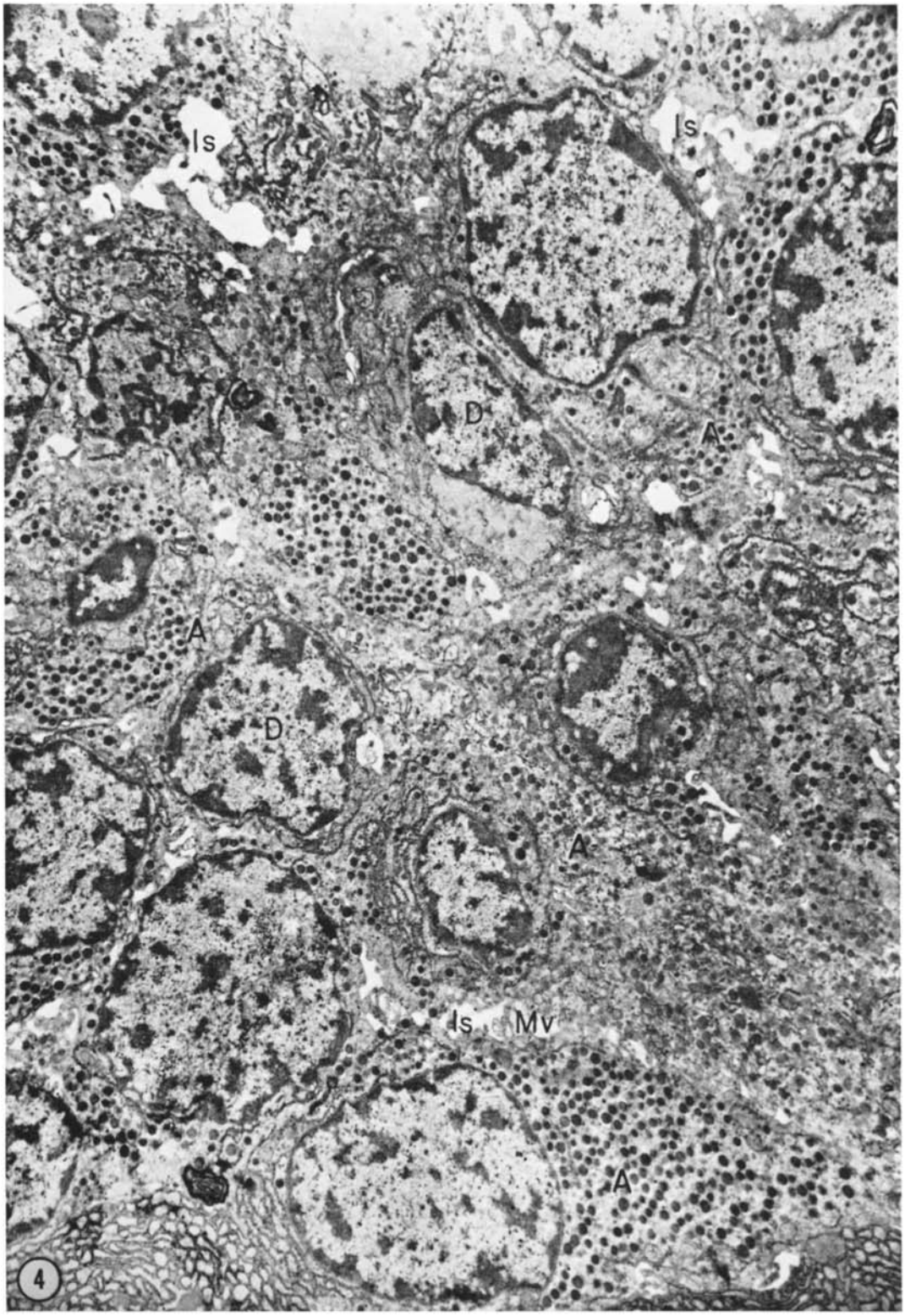

FIGURE 4 A and D cells of rabbit endocrine pancreas after 1½ hr of incubation with glucose. A cells (*A*); D cells (*D*); intercellular spaces (*Is*); microvilli (*Mv*). × 5400.

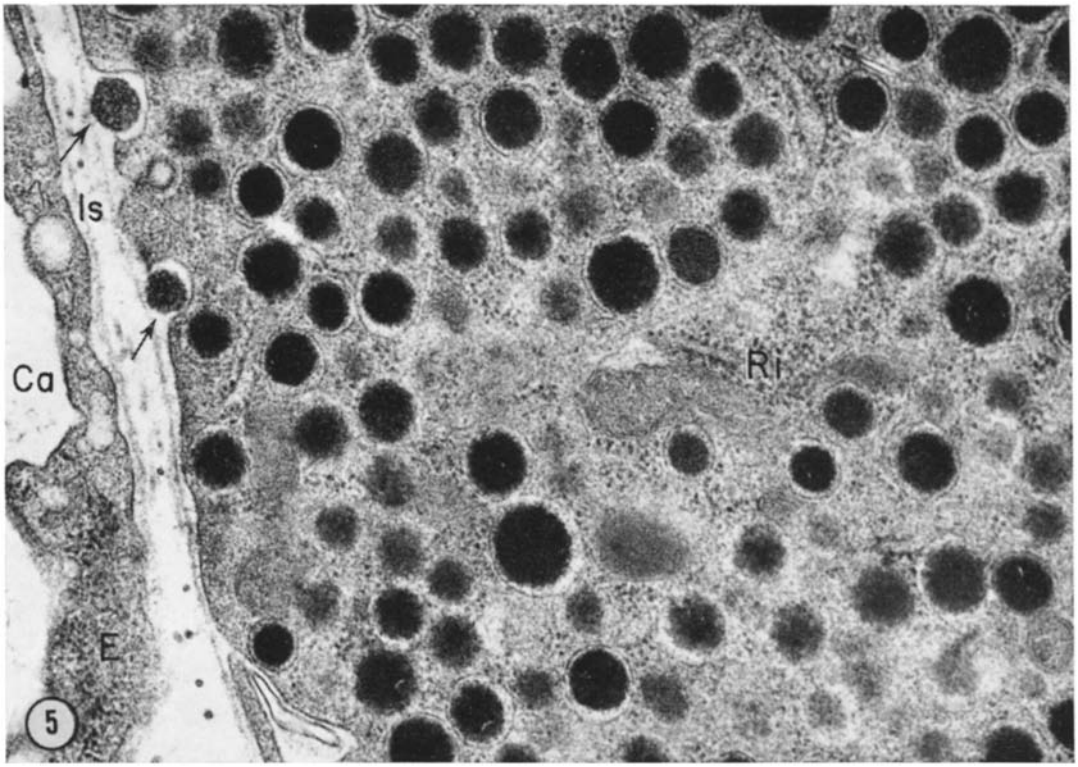

FIGURE 5 A cell of rabbit endocrine pancreas after 15 min of incubation without glucose. Emiocytosis of A granules is shown (→). Capillary lumen (*Ca*); endothelial cell (*E*); pericapillary space (*Is*); ribosomes (*Ri*). × 34,500.

the intercellular and pericapillary spaces, and few cells were partially degranulated.

At 60 min of incubation time, very few of the cells showed margination and emiocytosis of granules as described for the 15-min incubation. Most of the cells showed a large increase in the number of microvilli projecting into the intercellular and pericapillary spaces (Fig. 6). Occasionally, this phenomenon was rather marked (Fig. 7). We also observed quite often that some cells were moderately degranulated and showed an increase in the amount of lamellar ergastoplasm and free ribosomes (Fig. 8). In these cells, some granules were lying inside the flattened agranular sacs or in the vesicles of the Golgi apparatus (Fig. 9). No fragmentation of granules was observed in any of the cells examined.

A CELLS AFTER INSULINIC HYPOGLYCEMIA: A few of the A cells showed margination emiocytosis of the entire granule (Fig. 10). Although not many granules were being released, this phenomenon could be seen quite readily in many of the islets examined. For the most part, the A cells were filled with secretory granules, and only a few of the cells showed an increase in the number of microvilli at their junctions with adjacent cells and capillaries (Fig. 11).

MORPHOLOGY OF THE D CELLS IN VITRO: These cells, either after the 1st hr of incubation with 0.6 mg/ml of glucose in the medium or after further incubation with 3 mg/ml of glucose for another ½ hr, showed no alterations in their fine structure. All the cells studied were well granulated (Fig. 4), and only very rarely could we observe what appeared to be elimination of a D granule from the D cell by emiocytosis (Fig. 12). When D cells were incubated in an incubation medium without glucose, they showed no alterations apart from that described above.

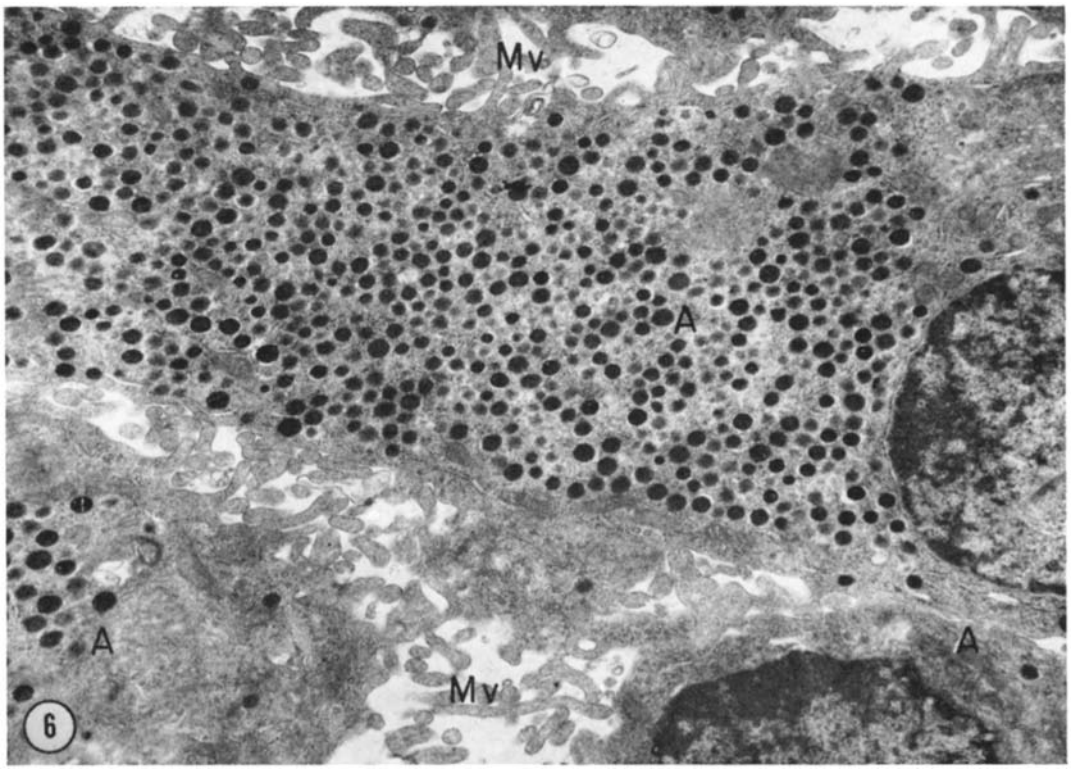

FIGURE 6 A cells of rabbit endocrine pancreas after 60 min of incubation without glucose. A cells (*A*); microvilli (*Mv*). × 11,500.

DISCUSSION

In a previous report (2), we described the behavior of the B cells of the rabbit endocrine pancreas incubated in vitro for 1 hr in a medium containing 0.6 mg/ml of glucose. The changes described consisted of spontaneous secretions of insulin with a partial regranulation of the B cells which had essentially stopped after approximately 60 min of incubation.

In contrast with the activity displayed by the B cells in vitro, the A cells when incubated in a medium containing glucose remained stable. However, when the A cells were incubated in a medium without glucose, there was an active release of A granules. Under our experimental conditions, the A granules were released from the A cells in the following way: margination, rupture of the fused granule-enveloping sac and plasma membrane, and extrusion of the entire granule into the intercellular and pericapillary spaces. A later manifestation of this process was the formation of microvilli at the cellular borders. This mechanism of release is, in essence, the same as that described previously for other endocrine cells such as the B cell of the islet (14). A difference occurs with respect to the B cell, however, in that the secretory granule is dissolved as soon as it is eliminated. The secretory granule of the A cell can be seen intact in the pericapillary and intercellular spaces. In our material, we have never observed secretory granules in the capillary lumen or in the endothelial cell cytoplasm. These findings do not agree with those on the mechanism of secretion of A cells as described by Munger (9). In that study, the author showed the morphological sequence of the secretory process in normal rabbit pancreas and at different intervals after synthalin administration. On the basis of his findings, he summarized the secretory cycle as follows: (1) formation of granules from prosecretory granules in the midst of the Golgi apparatus, (2) intracytoplasmic disintegration of A granules forming secretory particles, and (3) passage of secretory particles through the plasma membrane of the A cell, through the connective tissue, perivascular space, and endo-

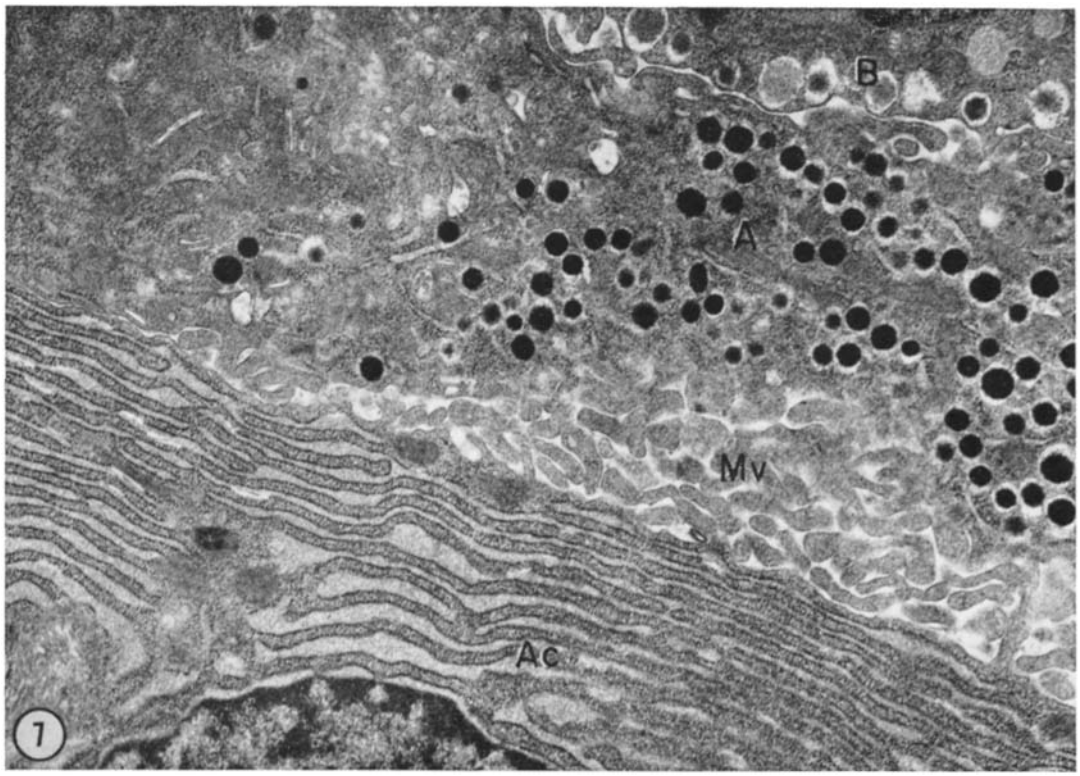

FIGURE 7 A cell after 60 min of incubation without glucose. A cell (*A*); microvilli (*Mv*); B cell (*B*); acinar cell (*Ac*). × 17,500.

thelial cell of the capillary, and finally release into the blood. He suggested that, although the A granules were formed in a manner analogous to that of other endocrine protein secretory granules, the mode of liberation of the secretory product was entirely different. In his study, Munger fixed the material with Dalton fixative (chrome-osmium). Nevertheless, in a second paper, Munger et al. (10) published a micrograph showing fragmentation of A granules in material fixed in glutaraldehyde and osmium tetroxide. In the animal in vivo after insulinic hypoglycemia, the release of the A granules in our material follows the same process as that described in vitro. This confirms that the mechanism described above is not an artefact of the conditions of the incubation in vitro.

The fact that the absence of glucose in the medium is coincident with slight-to-moderate release of the hormone suggests that the absence of this sugar is essential for the release of glucagon from the cells. It has also been demonstrated that the synthesis and secretion of A-cell hormone occurs in vitro in the same fashion as in vivo (9, 6), corroborating the adequacy of our system. Further studies along this line are now being followed up in our laboratory.

The D cells present no visible alterations in their fine structure. The occasional presence in these cells of granules in emiocytosis suggests that the mechanism of granule secretion for these cells is similar to that of the B and A cells. Since very little is known about the function of the D cells, their behavior in vitro makes them, in our opinion, a very good subject for further studies along these lines.

Part of this work was aided by a grant from Banco Español de Credito, Madrid (Spain), for which the authors are grateful.

The authors wish to express their gratitude to Miss Patrocinio Lopez and Mr. Eloy Blanco Marcos for their invaluable technical assistance. Acknowledgement is made to Dr. M. Rubio and personnel of the Electron Microscope Service of the Centro de Investigaciones Biologicas of Madrid for their assistance.

Received for publication 23 January 1967, and in revised form 20 September 1967.

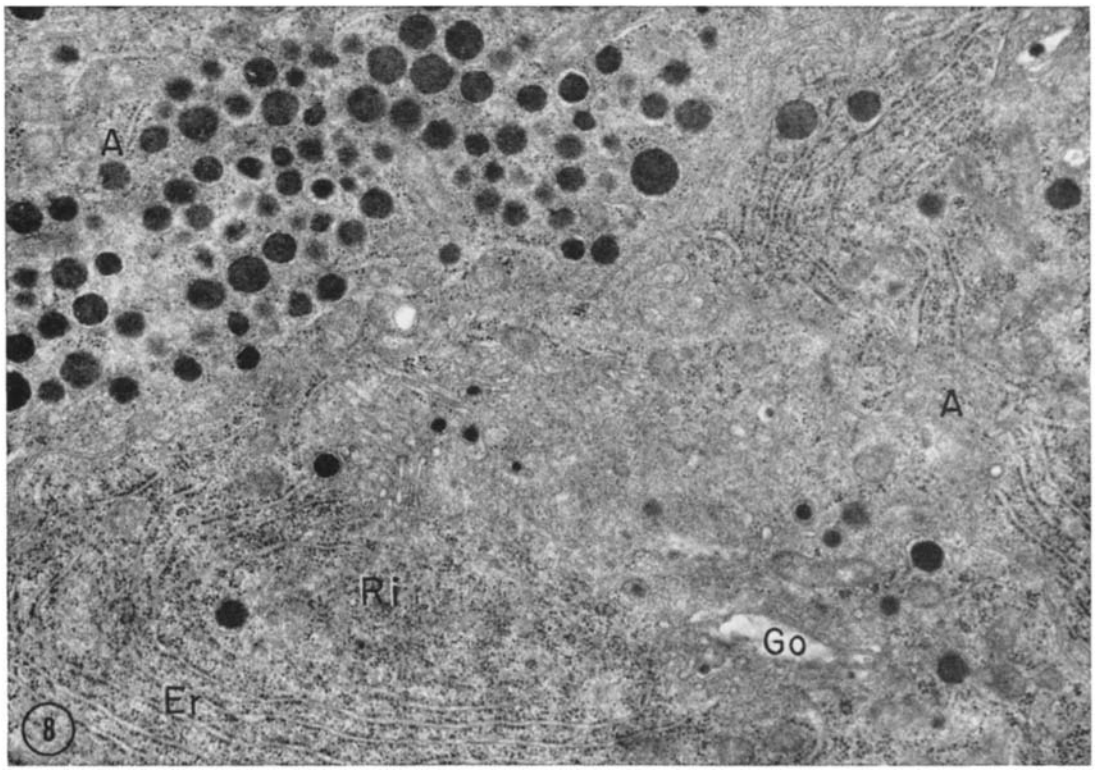

FIGURE 8 A cells after 60 min of incubation without glucose. A cells (*A*); lamellar ergastoplasm (*Er*); free ribosomes (*Ri*); Golgi apparatus (*Go*). × 21,000.

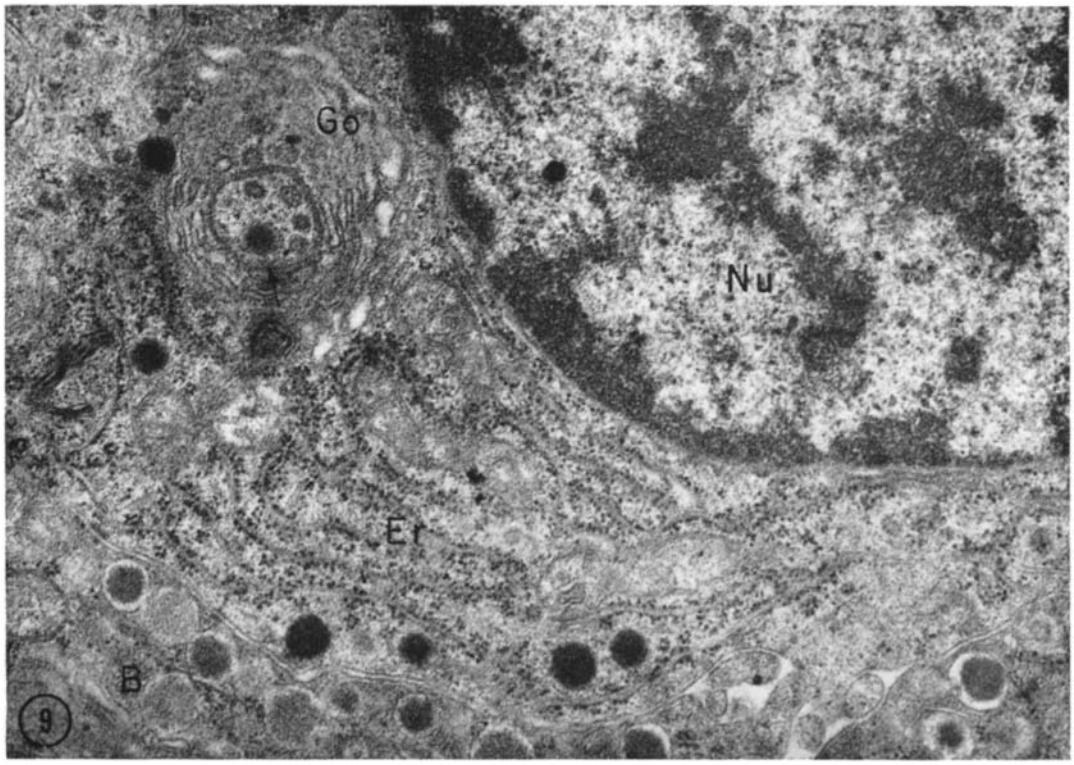

FIGURE 9 A cell after 60 min of incubation without glucose. Nucleus (*Nu*); lamellar ergastoplasm (*Er*); Golgi apparatus (*Go*); prosecretory granule (→); B cell (*B*). × 27,000.

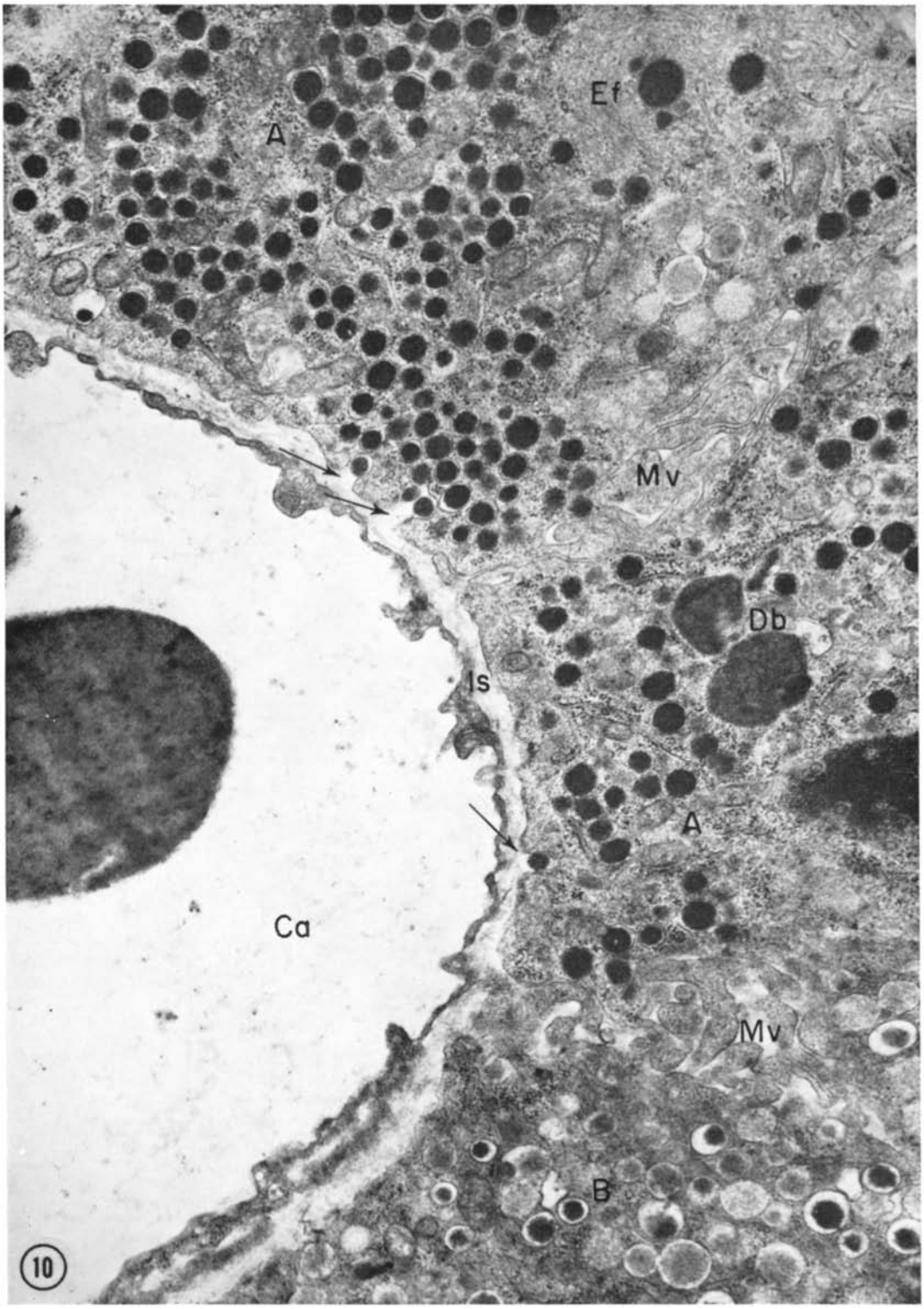

FIGURE 10 A cells, 1 hr after i.v. administration of insulin. A cells (*A*); capillary lumen (*Ca*); pericapillary space (*Is*); emiocytosis of A secretory granules (→); microvilli (*Mv*); dense bodies (*Db*); B cell (*B*); epithelial filaments (*Ef*). × 21,500.

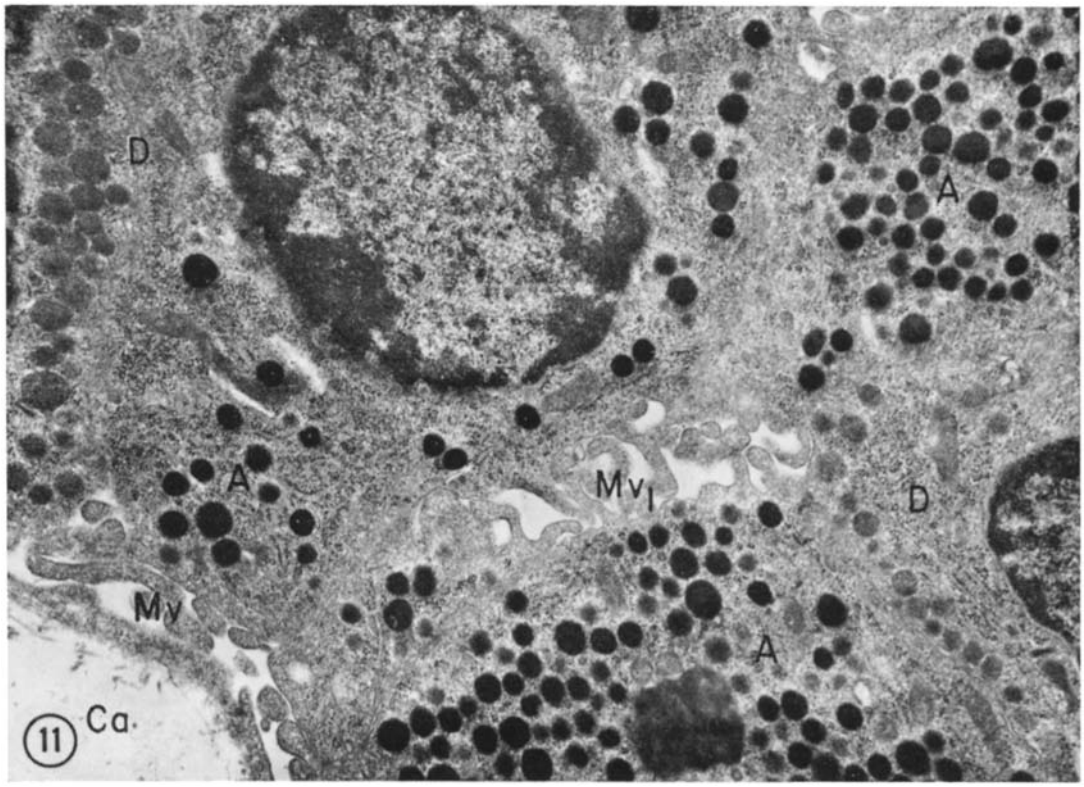

FIGURE 11 A cells, 2 hr after i.v. administration of insulin. A cells (*A*); microvilli projecting between cells (Mv_1) and between cell and capillary (*Mv*); capillary lumen (*Ca*); D cells (*D*). × 13,500.

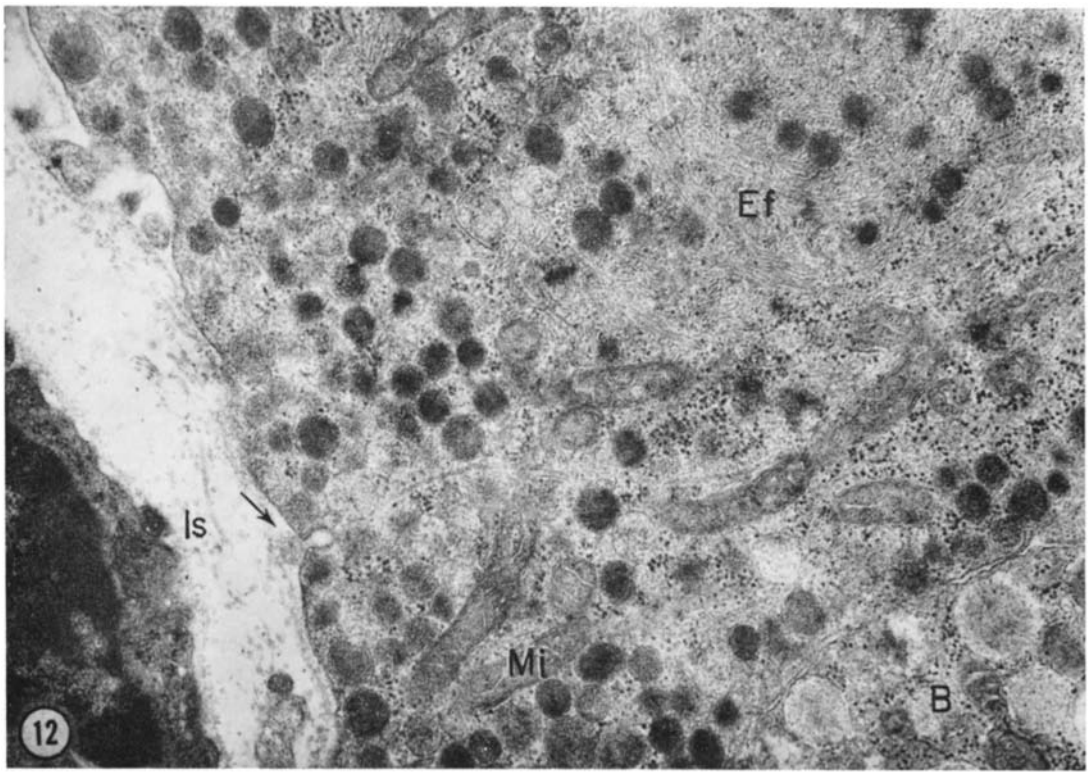

FIGURE. 12 D cell after 15 min of incubation with glucose. Pericapillary space (*Is*); emiocytosis of D secretory granule (→); mitochondria (*Mi*); epithelial filaments (*Ef*); B cell (*B*). × 28,000.

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