FINE STRUCTURAL CHANGES IN RESPONSE TO HORMONAL STIMULATION OF THE PERFUSED CANINE PANCREAS

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ABSTRACT

The dog pancreas isolated *in situ* was perfused with oxygenated dog blood and stimulated with pancreozymin, secretin, or both. There were no significant changes in the fine structure of the acinar, centroacinar, or duct cells attributable to the perfusion. Combined glutaraldehyde and osmium fixation gave good preservation of the secretory products of the acinar cell. Before stimulation, the lumen of the acini is filled with material similar in texture to the content of the zymogen granules, but of somewhat lower density. Release of secretion commonly takes place by coalescence of the limiting membrane of zymogen granules with the plasmalemma, but one granule opening at the surface may frequently be joined by others coalescing with its membrane and forming an interconnected series all with contents having the same texture as the released zymogen. Such a mechanism seems to permit a more rapid release of secretory product than discharge of individual granules. Pancreozymin stimulation caused marked depletion of zymogen granules, but no obvious changes in the Golgi apparatus. It is clear, therefore, that this hormone exerts its effect upon release of granules rather than upon their formation. Secretin stimulation of water and bicarbonate secretion caused a marked washing out of the luminal contents, but had little detectable effect on cellular structure.

INTRODUCTION

The exocrine pancreas of the guinea pig has been intensively studied by correlated biochemical and electron microscopic methods in different phases of its normal functional activity (16, 23–27). These investigations have established the respective functions of the various cell organelles in the synthesis, concentration, storage, and release of the zymogens. Hermodsson (7), studying the cat pancreas after stimulation with both secretin and pancreozymin, described a reduction in the number of zymogen granules in the acinar cells, appearance of large cytoplasmic vesicles, and striking alterations in the fine structure of the Golgi apparatus. Although the bulk of the fluid and electrolytes of the pancreatic juice are believed to be secreted by the centroacinar or duct cells, no fine structural changes in these elements after secretin stimulation have yet been described.

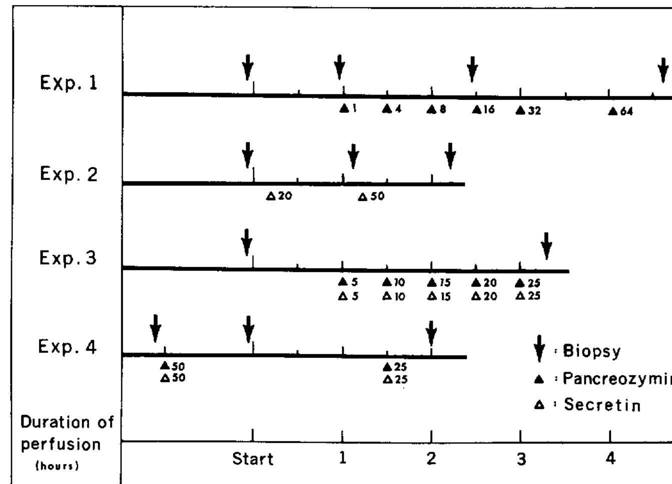
The present paper reports the cytological changes occurring in response to hormonal stimulation of the isolated canine pancreas maintained with a pump oxygenator for experimental periods up to 4 hours (13). This preparation permitted serial biopsy of the same gland at different time intervals after stimulation and made it possible to correlate the fine structure of the

various cell types with concurrent physiological and biochemical measurements of the secretory activity of the organ as a whole.

MATERIALS AND METHODS

The pancreas of 20- to 25-kg fasting mongrel dogs was left *in situ,* but isolated surgically from other structures and perfused with heparinized dog blood by means of a pump oxygenator connected to the celiac artery and portal vein. For details of the operative procedures and electromechanical devices employed in the preparation, the reader is referred to the paper by Nardi *et al.* (13) reporting the physiological aspects of the experiments. Beginning 1 hour after the initiation of perfusion, secretin (Boots or Vitrum) or pancreozymin (Vitrum) or both were injected at intervals in varying amounts into the tubing of the afferent blood supply. The dosages and time intervals used in the four experiments covered by this report are presented in Table I.

TABLE I

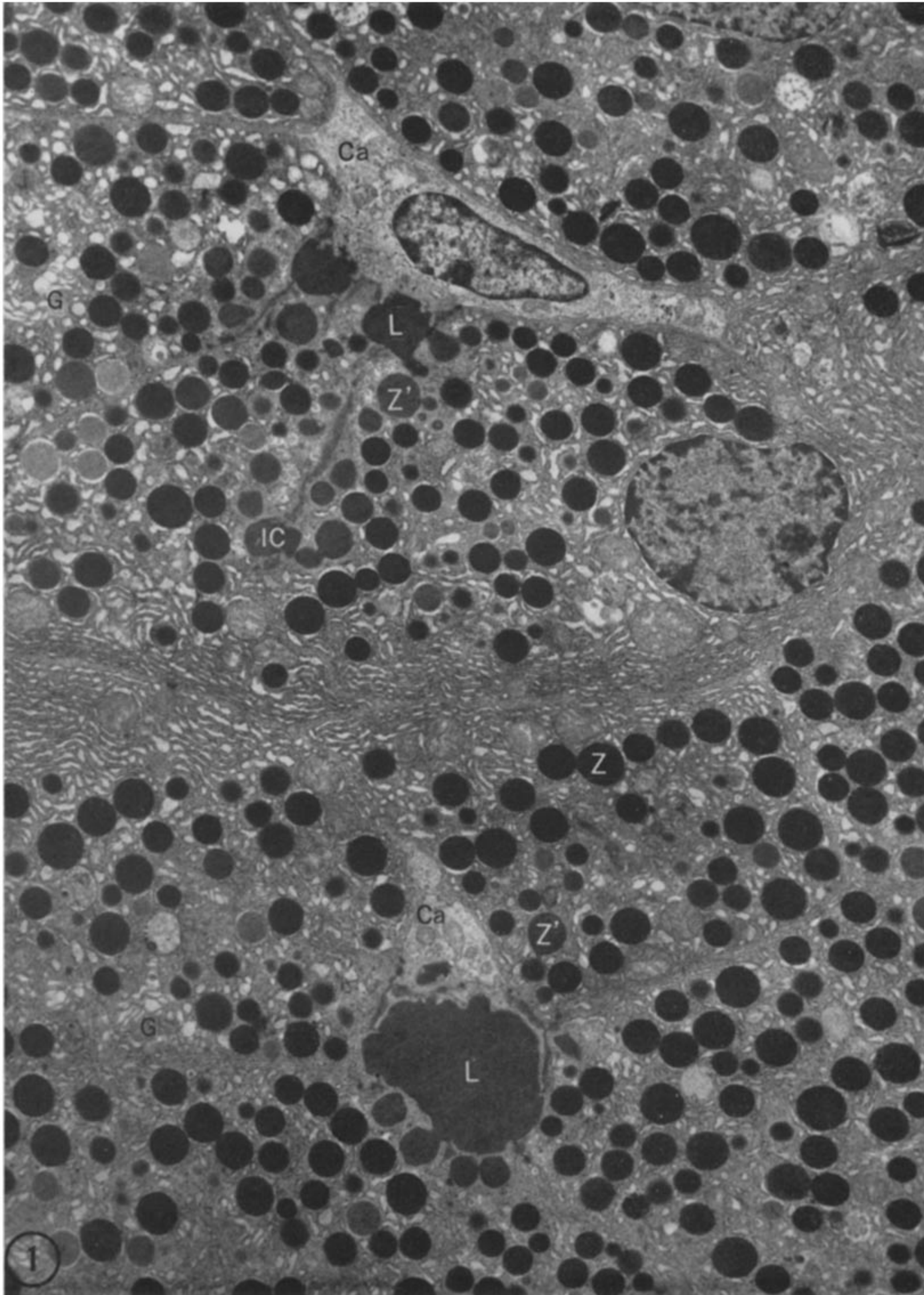


The items of the experimental procedures performed. The number indicates units of the hormone given. The initial stimulation of experiment 4 was performed by intravenous iniection prior to starting perfusion.

Small biopsies of pancreatic tissue were cut into smaller blocks and fixed for 2 hours in cold 1 per cent osmium tetroxide buffered to pH 7.4–7.5 with phosphate buffer (11). Other blocks were fixed in buffered 5 per cent glutaraldehyde, washed in buffer, and then postfixed in buffered 1 per cent osmium tetroxide (21). Dehydration was carried out rapidly in increasing concentrations of cold ethanol, and the tissues were embedded in Epon (9).

Thin sections showing yellow or gold interference colors were cut with glass knives on a Servall ultramicrotome and picked up on copper grids without formvar or celloidin support. The sections were stained with uranyl acetate (28) and lead citrate (19) and examined with an RCA-3E electron microscope. Micrographs were made at original magnifications of 1,250 to 10,600 times and enlarged photographically to the desired size. Thick sections (about 1 μ) were cut from the same blocks and stained

FIGURE 1 A low-power electron micrograph of a section through several pancreatic acini from an organ perfused with blood for 1 hour. The fine structure of these cells is indistinguishable from that of those fixed before perfusion. The lumen (*L*) of the acini is filled with material of the same density as some of the zymogen granules (*Z'*). Numerous zymogen granules occupy much of the apical cytoplasm and extend into the basal regions of the cells. The centroacinar cell (*Ca*) has a lower density than the surrounding pancreatic acinar cells. *G*, the Golgi apparatus; *IC*, intercellular canaliculus; *Z*, zymogen granules with highly dense content. Glutaraldehyde-osmium tetroxide fixation. × 4,800.


Ca
G
L
Z'
IC
Z
Ca
Z'
G
L
1

with toluidine blue (20) for examination by light microscopy.

OBSERVATIONS

The Fine Structure of the Perfused Pancreas

The perfused pancreas remained grossly normal in appearance. It continued to consume oxygen, and incorporated labeled amino acid into newly synthesized protein. It produced a small amount of pancreatic juice when unstimulated (13). There were no significant changes in the fine structure of the acinar, centroacinar, or duct cells in the first few hours of perfusion (Figs. 1, 5, 7, 8, and 10). The following description of the ultrastructure of these cell types is based on examination of glands perfused with oxygenated whole blood for 1 hour.

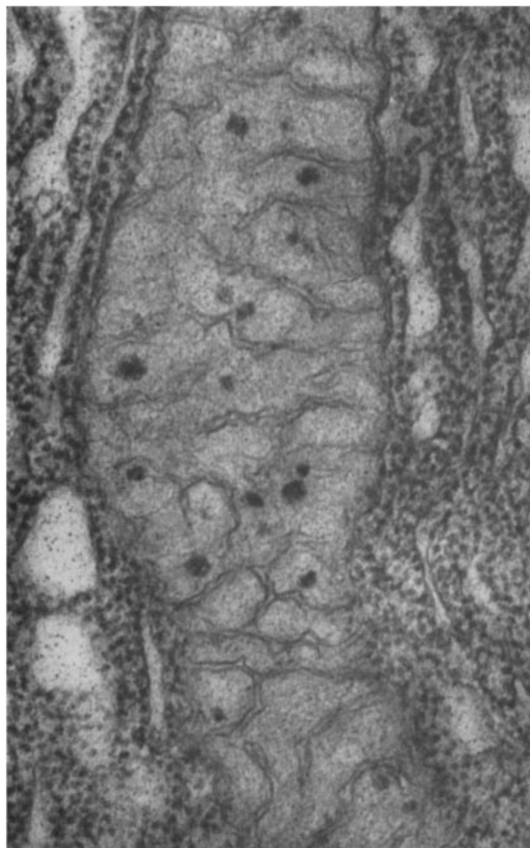


FIGURE 2 A mitochondrion in an acinar cell from a perfused, but unstimulated pancreas. Note the numerous angulations of the cristae and the dense intramitochondrial granules. Phosphate-buffered osmium tetroxide fixation. × 61,500.

The acinar cells have the same appearance as in tissue fixed before the onset of perfusion. The cells are pyramidal in form with a centrally placed, round, or slightly irregular nucleus. The basal cytoplasm is occupied by extensive cisternae of the endoplasmic reticulum. These are disposed parallel to each other and to the lateral and basal surfaces of the cell. The lumen of the cisternae is of variable width and often appears distended. The contents of the reticulum are of relatively low density with the specimen preparation techniques used. The cytoplasmic ground substance between cisternae is rather dense and crowded with ribosomes. In material fixed in osmium alone, "dark" and "light" acinar cells are sometimes distinguishable, but in tissue fixed first in glutaraldehyde, the cytoplasmic matrix of the acinar cells is of uniform density. This observation is consistent with the interpretation of the "dark cells" of classical cytology as artifacts of specimen preparation.

Elongated profiles of the filiform or bacilliform mitochondria are scattered throughout the basal two thirds of the cell body. The mitochondrial matrix contains numerous dense granules, and the cristae often exhibit periodic angulations of their

membranes. The neighboring cristae not infrequently coalesce at their points of angulation, dividing the matrix into numerous small compartments (Fig. 2).

A well developed Golgi complex is located in the supranuclear region. Its lamellar arrays of flat sacs show numerous dilatations and associated empty-appearing vesicles, in addition to the spherical vacuoles with a content of appreciable density which appear to be formative stages of zymogen granules.

The general appearance of the endoplasmic reticulum and Golgi complex in the acinar cells is frequently less orderly than we have come to expect from earlier published descriptions of the rat (3, 18) and bat (5) pancreas. It is not clear whether the relative prominence and irregularity of the lumen of the reticulum and the vacuolated appearance of the Golgi complex are characteristic of this species or are due to the technique of specimen preparation. It is clear, however, that these features are not regressive changes attributable to the perfusion, because tissue fixed before switching from the dog's own circulation to the pump oxygenator has the same appearance.

The zymogen granules in the apical region of the cell are spherical bodies, each enveloped by a smooth-surfaced membrane. The homogeneous content varies in density depending upon the fixation, appearing light after phosphate-buffered osmium tetroxide alone, and very dense after the combined glutaraldehyde and osmium tetroxide fixation. Material fixed by the latter procedure is particularly suitable for study of the mode of release of the zymogen from the acinar cells. The lumen of the acini is always filled with material

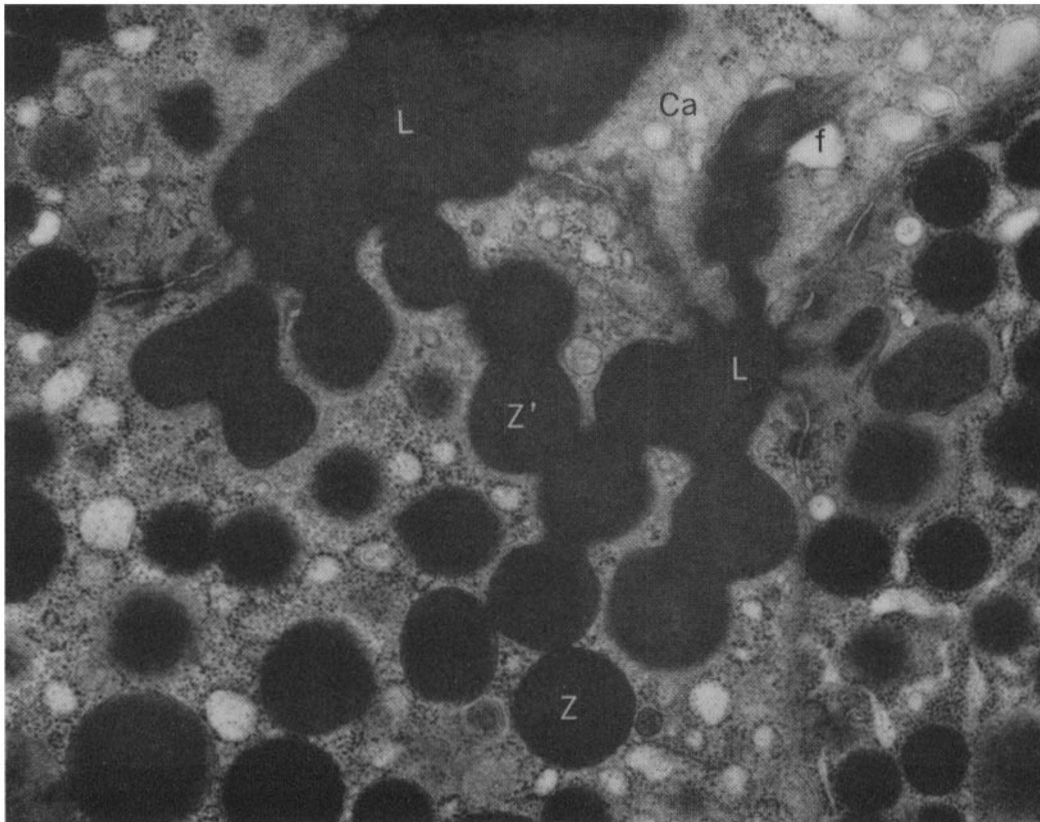


FIGURE 3 The apical portion of the acinus of pancreas perfused for 1 hour, showing the interconnected series of zymogen granules (*Z'*) with the same texture as the luminal content (*L*). Zymogen granules (*Z*) with no obvious connections to the lumen have a greater density. Part of a centroacinar cell (*Ca*) is shown with an oblique section of a flagellum (*f*). Glutaraldehyde-osmium tetroxide fixation. × 24,600.

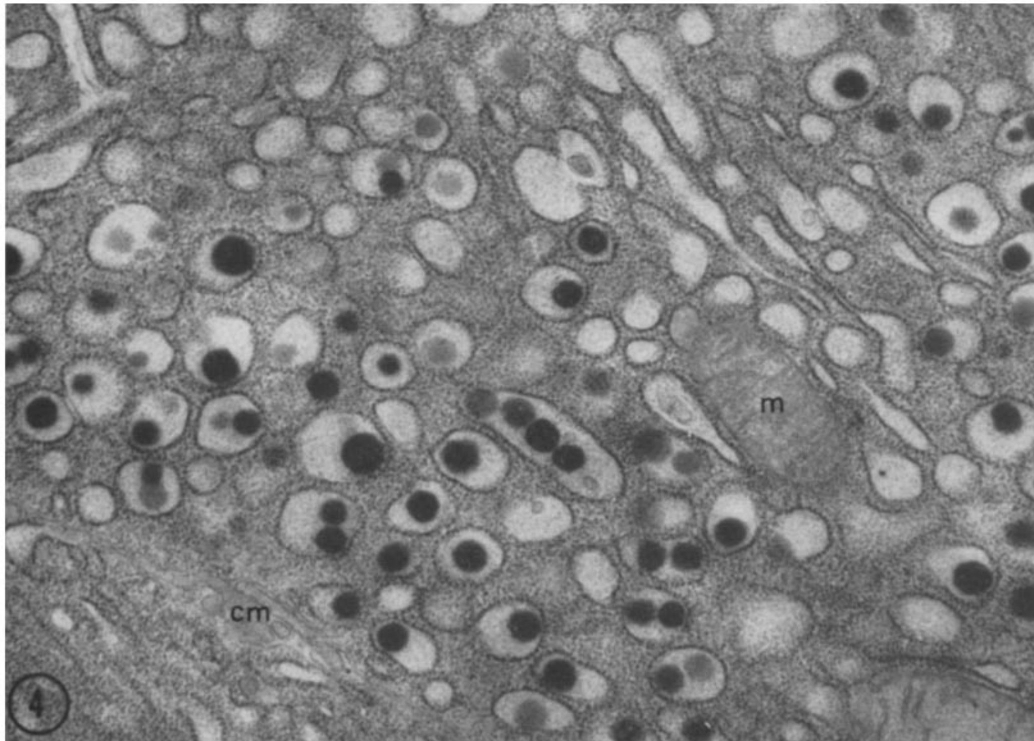


FIGURE 4 Intracisternal granules with a density resembling zymogen granules are found in certain pancreatic acinar cells. The granular endoplasmic reticulum of perfused unstimulated as well as stimulated organs have a small number of cells showing these granules. Note that they are of varying density. The significance of these intracisternal granules is not clear. *cm*, adjacent cell membrane; *m*, mitochondria. Glutaraldehyde-osmium tetroxide fixation. × 19,700.

which is similar in texture to the content of the stored zymogen granules, but of somewhat lower density. This evidently represents secretory material already released from the surrounding cells. Two categories of zymogen granules are distinguishable on the basis of their density. Those discharging granules the limiting membrane of which has fused with the plasmalemma are open to the lumen of the acinus, and their content, like the material in the lumen, is of lower density than the content of the deeper lying zymogen granules in storage stage (Fig. 3).

Single zymogen granules may discharge by coalescing with the plasmalemma, as described by Palade (15), and other granules may discharge by a process of fusion to form interconnections among several granules. A second zymogen granule may fuse with one which has already opened onto the cell surface, and a third may, in turn, fuse with it, so that beaded strings of interconnecting zymogen granules extend some distance downward into the apical cytoplasm. Even when their ultimate communication with the cell surface is out of the plane of section, members of such coalescent groups of zymogen granules can be distinguished from other zymogen granules by their lower density and by the presence of a thin homogeneous layer of the cytoplasm immediately surrounding them.

In some acinar cells, small round granules which are about 260 mμ in maximum diameter were occasionally observed within the distended cisternae of the granular endoplasmic reticulum (Fig. 4). They are homogeneous, but vary somewhat in density and appear to be identical to the intracisternal granules reported by Palade (14) in the guinea pig pancreas. This finding was obtained not only in the control gland, but also after pancreozymin stimulation, and no significant

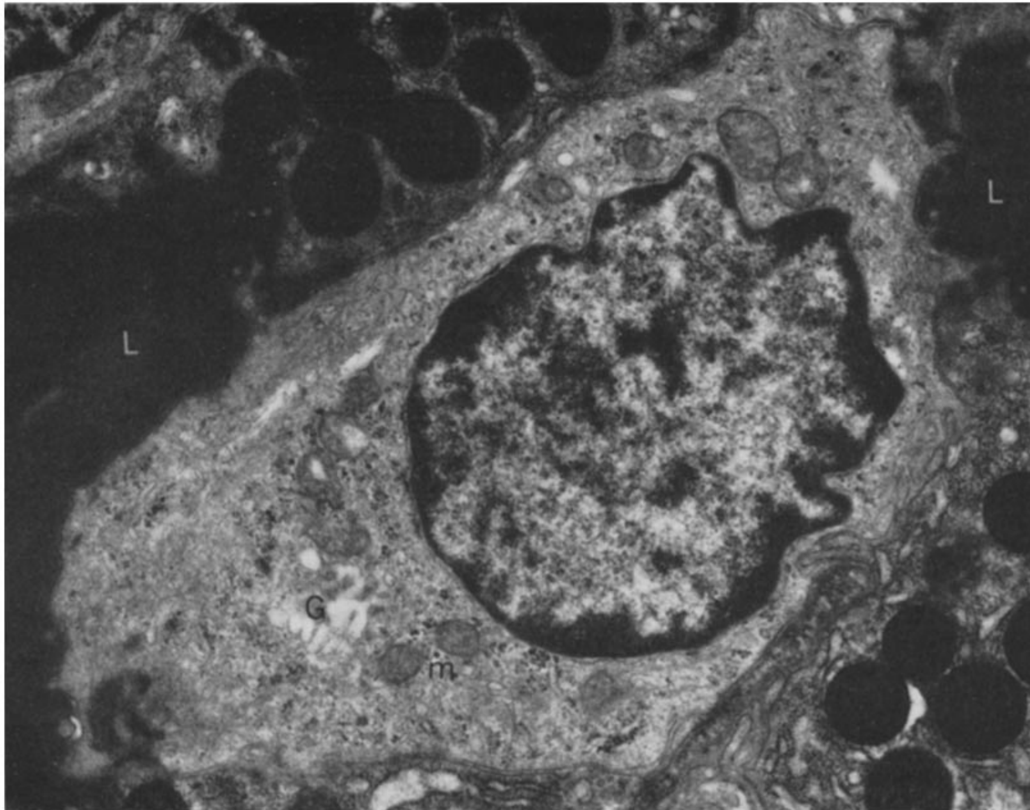


FIGURE 5 A centroacinar cell from a pancreas before stimulation. The cytoplasm is very light compared to that of the adjacent acinar cell. Scattered elements of the granular endoplasmic reticulum, a supranuclear Golgi complex (*G*), and a few mitochondria (*m*) are present. *L*, the lumen of the acinus. Glutaraldehyde-osmium tetroxide fixation. × 24,000.

changes in the appearance of these granules were detected during the experiments.

The centroacinar cells are characterized by their irregular angular shape conforming to the interstices between acinar cells, and by a cytoplasmic matrix of unusually low density (Fig. 5). These cells have a few short microvilli on their free surface and a single long flagellum ("Zentralgeissel" of Zimmermann) that extends into the lumen (Fig. 6). The mitochondria are smaller than those of the acinar cells and vary from cell to cell in their number and distribution. In some, they are randomly distributed in the cytoplasm, while in others they appear to be more numerous and are concentrated around the nucleus. Their cristae are sparse and irregularly oriented. The mitochondrial granules are few in number. A moderately well developed Golgi complex is situated between the nucleus and the free surface of the cell. The endoplasmic reticulum is represented by vesicular and tubular profiles widely scattered in the cytoplasm. Its limiting membranes are studded with small numbers of ribosomes in some areas, but much of the surface of the reticulum is agranular (Fig. 7). Occasional clusters of ribosomes and larger granules presumed to be glycogen are dispersed in the pale cytoplasmic matrix.

The cells of the intercalated ducts are generally similar to the centroacinar cells, but have relatively more mitochondria and a somewhat greater content of glycogen (Fig. 8). The lateral surfaces of the duct cells may be extensively interdigitated. At the interface between each pair of adjoining cells near the lumen, there is a very conspicuous junctional complex consisting of a zonula oc-

cludens and zonula adhaerens. Prominent bundles of tonofilaments converge upon zonula adhaerens from either side. Elsewhere on the surfaces of contact, the cells are attached by desmosomes (maculae adhaerentes).

The epithelial cells of the intralobular and interlobular ducts are similar to those just described in their mode of attachment and their cytological characteristics, but their mitochondria are few and their Golgi complex and endoplasmic reticulum are even less well developed (Fig. 10).

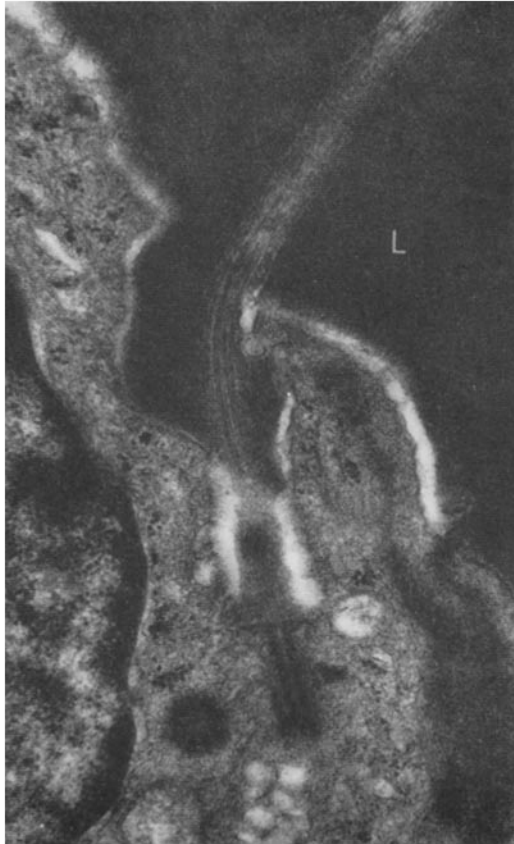


FIGURE 6 Part of a centroacinar cell with a flagellum extending into the lumen (*L*). Its basal body and an associated centriole are shown. Glutaraldehyde-osmium tetroxide fixation. × 25,500.

Changes in Cell Fine Structure after Stimulation with Pancreozymin

Experiment one was intended to study the effect of administration of pancreozymin given in increased doses over a period of 4 hours. Pancreozymin stimulation caused considerable increase in volume of the pancreatic juice output from 0.1 cc/min to 0.26 cc/min in the maximum. The protein content, which was initially 21 mg/ml, increased to 81 mg/ml after stimulation with 8 units of pancreozymin. Trypsinogen analyses of these secretions revealed an increase from 370 μg/ml to 2688 μg/ml (13). The dosages employed, the times of administration, and the intervals at which biopsies were taken are indicated in Table I. The biopsy at 2½ hours showed an appreciable discharge of zymogen, but the effect was by no means uniform throughout the gland; some acini contained as many granules as before stimulation, others contained relatively few. Clearly, the hormone was not equally and simultaneously effective for all acini in the perfused pancreas. The discharge of zymogen granules by coalescence with the cell membrane at the luminal surface was often observed after pancreozymin stimulation, but its frequency was not much greater than in the control. At later times in the experiment, particularly after the final stimulation with large doses

of pancreozymin, the great majority of the acinar cells were remarkably depleted of zymogen. Granules were entirely absent in many acini (Fig. 9), and present in very limited numbers in others. The acinar cells contain numerous vesicular profiles of the granular endoplasmic reticulum in the apical region and very few profiles of the agranular endoplasmic reticulum throughout the cytoplasm. The Golgi apparatus was not notably affected. Mitochondrial profiles seemed to be larger, relatively more numerous, and their matrix somewhat less dense than in unstimulated acinar cells. Some in the apical region of the cell appeared swollen and had central clear areas devoid of cristae. Elsewhere in the cell, the mitochondria were of more normal appearance, but the intra-mitochondrial dense granules were invariably absent.

In the apical region of depleted acinar cells, there were often a number of dense bodies of irregular shape and heterogeneous interior. These bodies, bounded by a close-fitting single membrane, conform to the general morphological description of lysosomes, but cannot be definitely identified as such without application of histochemical techniques to verify the presence of hydrolytic enzymes.

The centroacinar and duct epithelial cells do not show any marked change in their fine structure after pancreozymin (Fig. 11). More fat droplets were noted in their cytoplasm in the stimulated than in the control gland, but this may have been a matter of sampling rather than an effect of stimulation.

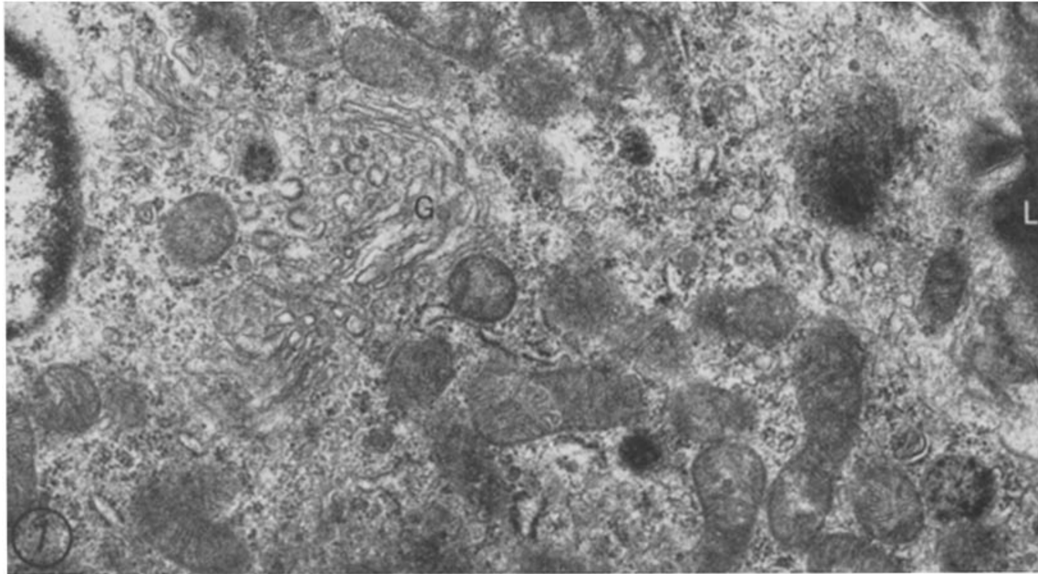


FIGURE 7 Apical part of a centroacinar cell, before stimulation. The Golgi apparatus (*G*) is moderately well developed and lies in the supranuclear region. Vesicular and canalicular profiles of the endoplasmic reticulum are distributed throughout the cytoplasm. Some of the reticulum in the apical cytoplasm shows smooth-surfaced membranes continuous with the granular endoplasmic reticulum. *L*, the lumen of the acinus. Glutaraldehyde-osmium tetroxide fixation. × 20,300.

Changes in Fine Structure after Secretin Administration

In experiment two, secretin was administered in two doses in the course of a 2½-hour perfusion (see Table I). Output of the pancreatic secretion, which is about 0.1 cc per minute before stimulation, increased rapidly to the maximum flow rate of 0.7 cc per minute after stimulation. On the other hand, spectrophotometric analysis showed that protein content of the pancreatic juice decreased from the normal value to almost zero after the final stimulation. Biochemical analyses of the enzyme activity revealed no free trypsin activity

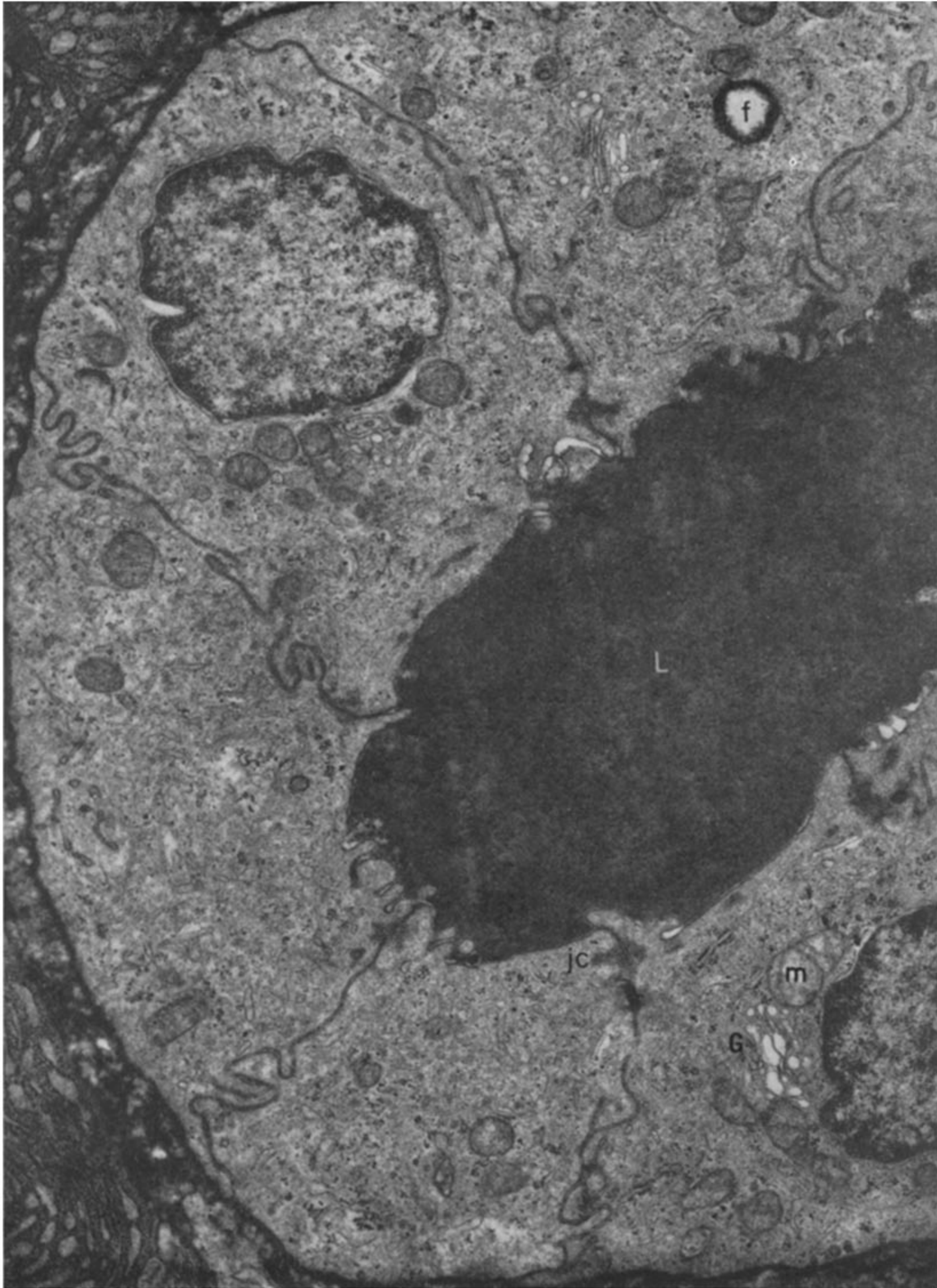


FIGURE 8 An intercalated duct of a perfused pancreas before stimulation. The duct cells are generally similar to the centroacinar cells and occasionally contain fat droplets (*f*) and small accumulations of glycogen granules in their cytoplasm. Junctional complexes (*jc*) and interdigitation between adjacent cell membranes are apparent. *G*, Golgi apparatus; *m*, mitochondria. Glutaraldehyde-osmium tetroxide fixation. × 22,000.

and a constant level of trypsin inhibitor after stimulation. In electron microscopy, no changes were observed in the abundance of zymogen in the acinar cells. Granules in the process of discharging into the lumen were very rarely encountered. The density of the contents of the lumen of the acini progressively decreased, with time after secretin administration suggesting dilution of the content of the secretory passages. The mitochondria of the acinar cells maintained their normal density and configuration, but the granules in the matrix were relatively few.

Because the centroacinar and intercalated duct cells are believed to be principally involved in contributing water and bicarbonate to the pancreatic juice, particular attention was given to these segments of the gland in the secretin experiment. The changes observed were remarkably slight. After large doses of secretin, the intercalated ducts appeared to decrease somewhat in diameter, and their epithelial cells became more flattened and showed complex interdigitations of their contact surfaces (Fig. 12). A small number of vesicles were often seen at the base of duct cells, and some of them are continuous with the cell membrane (Fig. 13). Such vesicles were seldom found before secretin stimulation. There was a decrease in glycogen content of the cells. Occasional fat droplets were seen in the cytoplasm. No significant changes were noted in the mitochondria, Golgi complex, or endoplasmic reticulum. There was no indication that these organelles play an important role in transport of the fluid and electrolyte to the duct lumen.

Fine Structural Changes after Administration of Both Secretin and Pancreozymin

Experiments three and four were designed to study the combined effects of secretin and pancreozymin. There was no evidence of synergism, and the results were simply a combination of the structural changes produced by the two hormones when given separately: namely, a depletion of zymogen granules in the acinar cells and disappearance of glycogen from the centroacinar and duct epithelia. The effects were less evident than in experiments one and two, probably because the doses of the hormones used were lower.

DISCUSSION

The observations reported here demonstrate that, despite the handling and necessarily long exposure of the pancreas in carrying out the surgery preparatory to perfusion, its cells remained in good cytological condition throughout the experimental period and exhibited clear morphological responses to hormonal stimulation. Thus, it can be said that the perfusion method used served well the intended purpose of providing an opportunity to correlate cytological changes in the pancreas with various degrees of induced functional activity.

Palade (15) first pointed out that, in the course of discharge of zymogen, the limiting membrane of the granule becomes continuous with the cell membrane at the apical pole of the acinar cell and the content of the granule then pours out into the lumen. It has generally been assumed that the zymogen granules are released in this manner, one after another. However, Ekholm *et al.* (3) noted that, on rare occasions, a zymogen granule was found to be continuous with another open to the lumen, and they suggested that zymogen granules can empty indirectly into the lumen. The present study has demonstrated that one granule opening at the surface is often joined to a second through a narrow constriction, and this with a third and even a fourth, forming a beaded strand of interconnected granules or vacuoles. Those in the releasing phase are surrounded, more or less, by a thin layer of homogeneous cytoplasm which is similar to the ectoplasm beneath the free surface of the plasma membrane. Therefore, these granules are easily distinguished from those in the formative or storage stages. Zymogen granules not in contact with the cell surface do not coalesce, however closely packed they may be. This suggests that when the membrane of a zymogen granule has fused with the cell membrane, it immediately takes on the properties of the plasmalemma that then enable other granules to coalesce with it, and the membranes of these, in turn, acquire the characteristics of surface membrane. Such a mechanism would seem to permit a more rapid release of secretory product than discharge of individual granules through the limited area of surface directly exposed to the lumen. How the redundancy of surface created by this mode of secretion is relieved by subsequent degradation of membrane or recirculation of its components remains to be elucidated.

The intracisternal granules in the pancreatic acinar cell have not been reported for species, other than the guinea pig. The present study shows that synthesized secretory products of the

dog pancreas may occasionally take the form of opaque granules in the cisternae under certain conditions of the acinar cell, even though the cell product usually has no appreciable density and no definite form until it has been concentrated within the Golgi apparatus. Whether this finding has a special significance in relation to the normal secretory pathway of the gland is not clear, because it can only be seen in a few cases.

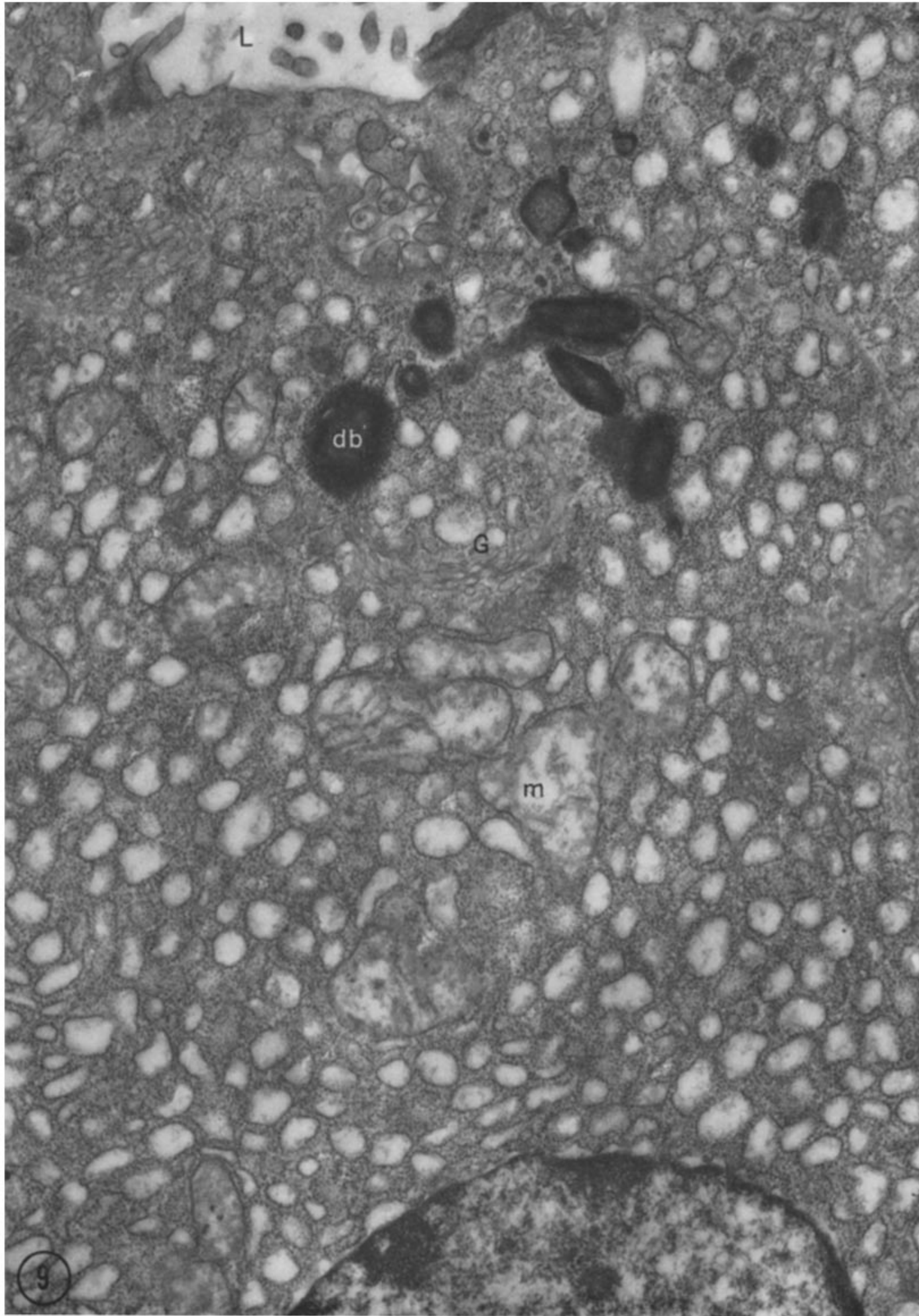
The only previous electron microscopic study of pancreatic tissue stimulated by secretin and pancreozymin is that of Hermodsson on the cat (7). The close topographical relation of the formative stages of zymogen granules to the Golgi complex was the principal focus of his attention, and the results obtained suggested to him a possible effect of these hormones on zymogen granule formation. In the work reported here, no significant changes were noted in the Golgi apparatus. Pancreozymin appeared to exert its effect upon release of granules, rather than upon their formation. This is in accord with the *in vitro* biochemical studies of Hokin and Hokin (8) which led them to conclude that pancreozymin is concerned with transfer of the digestive enzymes across the acinar cell membrane, but is not directly concerned with intracellular protein synthesis. In the materials fixed after stimulation with 8 units of pancreozymin, the frequency of occurrence of images of discharging granules was not much greater than in the control. This may be attributable to the fact that the sampling was made 20 minutes after pancreozymin injection. If the sample had been taken out immediately after stimulation, morphological evidence of massive discharge might have been found more frequently.

A decrease in the number of intramitochondrial granules after pancreozymin administration was observed in all experiments. This is probably correlated with the hormone's known stimulation of pancreatic metabolism (1) and is a further indication that the number of mitochondrial granules may be a rough indicator of mitochondrial activity. A difference in abundance of granules in the light- and dark-adapted retina has been reported, the granules being more numerous in the dark-adapted state (6). The granules were also entirely absent in a human case of hypermetabolism attributed to a disorder of mitochondrial function (10). The function of the intramitochondrial granules is not well understood, but Peachey (17) has recently presented experimental evidence suggesting that they are cation-binding sites involved primarily in divalent cation accumulation. Observations on changes in their abundance in different states of cell activity may provide morphological clues that will lead ultimately to a better understanding of their biochemical significance in the secretory process.

The apparent swelling of the mitochondria and the appearance of areas of rarefaction in the mitochondrial matrix, changes which were observed in the apical cytoplasm of the acinar cells after very large doses of pancreozymin, have their counterparts in other tissues subjected to excessive stimulation. Localized areas of low density in the mitochondria were observed in sweat glands stimulated to secrete by iontophoresis of neurohumoral agents (12). Similar changes were observed in mitochondria of the parietal cells of the dog stomach stimulated by insulin, vagal stimulation, or administration of histamine (22). Although there seem to be no detectable morphological changes in mitochondria in the course of yielding energy for the normal physiological activities of cells, excessive demands may result first in a disappearance of granules, and ultimately in visible changes in the matrix. The latter may be a consequence of depletion of some matrix components or alterations in mitochondrial membrane permeability attended by imbibition of water.

Ekholm *et al.* (4) reported great morphological

FIGURE 9 The upper half of an acinar cell after the final stimulation of pancreozymin. The cell no longer contains zymogen granules. The granular endoplasmic reticulum shows many more vesicular and canalicular profiles. The mitochondria (*m*) appear to be swollen with disorganized cristae and few intramitochondrial granules. The functional significance of the dense bodies (*db*) which are found in the apical region only after depletion of secretory granules is unknown. After extensive stimulation and depletion of dense secretory material from the lumen (*L*), the microvilli are more apparent. Glutaraldehyde-osmium tetroxide fixation. × 15,000.


L
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9

similarities between centroacinar and duct cells, but noted that microvilli were more abundant on the former and that the latter tended to have small blebs projecting from their luminal surface. Even these minor differences were not confirmed in the present study. No blebbing of the surface of the duct cells was seen, with the methods of specimen preparation employed here, and the variation in numbers of microvilli was so great within the same category of cells that no significant differences could be detected between epithelia of the various segments of the duct system. The presence of a single flagellum on many of the cells was repeatedly verified. This is a cytological feature of the duct epithelia that seems to have been largely overlooked by light microscopists. Whether these motile cell processes serve some useful purpose by agitating the secretion within the lumen, or whether they contribute to the movement of the secretory product through the small ducts is not known. The presence of dark and light cells in the intercalated ducts reported by Ekholm and Edlund (2) for the human pancreas, could not be confirmed in the dog, and may depend upon the differences in technical methods employed in the two studies.

The cells of the centroacinar and intercalated duct epithelia were alike in their fine structure in both normal and experimental conditions. No definite and reproducible changes in their organelles were detected after secretin stimulation, even though there was a marked washing out of the contents of the lumen and simultaneous measurement of the volume of secretion of the same pancreas demonstrated a marked physiological response to the hormone. Small vesicles encountered at the base of duct cells are considered to demonstrate the existence of a pinocytotic mechanism in these cells. However, the number of these vesicles seems to be far too small to account for the production of the large amount of water and electrolytes present in the pancreatic juice. Thus, if the centroacinar cells and intercalated duct cells are indeed responsible for contributing the water and bicarbonate ion of the pancreatic juice, the active transport of these substances requires very little structural specialization of the cells and is attended by no cytological changes detectable by current electron microscopic methods.

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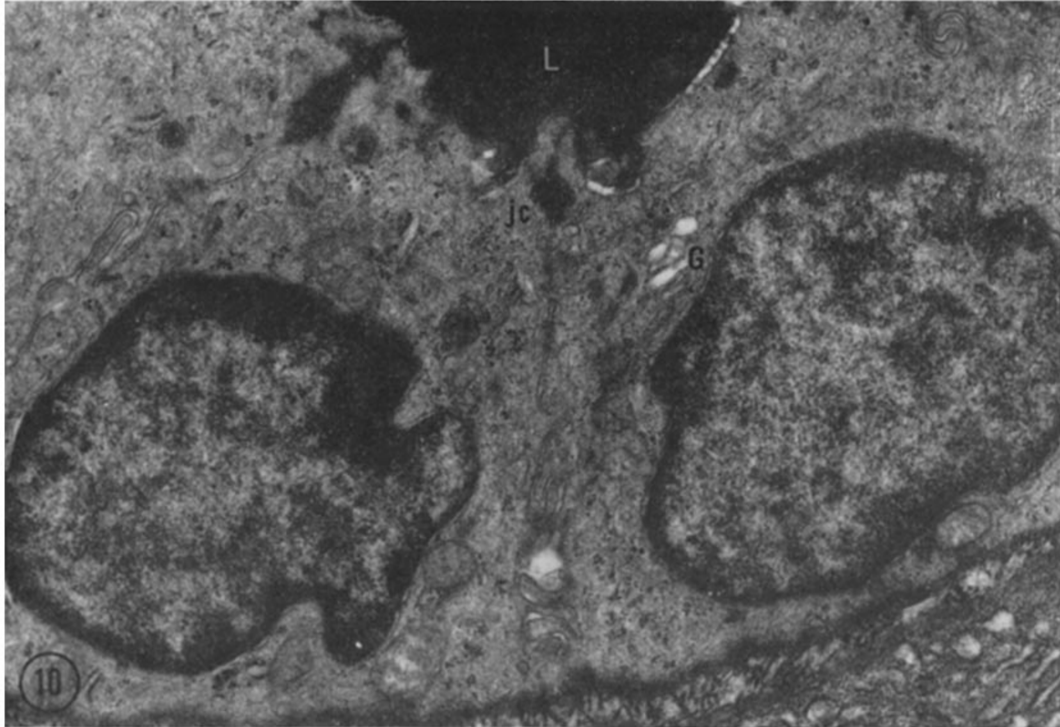
REFERENCES

1. Davies, R. E., Harper, A. A., and Mackay, I. F. S., A comparison of the respiratory activity and histological changes in isolated pancreatic tissue, *Am. J. Physiol.*, 1949, **157**, 278.
2. Ekholm, R., and Edlund, Y., Ultrastructure of the human exocrine pancreas, *J. Ultrastruct. Research*, 1959, **2**, 453.
3. Ekholm, R., Zelander, T., and Edlund, Y., The ultrastructural organization of the rat pancreas. I. Acinar cell, *J. Ultrastruct. Research*, 1962, **7**, 61.

Figure 10 The intralobular duct of the perfused pancreas before stimulation. The duct cells contain a small Golgi apparatus, a few small mitochondria, and sparse granular endoplasmic reticulum. However, the lumen (*L*) is always filled with dense secretory material. *jc*, junctional complex. Glutaraldehyde-osmium tetroxide fixation. × 10,500.

Figure 11 The intralobular duct of the perfused pancreas after pancreozymin stimulation. No change in the fine structure of the duct cells is seen but the lumen (*L*) appears empty. Glutaraldehyde-osmium tetroxide fixation. × 11,000.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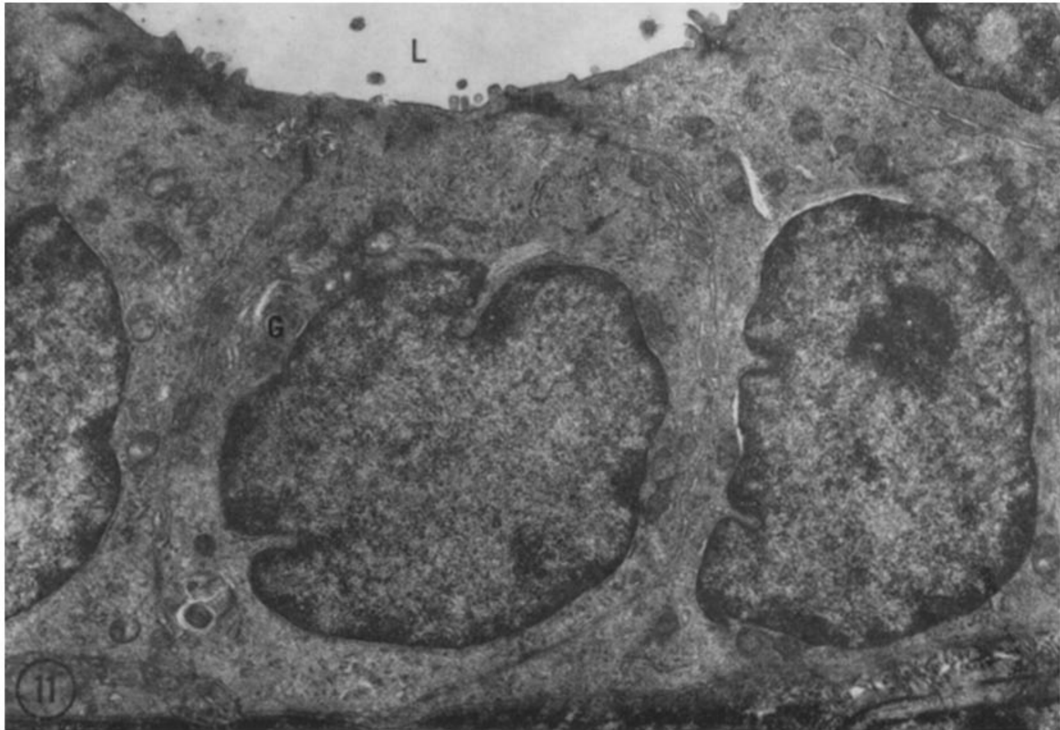
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11

FIGURES 12 and 13 A transverse section through an intercalated duct after secretin stimulation. The cuboidal cells lie on a basement lamina (*bl*) reinforced with collagen fibers. The lateral boundaries of the cell show numerous interdigitations. No striking difference in morphology is apparent that might suggest fluid secretion. However, pinocytotic vesicles (arrows in figure 13) are more frequently encountered after secretin stimulation and the lumen of the duct (*L*) appears empty. Glutaraldehyde-osmium tetroxide fixation. Fig. 12, × 16,400. Fig. 13, × 49,000.

4. Ekholm, R., Zelander, T., and Edlund, Y., The ultrastructural organization of the rat pancreas. II. Centroacinar cells, intercalary and intralobular ducts, *J. Ultrastruct. Research*, 1962, **7**, 73.
5. Fawcett, D. W., The membranes of the cytoplasm, *Lab. Invest.*, 1961, **10**, 1162.
6. Fernández-Morán, H., The fine structure of vertebrate and invertebrate photoreceptors as revealed by low temperature electron microscopy, *in* The Structure of the Eye, (G. K. Smelser, editor), New York, Academic Press, Inc., 1961, 521.
7. Hermodsson, L. H., Changes in the ultrastructure of the exocrine pancreas of the cat after stimulation of secretin and pancreozymin, *in* Electron Microscopy, New York, Academic Press, Inc., 1962, YY-11.
8. Hokin, L. E., and Hokin, M. R., Studies of pancreatic tissue *in vitro*, *Gastroenterology*, 1959, **36**, 368.
9. Luft, J. H., Improvement in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
10. Luft, R., Ikkos, D., Palmieri, G., Ernster, L., and Afzelius, B., A case of severe hypermetabolism of non-thyroid origin with a defect in the maintenance of mitochondrial respiratory control: A correlated clinical, biochemical and morphological study, *J. Clin. Invest.*, 1962, **41**, 1776.
11. Millonig, G., Advantages of a phosphate buffer for osmium tetroxide solutions in fixation, *J. Appl. Physics*, 1961, **32**, 1637 (abstract).
12. Munger, B. L., The ultrastructure and histophysiology of human eccrine sweat gland, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 385.
13. Nardi, G. L., Greep, J. M., Chambers, D. A., McGrae, C., and Skinner, D. B., Pathophysiologic peregrinations in pancreatic perfusion, *Ann. Surg.*, 1963, **158**, 830.
14. Palade, G. E., Intracisternal granules in the exocrine cells of the pancreas, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 417.
15. Palade, G. E., Functional changes in the structure of cell components, *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press, 1959, 64.
16. Palade, G. E., and Siekevitz, P., Pancreatic microsomes. An integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 671.
17. Peachey, L. D., Electron microscopic observations on the accumulation of divalent cations in intramitochondrial granules, *J. Cell Biol.*, 1964, **20**, 95.
18. Porter, K. R., Electron microscopy of basophilic components of cytoplasm, *J. Histochem. and Cytochem.*, 1954, **2**, 346.
19. Reynolds, E. S., The use of lead citrate at high pH as an electron-opaque stain in electron microscopy, *J. Cell Biol.*, 1963, **17**, 208.
20. Richardson, K. C., Jarett, L., and Finke, E. H., Embedding in epoxy resins for ultrathin sectioning in electron microscopy, *Stain Technol.*, 1960, **35**, 313.
21. Sabatini, D. D., Bensch, K. G., and Barrnett, R. J., Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation, *J. Cell Biol.*, 1963, **17**, 19.
22. Sedar, A. W., and Friedman, M. H. F., Correlation of the fine structure of the gastric parietal cell (dog) with functional activity of the stomach, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 349.
23. Siekevitz, P., and Palade, G. E., A cytochemical study on the pancreas of the guinea pig. I. Isolation and enzymatic activities of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 203.
24. Siekevitz, P., and Palade, G. E., A cytochemical study on the pancreas of the guinea pig. II. Functional variations in the enzymatic activity of microsomes, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 309.
25. Siekevitz, P., and Palade, G. E., A cytochemical study on the pancreas of the guinea pig. III. *In vivo* incorporation of leucine-1-C^{14} into the proteins of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 557.
26. Siekevitz, P., and Palade, G. E., A cytochemical study on the pancreas of the guinea pig. V. *In vivo* incorporation of leucine-1-C^{14} into the chymotripsinogen of various cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 617.
27. Siekevitz, P. and Palade, G. E., A cytochemical study on the pancreas of the guinea pig. VI. Release of enzymes and ribonucleic acid from ribonucleoprotein particles, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 631.
28. Watson, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.