# ULTRASTRUCTURAL CHANGES IN THE PANCREATIC ACINAR CELL PRODUCED BY STAPHYLOCOCCAL TOXIN

by

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# INTRODUCTION

Acute pancreatitis is an extremely painful disease seen in man and domestic animals. It is characterized by an abrupt onset of vomiting and severe abdominal distress. Pancreatitis is an extremely difficult disease to diagnose without exploratory celiotomy. Once diagnosed it is equally difficult to treat. Treatment can vary from supportive measures to surgery. Death is not uncommon following an attack of acute pancreatitis.

Very little is known about the etiology of pancreatitis. In man it appears to be associated with alcoholism or biliary obstruction. Neither of these is the cause in dogs. In the canine, acute pancreatitis is more commonly seen in the obese middle aged dog, particularly after eating a large fatty meal.

Most of the research in the field of pancreatitis has dealt with either the physiology of the pancreas or with treatment of spontaneous pancreatitis. Only a small amount of work has been done using the electron microscope to study the pancreas. Most of what has been reported has been on the normal pancreas of the rat, mouse, rabbit, human, and guinea pig.

The purpose of this study is to compare the fine structure of the pancreas of test dogs with the fine structure of the pancreas of control dogs.

# LITERATURE REVIEW

## Light microscopy

The work in this paper is a detailed study of one phase of a Ph.D. dissertation by Neil V. Anderson (1968). Anderson produced pancreatitis in 47 dogs by means of intraductal infusion of crude staphylococcal alpha toxin and studied its progression from 1 hour to 180 days using the light microscope. The intervening periods of time were 4 hours, 12 hours, 24 hours, 48 hours, 72 hours, 5 days, 10 days, 20 days, 30 days, 60 days, 90 days, and 120 days.

Anderson used staphylococcal alpha toxin because it produced experimental pancreatitis which resembles the type of spontaneous pancreatitis seen in man and dogs. Furthermore, he used .02 ml/kg of 1:3 dilution of toxin and did not ligate the ventral duct because ductal obstruction at the duodenal wall is not a recognized feature of spontaneous pancreatitis in dogs.

Gross lesions in the four-hour dogs were described as having pink-tinged interstitial exudate producing a separation of lobules. Frank hemorrhage was not described until twelve hours or later.

The histopathologic changes seen at four hours by Anderson (1968) were increased exudate, interstitial accumulation of neutrophils and eosinophils, and severe lymphatic congestion. Furthermore, he noted some focal acinar necrosis, and ductal obstruction. Most of the change seen at four hours was interstitial. There were more eosinophils present at four hours than at any other time interval that he studied.

Another interesting study utilizing the light microscope was that done by Morris (1964). He exteriorized a mouse pancreas, with blood supply intact, and studied the progression of pancreatic necrosis following ductal ligation. The most important irreversible change he found was edema of the intercalated ducts.

Using a similar but much more elaborate technique, Heisig (1968) has developed a chamber which allows an in situ study of the microcirculation of the rabbit pancreas. He measured its microcirculation in relation to glandular activity.

Further work on microcirculation and the lymphatic system in the dog was done by Anderson and Schiller (1967). They described a periacinar space located at the basal region of the acinar cells. Anderson stated that this space has direct communication with both lymphatic and circulatory systems and that when the lymphatic system was open much material was removed by this route. During pancreatitis these spaces became congested and erythocytes filled the areas.

Normal fine structure

Several papers have been written on the normal fine structure of the pancreas. Ekholm, et al. (1962a, b), described in detail the acinar cells, the centroacinar cells, and the intercalary and intralobular ducts of the rat. In one paper (1962a) they mentioned four things of importance: presence of microvilli, Golgi vacuoles, immature zymogen granules, and zymogen granules discharging their contents into the acinar lumen. In a second paper (1962b), they described the centroacinar cells, intercalary, and intralobular duct cells as being similar in that all three have sparse endoplasmic reticulum, few mitochondria, and Golgi apparati. In a paper by Zelander et al. (1964), they described capillaries, arterioles, and nerve fibers of the interacinar and intermicrolobular spaces.

Before describing the pancreas of the rat, Ekholm and Edlund (1959) wrote on the ultrastructure of the human exocrine pancreas. This paper was the first one to describe a human acinar cell in its entirety. Celle and Cuneo (1964) confirmed the observations of Ekholm et al. and those of Fedou (1963).

One of the outstanding contributions to the electron microscopic study of the pancreas is that by Sjostrand (1962). He stated that the exocrine pancreatic cells are simple in morphology. In his study of the cat pancreas, he described the parts of the acinar cell, i. e., endoplasmic reticulum, Golgi apparatus, mitochondria, nucleus, and plasma membrane. He also summarized the contributions of other authors.

Palade et al. (1962), have done outstanding studies on the ultrastructure of guinea pig pancreas. They divided their paper into four parts: fine structure, cell fractionation, protein synthesis in the pancreactic exocrine cell, and intracellular transport and storage. Almost the entire section on fine structure was a description of endoplasmic reticulum. They stated that rough and smooth endoplasmic reticulum are continuous and that ribosomes are not precipitation artifacts.

Zymogen granule synthesis

There has been much investigation of the synthesis and migration of proteins in cells of the exocrine pancreas. Warshawsky (1963) took radioautographs of the pancreatic acinar cell from rats and mice after injection with radioactive proteins. At 2-5 minutes newly-synthesized protein appeared in the ergastoplasmic region. From there it migrated to and accumulated in the Golgi zone. The protein was then seen in the zymogen granule region of the cell. It was theorized that there are two types of synthesized proteins: a slowly moving one and a rapidly moving one.

Van Heyningen (1964) used the electron microscope to take radioautographs of rat pancreas and consequently confirmed the work done by Warshawsky (1963).

Palade (1966) has described in detail the secreting cycle within the pancreatic acinar cell of the guinea pig. He provides the following sequence:

1) attachment of ribosomes which contain newly synthesized protein to the rough endoplasmic reticulum

2) transportation across the membrane (ER) to the intracisternal space

3) diffusion in this space to the ER (part rough and part smooth) at the periphery of the Golgi apparatus

4) transport from the outer Golgi vesicles to the inner ones

5) concentration and condensation within the Golgi

6) intracellular storage of zymogen granules

7) fusion of zymogen granules with apical cell membrane and their discharge into the lumen.

Further work done in Palade's laboratory (Jamieson & Palade, 1968a) led to the conclusion that (1) transport and protein synthesis are separable processes, (2) transport is not the result of continuous delivery of protein, and (3) transport is not dependant on synthesis of "specific" nonsecretory proteins.

Jamieson and Palade (1968b) in an additional study of guinea pig pancreatic slices were able to learn the metabolic requirements of intracellular transport. The results indicated that transport is insensitive to glycolytic inhibitors and is blocked by inhibitors of oxidative phosphorylation and by respiratory inhibitors.

Dietary lesions

Ultrastructural changes have been seen following dietary changes. Weisblum et al. (1962) fed a protein free diet for 10 days to rats and noted the following changes: coarsening of nuclear matrix, depletion of zymogen granules, loss of ribosomes, and dilatation of intracisternal spaces. These authors also described regeneration of the cell. For further cell regeneration studies, the reader is referred to Herman et al. (1962b), Tiscornia et al. (1966), Fitzgerald et al. (1966), and Fitzgerald et al. (1968). The above papers are

excellent; however, because the scope of this paper is limited, one can only mention them.

Paradisi (1965), using a phase microscope, examined pancreas from rats fed three different diets - fasted, non protein, excess cholesterol. After fasting the rats for 48 - 150 hours, he noticed cytoplasmic damage. Following feeding a non protein diet, he observed a decrease in size and an eventual disappearance of nucleoli. As a result of feeding excess cholesterol he reported nuclear lesions followed by severely affected cytoplasm.

Scott (1966) deprived rats of lysine and demonstrated a reduction in zymogen granules; fragmentation of zymogen granules; unaltered Golgi apparati, cell nuclei, and ergastoplasm; large clusters of lipid droplets, and swollen mitochondria. Myelin figures were a sequela to the swollen mitochondria.

Fine structure of the pancreas following the introduction of foreign materials

Herman et al. (1962a) injected rats intraperitoneally with ethionine and noted changes in the pancreas. The greatest morphological change was in the ergastoplasm. There was widening of the cisternae and a decrease in ribosomes associated with their membranes. Coarsening and clumping of the nuclear chromatin occurred. Changes in zymogen granules, mitochondria and Golgi did not occur until late in the experiment.

B-3-thienylalanine was fed to rats by Hruban, Swift, and Wissler (1962) and early findings were localized cytoplasmic necrosis and excess numbers of prozymogen granules. Larger doses for longer periods resulted in complete stoppage of zymogen granule formation, presence of smooth walled vacuoles and a hyperplasia of Golgi apparatus.

In another study by Hruban, Swift, and Slessers (1965a), rats were fed or injected intraperitoneally with triparanol, or were force fed diethanolamine.

Myeloid bodies, which appeared to be related to ergastoplasm, were seen in animals given triparanol. A reduction of endoplasmic reticulum with free ribosomes was seen following diethanolamine. Both treatments resulted in inhibition of zymogen granule synthesis and hypoplasia of the Golgi apparatus.

In still another study of Hruban, Swift, and Dunn (1965), rats were force fed B-3-furylalanine, an analog of phenylalanine. The procedure resulted in focal cytoplasmic degradation, Golgi hyperplasia, inhibition of zymogen granule formation, accumulation of intracisternal granules, and changes in mitochondrial structure.

One further study by Hruban et al. (1965b) involved the intraperitoneal injection of azaserine into rats. Briefly, the results were alterations of the rough endoplasmic reticulum, large areas of focal degradation and formation of osmiophilic plaques within the acinar cells.

Imai (1966) did an electron microscopic study comparing therapeutic doses of tetracycline and chloramphenicol with overdoses of these two drugs. In guinea pigs that received therapeutic doses, only slight differences from the controls were noted: segmental dilatation of the cisternae and a slight reduction in intracisternal granules. Important changes were seen in the pancreas taken from guinea pigs receiving an overdose of drugs. The rough endoplasmic reticulum was fragmented and vesiculated and RNP particles were found free in the cytoplasm. Intracisternal granules were reduced in numbers as were the zymogen granules. A number of mitochondria were found surrounding a large vacuole.

Watari and Noriomi (1968) wrote a paper on the affect of alloxan, pilocarpine, and dehydroascorbic acid on the pancreatic acinar cell. It was of particular interest in that it involved ultrastructure of the canine pancreas. The nuclei appeared

normal until late in the experiment when they appeared densely stained. Zymogen granules were fewer in number and appeared to fuse with the plasma membrane. The rough endoplasmic reticulum was vesiculated and fragmented. The Golgi was remarkably enlarged. Mitochondria were essentially normal. They described complex dense bodies as having increased numbers and as having originated from Golgi, mitochondria or autophagic vacuoles. They mentioned two more items of interest: 1) fat droplets are seldom seen in acinar cells, and 2) glycogen is not seen in the normal acinar cell of the dog.

Dense complex bodies

Several authors have described "abnormal" structures seen in the pancreas. These have been called various things such as corpuscles and dense complex bodies. Fedou et al. (1966) described a number of intracytoplasmic crystalline formations seen in the pancreas of the dog. Although they were rarely seen in normal dogs, they were greatly increased in numbers after injection of rabbit serum containing anti-dog pancreas antibodies. They were composed of three different types of elements -- tridimensional crystals, lipid appearing material, and a heterogenous granular matrix. They were merely described.

Legg (1968) reported what he called compound tubular bodies found in the exocrine pancreas of normal cats. They were found in the apical regions of acinar cells in association with zymogen granules. The tubules appeared to differ from both microtubules and intramitochondrial tubules. Legg has theorized that this organelle is probably related to secretory function.

Alousi et al. (1968) injected mice subcutaneously with 1% neutral red dye. They noticed "abnormal" structures which they named three different things depending on the stage of development. The early stages were called autophagic vacuoles and contained identifiable segments of endoplasmic reticulum.

The intermediate stages were classified as composite bodies and held partially digested segments of endoplasmic reticulum and mitochondria. The latest stages were called residual bodies; they contained material that could not be identified These bodies disappeared by 18 hours after injection.

Frexinos et al. (1968) authored a paper on lysosomes of the exocrine pancreas. Their paper was actually a summarization of the literature. They stated that lysosomal structures have appeared in the acinar cell after experiments of very different types were conducted. They reported that the changes seen by other authors were very similar to those seen in chronic pancreatitis of man.

Nagata, et al. (1968) studied the exocrine pancreas of mice by means of electron microscopy and noted inclusion bodies. They noted lipase activity in these inclusions, both in the cisternae and in the zones between the cytoplasmic matrices and tubular elements which surround the crystalloids. They suggested that these inclusion bodies could store and discharge lipids depending on the needs of the animal.

Miscellaneous papers on ultrastructure of pancreas

Before this literature review can be complete, five "miscellaneous" papers, all dealing with electron microscopy of the pancreas, must be included. Zelander et al. (1963) ligated the pancreatic ducts of rats and described subsequent changes. The acinar lumina were dilated and the acinar and centroacinar cells were reduced in height. The Golgi apparati had enlarged cisternae. Often there were large vacuoles within the cells. Furthermore, there were fewer zymogen granules; the endoplasmic reticulum had lost its normal organization; and the mitochondria were swollen and were losing their inner membranes.

Volk et al. (1966) subjected the exteriorized dog pancreas to irradiation and then described the subsequent changes. There were large numbers of

membrane-bound bodies which contained altered cytoplasmic components as well as electron dense structures, vacuoles, and granules. Moreover the number and size of mature zymogen granules was reduced.

Ichikawa (1965) studied the perfused canine pancreas by means of the electron microscope. He noticed no change between the perfused pancreas and normal pancreas. After stimulation with pancreozymin, there was an increase in discharge of zymogen granules. This affect, however, was not uniform, as some cells retained their granules. There was little affect on the Golgi apparatus. Stimulation with secretin had little affect on the cells - acinar or centroacinar.

Tigyi et al. (1967) classified the arrangement of the ergastoplasm. Annular endoplasmic reticulum is that which is scattered loosely, saccular endoplasmic reticulum is intermediate in type, and the third or lamellar endoplasmic reticulum is parallel or concentric in arrangement. They also claimed that there is no correlation between the Golgi apparatus and the functional state of the cell. In addition, they said that the mitochondria are long in fasted animal and short in fed animal.

Tasso (1967) studied eight cases of chronic pancreatitis in man. The changes in the pancreatic acinar cell involved the endoplasmic reticulum, the Golgi apparatus, and secretory granules. In addition to these changes, there were many inclusions.

## MATERIALS AND METHODS

The dogs used for this procedure were healthy alert mongrel dogs, mature as determined by presence of permanent teeth, and weighing between 10 - 15 kg. Six dogs (3 females and 3 males) were held for 2 weeks prior to surgery. During this period of time they were fed commercial dog food* and had free access to water. The dogs varied in weight from 9.1 kg to 15 kg. One control dog had a temperature of 104°; the others fell within a normal range. Four dogs received an intraductal infusion of crude staphylococcal alpha toxin into the ventral pancreatic duct, and two dogs were used as surgical controls.

The dogs were fasted for 24 hours and then anesthetized with sodium pentobarbitol, injected into the cephalic vein at a dosage of 1 gr/5 lbs. A preanesthetic was not given, nor were the animals given fluids during or following surgery. The animals were shaved, cleaned, and draped for surgery.

A long ventral midline incision was made, extending from the xiphoid cartilage to a point midway between the unbilicus and pubis. The descending duodenum and pancreas were found and exteriorized. A longitudinal incision was made into the duodenum opposite the ventral pancreatic duct. A size PE 10 catheter attached to a tuberculin syringe was then inserted into the ventral pancreatic duct and directed cranially into the left lobar duct.

In the four experimental dogs, .02 ml/kg of a 1:2 dilution of crude staphylococcal alpha toxin (1020 hemolytic units/ml) was slowly injected into the left lobe of the pancreas. The procedure was the same for the two surgical controls, except that 1 cc of sterile physiological saline was used in place of the toxin. (Anderson, 1968)

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* Purina Dog Chow

Following injection, the duodenum was sutured with a Connell-Cushing suture pattern using 00 catgut. The abdomen was closed with a simple continuous suture pattern using cotton suture. The dogs were maintained under anesthetic for 4 - 4 1/2 hours following surgery.

At the end of 4 hours, the abdomen was re-opened and the pancreas was relocated. Using a 2 cc syringe and a 25 gauge disposable needle, 1/2 - 1 cc of 2% buffered osmium was injected into a periductal region of the left lobe. After 2 - 3 minutes, or when the tissue had turned brown, a small piece was removed. This piece was further sectioned under a dissecting scope into cubes no larger than 0.5 mm. They were then put into fresh 2% osmium buffered with 0.1 M cacodylate pH 7.35. The fixative was on ice during this procedure. After removal of the pancreatic tissue, the dog was euthanatized.

The vials of fixative containing the tissue were kept in the refrigerator from 1 to 4 1/2 hours. Following this time interval, they were removed and dehydrated without rinsing. This procedure was done at room temperature, in increasing concentrations of ethanol: 5 minutes in 50% twice, 5 minutes in 70% once, 5 minutes in 90% once, and 5 minutes in 100% thrice.

Following dehydration, the tissues were infiltrated with Epon overnight at room temperature. The next day they were flat embedded in Epon according to the procedures of Luft (1961). They were hardened in 35°, 45°, and 60° C. ovens for one day each.

Once the Epon blocks were hardened, they were trimmed and were then ready to be sectioned. One-half to one micron thick sections were stained with 2% toludine blue and 2% sodium borate. Once it was certain that pancreatic acinar sections were being cut, thin sections (600-900 Å) were taken. All sectioning was done using a diamond knife in a Westfall-Healy section mounter. Once the sections were cut they were lowered onto 100 mesh specimen screens which were double coated with parlodion and carbon.

Thin sections, mounted on grids, were stained with uranyl acetate (Watson, 1958) for 45 minutes at 37° C. They were then rinsed with distilled water and stained with lead citrate (Reynolds, 1963) for 20 minutes. Following this step they were rinsed with 0.2 N sodium hydroxide followed by distilled water. Once dry the grids were studied using a RCA-EMU-3G electron microscope.

# RESULTS

## I. Saline Control Dogs

A saline injection into two control dogs produced similar results. The following is a description of the fine structure seen in these two dogs. Results obtained are summarized in Table I and are illustrated by Figures 1 through 6.

### Acinar organization

From three to six pancreatic acinar cells, with or without centroacinar cells, can be seen around a centrally located lumen (Fig. 1). Each acinus is surrounded by a periacinar space.

A trilaminar plasma membrane bounds each cell. Cells are separated from each other by a narrow intercellular space. Cells are attached to each other near their apices by a junctional complex composed of a zonula occludens, a zonula adherens, and a macula adherens (desmosome). Basally they are held together by invaginations, or undulations, in cell membranes as well as by desmosomes. Intercellular bridges, normal openings in the cell membrane, are seen (Fig. 2).

Individual acinar cells have a basally located nucleus, apically located zymogen granules as well as rough endoplasmic reticulum, mitochondria, Golgi apparatus, and microfilaments (Fig. 2).

The nuclei of the acinar cells, with few exceptions, appear round and have distinct nucleopores connecting the outer nuclear membrane with the rough endoplasmic reticulum. The nucleoplasm is essentially homogenous in appearance. One distinct, or not uncommonly, two distinct nucleoli are present. Figure 2 illustrates one of two cells seen in an acinus which has two nuclei.

Rough endoplasmic reticulum

The rough endoplasmic reticulum, membranes with attached ribosomes, has a parallel lamellar arrangement (Fig. 2). In all cases the cisternae, membrane bound cavities, are somewhat dilated. In those cells with a lot of cisternal enlargement, some of the parallel lamellar arrangement is lost. One tissue block differs from the others in that the endoplasmic reticulum is vesiculated (rounded). Free ribosomes, those that are no longer attached to their membranes, were seen in an occasional cell. Intrascisternal granules (Fig. 3), densely staining protein found in the cisternae of the rough endoplasmic reticulum, are present in all except one of the tissues sectioned.

Zymogen granules

Zymogen granules are seen mostly in the apex of the cell and vary in number from cell to cell. They are round with a distinct membrane and range in size from 0.4 to 1.0 microns. In some cells the zymogen granules appear to have lost their limiting membranes (Fig. 2); in other cells there is an increase in numbers of prozymogen granules (Fig. 4). Prozymogen granules are those in which the secretory protein does not reach the limiting membrane.

Mitochondria

The mitochondria are located between the nucleus and apex of the cell. With the exception of a few long slender ones, the rest are small and ellipsoidal in shape. They vary somewhat in density from light staining swollen mitochondria with short cristae (Fig. 3) to typical mitochondria with distinct cristae (Fig. 4). Cells from one dog have distinct mitochondrial granules; whereas, the cells from the other dog have few if any (Table I).

Golgi apparatus

The Golgi apparatus varies from a typical arrangement, having lamellae of

flattened cisternae with associated vesicles and vacuoles (Fig. 2), to a more vesicular appearance in which the cisternae are dilated (Fig. 5). The latter type, with its cisternae in various stages of dilatation, is seen in most cells.

Miscellaneous intracytoplasmic structures

Microfilaments (Fig. 4) are present in most of the acinar cells. They are seen most commonly in the apex of the cell but are also seen along the cell membrane and adjacent to the nuclear membrane.

Dense complex bodies are seen in a number of cells (Figs. 2, 3, 6). They range in size from 0.85 microns to 3.0 microns. All appear to be membrane bound; however, they vary as to morphology. One appears to have crystals and a dense granule (Fig. 3), several posses myelin figures or a homogenous matrix (Fig. 6).

Centroacinar cells

The centroacinar cells (Fig. 1) are lightly stained and contain a nucleus and numerous mitochondria. Rough endoplasmic reticulum and zymogen granules are absent.

## II. Staphylococcal Alpha Toxin Dogs

Although the results obtained in the two control dogs are similar to each other, such is not the case with the four dogs injected with toxin. There was a good deal of variation between dogs and equally as much variation between acinar cells. Results are summarized in Table II and are illustrated in Figures 7 through 17.

Acinar organization

As in the control dogs, the acinar cells are arranged in groups around a central lumen. The apical areas contain dense complex bodies, disintegrating

mitochondria and abnormal appearing zymogen granules which show a loss of their limiting membranes (Fig. 7). The acinar cells are joined to each other by desmosomes and by undulations in the cell membrane. Although the plasma membranes in Fig. 8 are very clear, other cell membranes are not distinct. Disruptions in the cell membranes (Figs. 9 and 10) are seen most often near the periacinar space. Numerous intercellular bridges are found between acinar cells (Figs. 7 and 11).

The nuclei are round, oval or indented and reveal distinct nucleopores and nuclear membranes (Fig. 12). At least one nucleolus is present in the majority of the nuclei. It is not uncommon to see clumping of chromatin material (Fig. 10).

Rough endoplasmic reticulum

In twelve (80%) of the fiften tissues sectioned, the rough endoplasmic reticulum is arranged in concentric lamellae (Fig. 8). In the other three tissues (20%), the rough endoplasmic reticulum is vesiculated (Fig. 12, 13). The cisternae are dilated to some degree in all of the cells studied. This dilatation varies from almost none (Fig. 8) to a complete loss of parallel arrangement of lamellae (Fig. 12). Intracisternal granules are present in only a very few cells (Table II).

Zymogen granules

Zymogen granules in some cells are distinct, large and numerous (Fig. 8). In other cells they are irregular dense cores which have separated from their limiting membranes (Figs. 7 and 11). It is not uncommon to see some degree of adhesion between granules (Fig. 7). Some cells have few or no zymogen granules (Figs. 10 and 14).

Since there appear to be fewer zymogen granules in the acinar cells of

test dogs compared to control dogs, and more irregular granules (those separated from their limiting membrane), the following two hypotheses were tested:

1) the median number of total zymogen granules from the control dogs equals the median number of total zymogen granules from the test dogs

2) the median number of irregular zymogen granules from the control dogs equals the median number of irregualr zymogen granules from the test dogs.

The data were obtained by counting the zymogen granules - total numbers and irregular granules - from eleven cells of test dogs and from eight cells of controls. The data are as follows:

Test Dog Pancreas

| Cells | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Total number of zymogen granules | 68 | 61 | 35 | 29 | 33 | 43 | 36 | 23 | 44 | 96 | 68 |
| Irregular zymogen granules | 6 | 8 | 2 | 1 | 1 | 4 | 4 | 22 | 42 | 72 | 59 |

Control Dog Pancreas

| Cells | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Total number of zymogen granules | 13 | 51 | 37 | 20 | 38 | 65 | 89 | 44 |
| Irregular zymogen granules | 10 | 2 | 6 | 15 | 2 | 17 | 11 | 2 |

By using the Wilcoxson Rank Sum Test with alpha set at .1, the above two hypotheses failed to be rejected.

Mitochondria

Mitochondria are numerous and are present in the subnuclear area of the acinar cell as well as in the apex. They are basically of two shapes, one being long and thin, the other being short and rounded. All of them have

cristae. The mitochondria vary in density from light staining swollen ones (Figs. 9, 12, 13) to mitochondria that are so densely stained that they resemble the density of zymogen granules (Fig. 10). One mitochondrion (Fig. 10) has surrounded a piece of ergastoplasm. Large distinct mitochondrial granules (Fig. 11) are seen in acinar cells of three dogs; whereas none are seen in one dog (Table II).

Golgi apparatus

The Golgi apparatus, in one cell having few zymogen granules (Fig. 14), has a neat parallel arrangement of smooth endoplasmic reticulum. The cells with numerous zymogen granules (normal appearing or non-normal appearing) have few if any similarities in their Golgi apparati. The Golgi cisternae are in various stages of dilatation and range in numbers from a few vacuoles to several dozen (Figs. 7, 9, 10, 12, 15).

Miscellaneous intracytoplasmic structures

Microfilaments occur in almost all of the acinar cells, (Figs. 10, 11, 14). As in the control dogs, they are seen in greater numbers in the cell apices and around the nuclear and plasma membranes. They are also seen near desmosomes (Fig. 11).

Vacuoles of two types are present. One type is light staining and "empty" (Fig. 16); the other is similar in size except that it is filled with a homogenously dark staining material.

Complex dense bodies (Fig. 9, 15, 17) are present in the apices of most of the pancreatic acinar cells from test dogs. Although they are all about equal in size, 0.8 microns, they vary as to appearance. One appears to contain two mitochondria within a membrane (Fig 15), others reveal myelin figures and granular matrices within a membrane (Fig. 17).

Lipid droplets, irregular in shape and approximately 1.0 micron in size were seen in several cells. One is shown in Fig. 10. They are frequently adjacent to mitochondria.

Centroacinar cells

The centroacinar cells contain mitochondria that are swollen and have short cristae. Several centroacinar cells have microfilaments. A few cells have lipid droplets, and one has an "autophagic vacuole."

# DISCUSSION

## I. Similarities in Acinar Morphology of Control and Test Dogs

### Acinar cells

One can see from the results and illustrations that the basic structure of the acinus is the same in both test and control dogs. Acinar cells from both test and control dogs have rounded nuclei with prominent nucleoli and nucleopores. They are both joined by a junctional complex and by undulations of the cell membranes. Acinar cells from test and control dogs are separated between junctions by a narrow intercellular space, and are surrounded by a periacinar space.

Intercellular bridges are seen occasionally between cells within an acinus in both test and control dogs. These are normal in that the plasma membrane gaps are small with free exchange of cytoplasm and there is no apparent disorganization of the membranes or adjacent cytoplasm.

### Organelle structure not related to the condition of the cell

A more or less parallel lamellar arrangement of the rough endoplasmic reticulum is present in test and control dogs. In fact, the cells that had the most "classic" concentrically arranged lamellae were from test dogs. The ergastoplasm was vesiculated in one control and two test animals. Hruban et al. (1965) showed a similar occurrence in rats. He reported this vesiculation as a fragmentation of cisternae. He suggested such vesiculation to be related to impending death of the cell. In other words, both ergastoplasm in a parallel lamellar arrangement and vesiculated ergastoplasm are common to test and control dogs. Therefore, one can question the importance of the numerous reported descriptions of changes in the endoplasmic reticulum.

Furthermore, one can question the changes in mitochondria reported in the

literature. In this study, all animals exhibit normal appearing mitochondria along with some which are swollen, of light density, and starting to lose their cristae. As is seen with the rough endoplasmic reticulum, variations are between cells rather than between test and control dogs.

Tigyi et al. (1967) stated that there was no correlation between configuration of the Golgi apparatus and the functional state of the cell. The author of this paper, based upon her work, is inclined to agree with this statement with one important exception. In cells with no zymogen granules (Fig. 14), the Golgi is quite small and inactive in appearance; whereas, in cells with zymogen granules the Golgi complex varies in shape and size.

Microfilaments

Large numbers of microfilaments are present in the apical region of certain cells, although they are apparently absent in others. Their frequency of occurrence seems equal in test and control dogs.

Centroacinar cells

With the exception of a loss of cristae in the mitochondria, there was little difference between the centroacinar cell of the test dogs and that of the control dogs.

II. Differences in Acinar Morphology Between Control and Test Dogs

Absence of intracisternal granules in test dogs

The most interesting observation seen in this study is the presence of intracisternal granules in the rough endoplasmic reticulum of control dogs and the absence of them in the test dogs.

Palade (1956) reported these granules in normal pancreas of guinea pigs, and Ichikawa (1965) reported them in the normal pancreas of dogs. Ekholm (1962) stated that he was unable to find these granules in normal pancreas of rats

and humans. He reported that he saw them in rats treated with ethionine. Subsequently, he suggested that perhaps they are normal only in guinea pigs. Sjostrand (1962) stated that he did not observe them in cats, rats, or mice.

Because of their presence in the control dogs and their absence in the test dogs, it can be postulated that an absence of intracisternal granules indicates the initial step in the reduction of numbers of zymogen granules. In support of this idea, Imai (1966) noted fewer intracisternal granules and fewer zymogen granules in guinea pigs which were given an overdose of tetracycline and chloramphenicol.

In opposition to this theory, Hruban et al. (1965b) reported an accumulation of intracisternal granules following force feeding of B-3-furylalanine to rats. He did not find them in control rats.

Hruban et al. (1962; 1965; 1965a), Imai (1966), Watari et al. (1968), Zelander et al. (1963), and Volk et al. (1966) noted a decrease or complete absence of zymogen granules after the production of experimental pancreatitis. Although the statistically checked hypotheses in this paper (page 18), indicate no difference in numbers of zymogen granules between the test and control dogs, the test sample was quite small. Furthermore, there are definitely cells (Figs. 10, 14) from the test dogs which contain few or no zymogen granules

These papers would further support the theory that pancreatitis results in a reduction of zymogen granules. Therefore, an absense of intracisternal granules in the test dogs could easily represent the initial step in decreased zymogen granule formation.

Mitochondria seen surrounding ergastoplasm

As stated previously, the changes seen in the mitochondria vary from cell to cell rather than between control and test dog. However, there is one difference (Fig. 10). In this cell several mitochondria appear to surround

clusters of ergastoplasm. Alousi (1968) illustrated a similar example in mice after injection with 1% neutral red dye.

Free ribosomes

In the test dogs, areas of free ribosomes appear much more frequently than in the control dogs. This finding is in agreement with the work, described in the literature review, of Herman et al. (1962), Hruban et al. (1965), and Imai (1966).

Dense complex bodies, vacuoles, and lipid occur more often in acinar cells from test dogs

One thing that many investigators of experimental pancreatitis have reported is the occurrence of intracytoplasmic inclusions - corpuscles, dense complex bodies, and autophagic vacuoles. The reader is referred to the literature review, under the heading dense complex bodies, for a summary of their interpretations. Most authors described the abnormalities formed; however, few ventured any hypotheses as to their origin or function.

The author of this paper found these intracytoplasmic inclusions in both the test and control dogs. They were, however, more numerous in the test dogs. Without the aid of histochemical techniques, it is not possible to determine the structure of these bodies. However, because of their association with and their resemblance to mitochondria, it can be postulated that many of them are degenerating mitochondria.

Vacuoles may result from a malfunctioning Golgi apparatus or an extremely dilated cisterna. Because these vacuoles are large, light staining, and homogenous in composition they certainly do not seem to be associated with the dense complex bodies mentioned above.

Lipid was present in several cells. The droplets were quite large in size and were seen more often in the test than control dogs. In agreement

with Palade (1958) and Imai (1966), lipid droplets occasionally appeared to be surrounded by mitochondria.

Disruption of cell membranes and accumulation of degenerate organelles in cell apices

It is probable that the toxin leaves the duct and enters the acinar cell by either of two paths. The first postulated route is one in which the toxin leaves the duct, travels up the intercellular spaces and enters the periacinar space. The second assumed route is one in which the toxin leaves the duct and enters the adjacent apical region.

In support of the first idea, one can see disruptions along the periacinar space (Fig. 10) and near what is most probably an intercellular bridge (Fig. 9). Anderson (1967) has shown that when india ink was injected into the ventral pancreatic duct of dogs, and particles left the duct, entered the intercellular spaces and accumulated in the periacinar space. Therefore, the toxin could accumulate in the periacinar space and affect the cell through its peripheral membrane.

In defense of the second postulated route, the toxin may pass between the centroacinar cells and into the cell apices. It is probable that the centroacinar cells remain undamaged because they are more resistant than acinar cells. Since there is an accumulation of inclusions and damaged organelles in the apical region, it is possible that the toxin had a direct affect on them.

This accumulation of degenerate organelles in the apices of acinar cells brings several unanswered questions to mind. Are these degenerate organelles there because the toxin has affected the apical region of the acinar cell? Are they absent in control animals because the normal cell discharges its degenerate organelles? Does a damaged cell no longer secrete or excrete, thus resulting in an accumulation of degenerate organelles? More work combining

morphology with function will have to be done before these questions can be answered.

# CONCLUSIONS

Pancreatic acinar cells from control and test dogs have many similarities. Acinar cells from both are connected by a junctional complex and undulations in cell membranes. They are separated between junctions by a narrow intercellular space and are surrounded by a periacinar space.

Loss of parallel lamellar arrangement of endoplasmic reticulum, changes in structure of mitochondria, and changes in configuration of Golgi apparatus have been reported in the literature as pathological. This paper shows that changes in endoplasmic reticulum, mitochondria, and Golgi occur between individual cells and dogs, not between control and test animals.

There are several differences noted between the acinar cells from control and test dogs. The most interesting observation is the absence of intracisternal granules in acinar cells of test dogs and their presence in control dogs. This observation may indicate a cessation of protein synthesis.

Dense complex bodies, vacuoles, and lipid were more numerous in acinar cells of the test dogs than in the control dogs. Also seen in a test dog was a mitochondrion surrounding a cluster of ergastoplasm. Areas of free ribsomes were numerous in the acinar cells of test dogs.

Further work in this field is indicated. Study of numerous samples of normal canine pancreas would establish a pattern, or lack of one, for the appearance of rough endoplasmic retriculum, arrangement of Golgi apparatus, and the structure of mitochondria in acinar cells.

It would be beneficial to study pancreatitis at various time intervals to determine the progression of the disease. Furthermore, much work could be done in the field of histochemistry in order to determine the composition of the dense complex bodies.

Lastly it would be interesting to see more definitive work done on

intracisternal granules in order to determine if they are always present in normal pancreas and to prove that their absence is the first step in reduction of protein synthesis.

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# APPENDIX

TABLE I

SURGICAL CONTROL DOGS

| dog | tissue block | Mitochondria | | Endoplasmic Reticulum | | | cell membrane continuity |
|---|---|---|---|---|---|---|---|
| | | cristae | granules | parallel lamellar arrangement | dilated cisternae | intracisternal granules | |
| 3 | A | + | - | ± | + | + | + |
| 3 | B | + | - | + | + | ± | + |
| 3 | C | + | - | + | + | - | + |
| 3 | D | + | - | + | + | + | + |
| 4 | A | + | + | + | - | + | - |
| 4 | B | + | + | - | + | + | - |
| 4 | C | + | + | + | + | + | + |
| 4 | D | + | + | + | + | + | - |

+ = presence of

- = absence of

TABLE II

EXPERIMENTAL DOGS

| dog | tissue block | Mitochondria cristae | Mitochondria granules | Endoplasmic Reticulum parallel lamellar arrangement | Endoplasmic Reticulum dilated cisternae | Endoplasmic Reticulum intracisternal granules | cell membrane continuity |
|---|---|---|---|---|---|---|---|
| 1 | A | ± | + | ± | + | ± | + |
| 1 | B | ± | + | ± | + | - | - |
| 1 | C | + | + | + | + | - | - |
| 1 | D | + | + | ± | + | - | - |
| 2 | A | + | ± | - | + | - | ± |
| 2 | B | ± | + | + | – | – | – |
| 2 | C | + | - | - | + | - | ± |
| 2 | D | + | + | + | ± | – | ± |
| 5 | B | + | - | ± | ± | ± | - |
| 5 | C | + | - | + | + | - | - |
| 5 | D | + | - | + | + | + | - |
| 6 | A | + | + | + | ± | - | - |
| 6 | B | + | + | + | ± | - | - |
| 6 | C | + | + | - | + | - | + |
| 6 | D | + | + | + | + | - | + |

PLATE I



Fig. 1 Pancreatic acinus from control dog. Two acinar (A) and three centroacinar cells (CA) are adjacent to a common lumen (L). Microfilaments (F), Golgi apparatus (G), mitochondrion (M), nucleus (N), zymogen granule (Z). X 7,250.

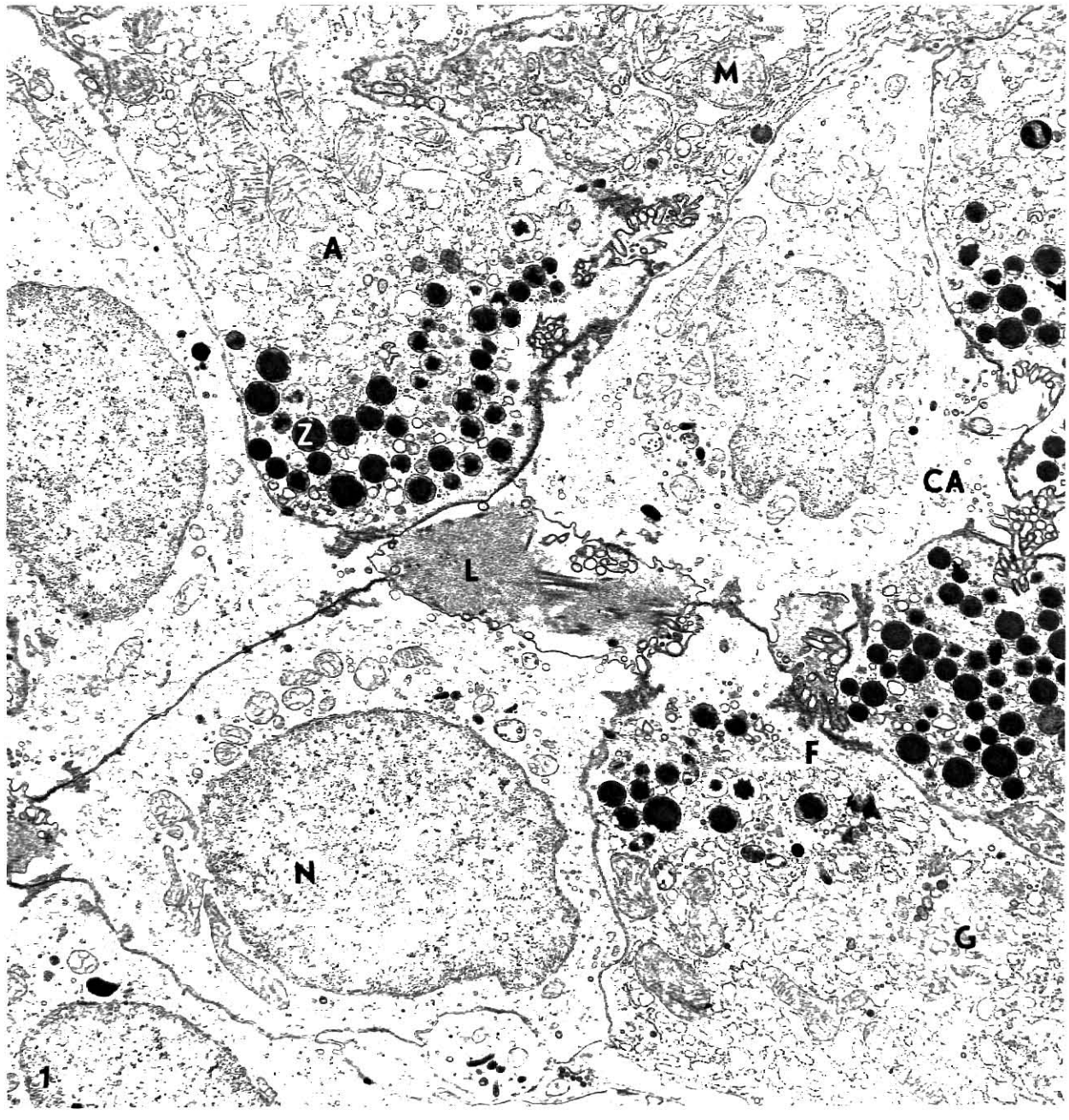

M
A
Z
CA
L
F
N
G
1

PLATE II

Fig. 2 Double nucleated pancreatic acinar cell from control dog with a prominent Golgi apparatus (G), and intercellular bridge (arrow). Desmosomes (D), dense complex body (DB), rough endoplasmic reticulum (ER), lumen (L), mitochondrion (M), nucleus (N), zymogen granule (Z). X 12,150.

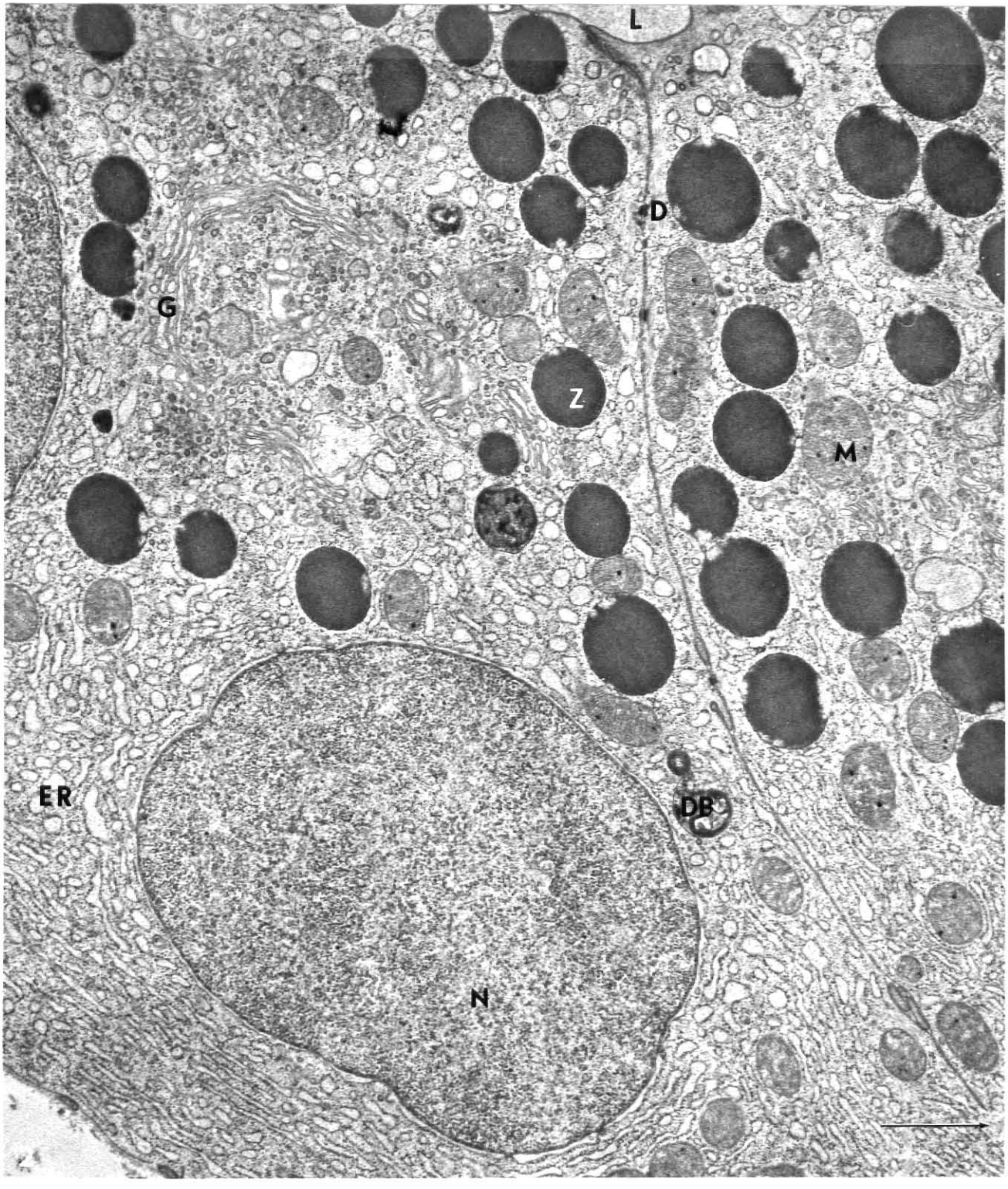

L
D
G
Z
M
ER
DB
N

PLATE III

Fig. 3 Intracisternal granules (IG) and a large dense complex body (DB) in a pancreatic acinar cell from control dog. Cell membranes (CM), Golgi apparatus (G), mitochondrion (M), zymogen granule (Z). X 12,150.

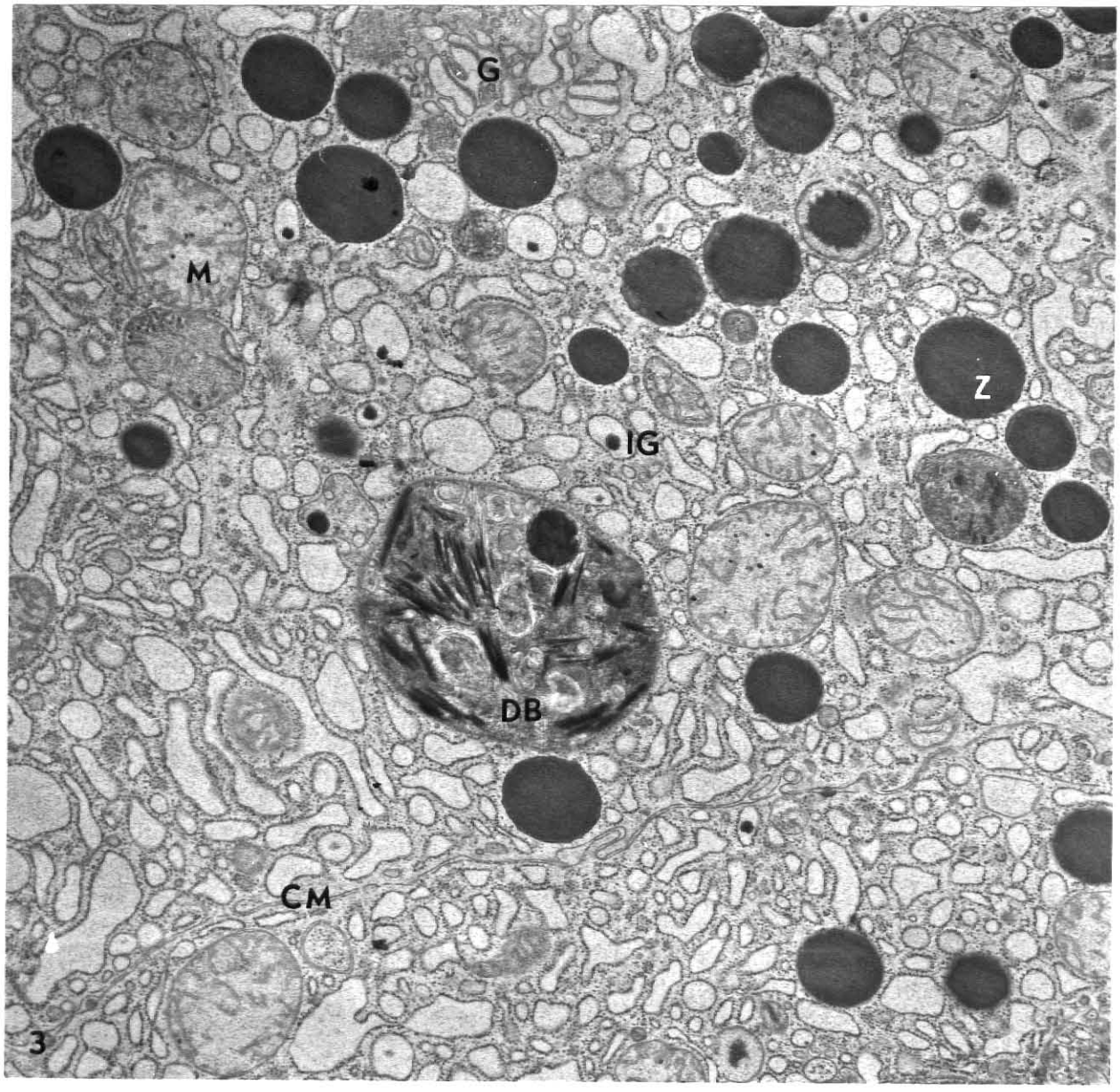
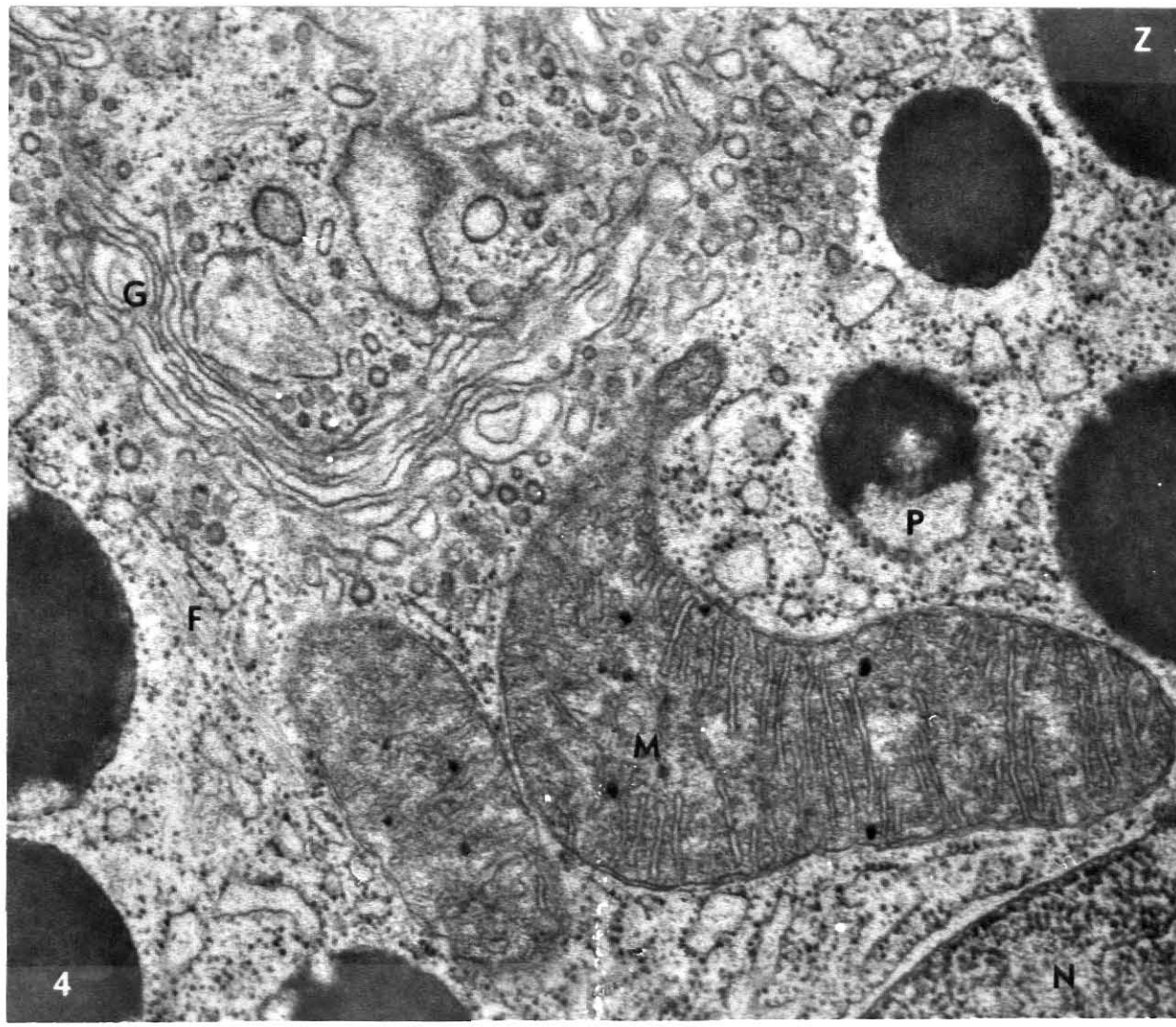

G
M
Z
IG
DB
CM
3

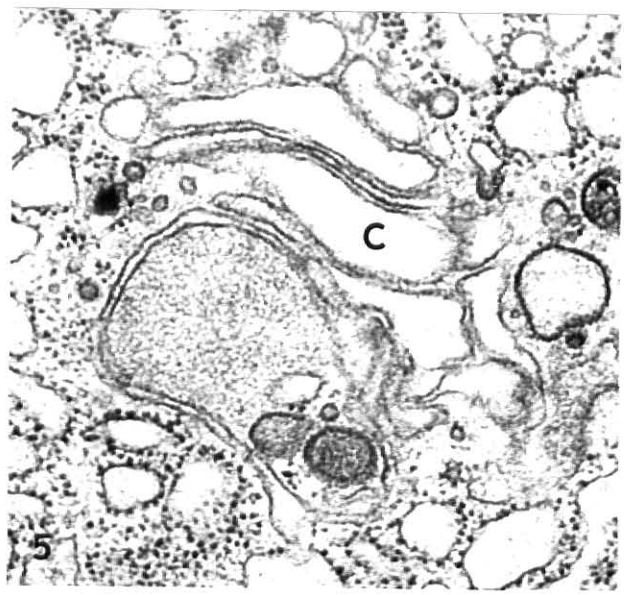
PLATE IV

Fig. 4 Mitochondria (M) with distinct mitochondrial granules are near a Golgi apparatus (G) in a pancreatic acinar cell of a control dog. Microfilaments (F), nucleus (N), prozymogen granule (P), zymogen granule (Z). X 39,550.

Fig. 5 Golgi apparatus with dilated cisternae (C) in a pancreatic acinar cell from control dog. X 39,550.

Fig. 6 Three dense complex bodies (DB) and a zymogen granule (Z) in a pancreatic acinar cell from a control dog. X 39,550.


Z
G
P
F
M
N
4


C
5

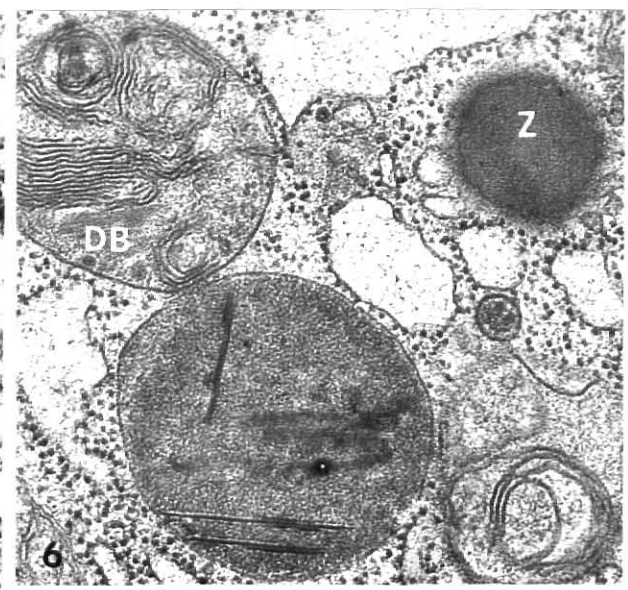

Z
DB
6

PLATE V

Fig. 7 Pancreatic acinus from a test dog. Four acinar cells are bordering a common lumen (L). There are several intercellular bridges (arrows). Golgi apparatus (G), mitochondrion (M), periacinar space (PS), rough endoplasmic reticulum (ER). X7,250.

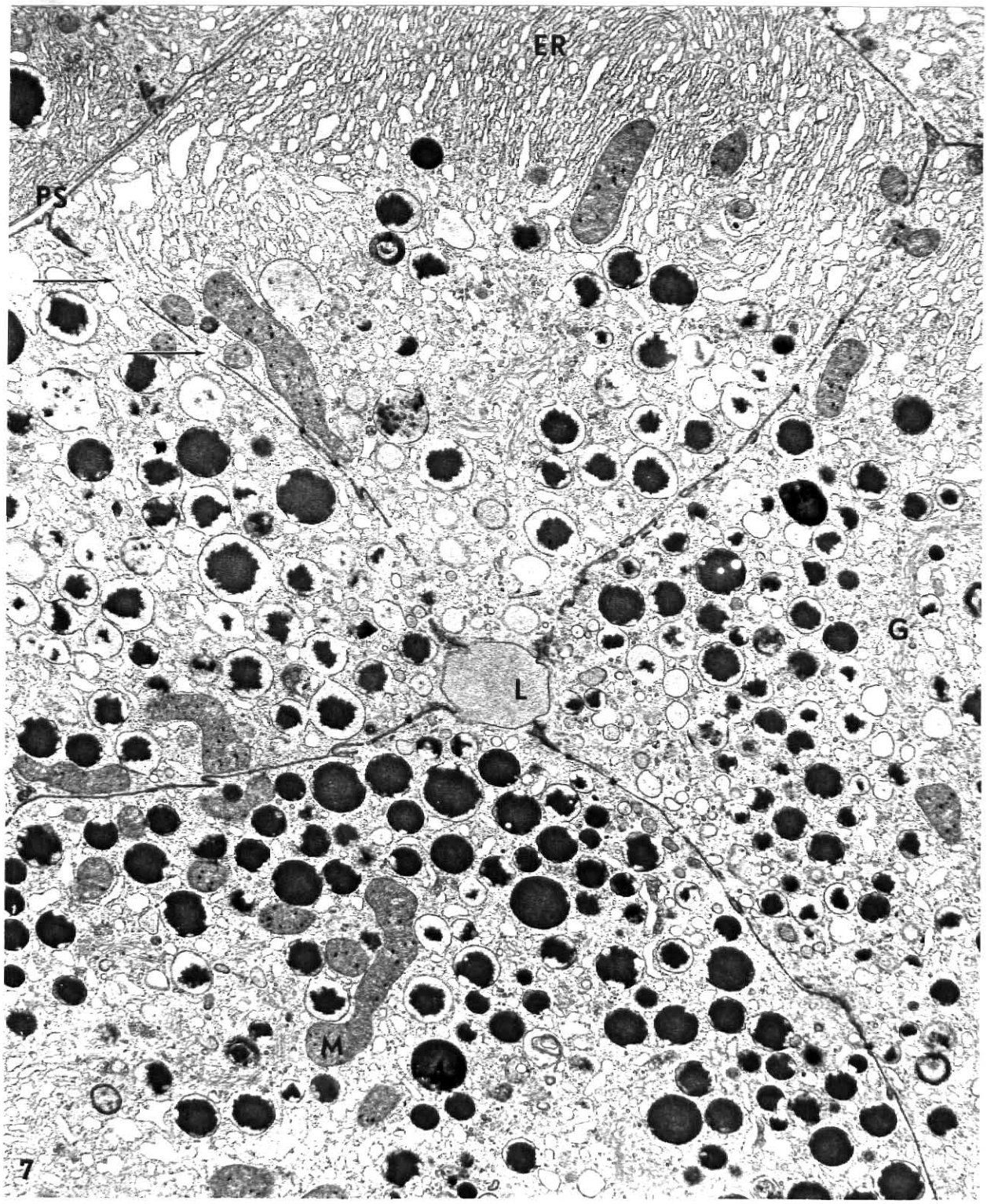

ER
PS
G
L
M
7

PLATE VI

Fig. 8 Distinct cell membranes (CM) separate parallel lamellar arrangement of rough endoplasmic reticulum (ER) seen in two pancreatic acinar cells from a test dog. Dense complex bodies (DB), mitochondrion (M), zymogen granule (Z). X 12,150.

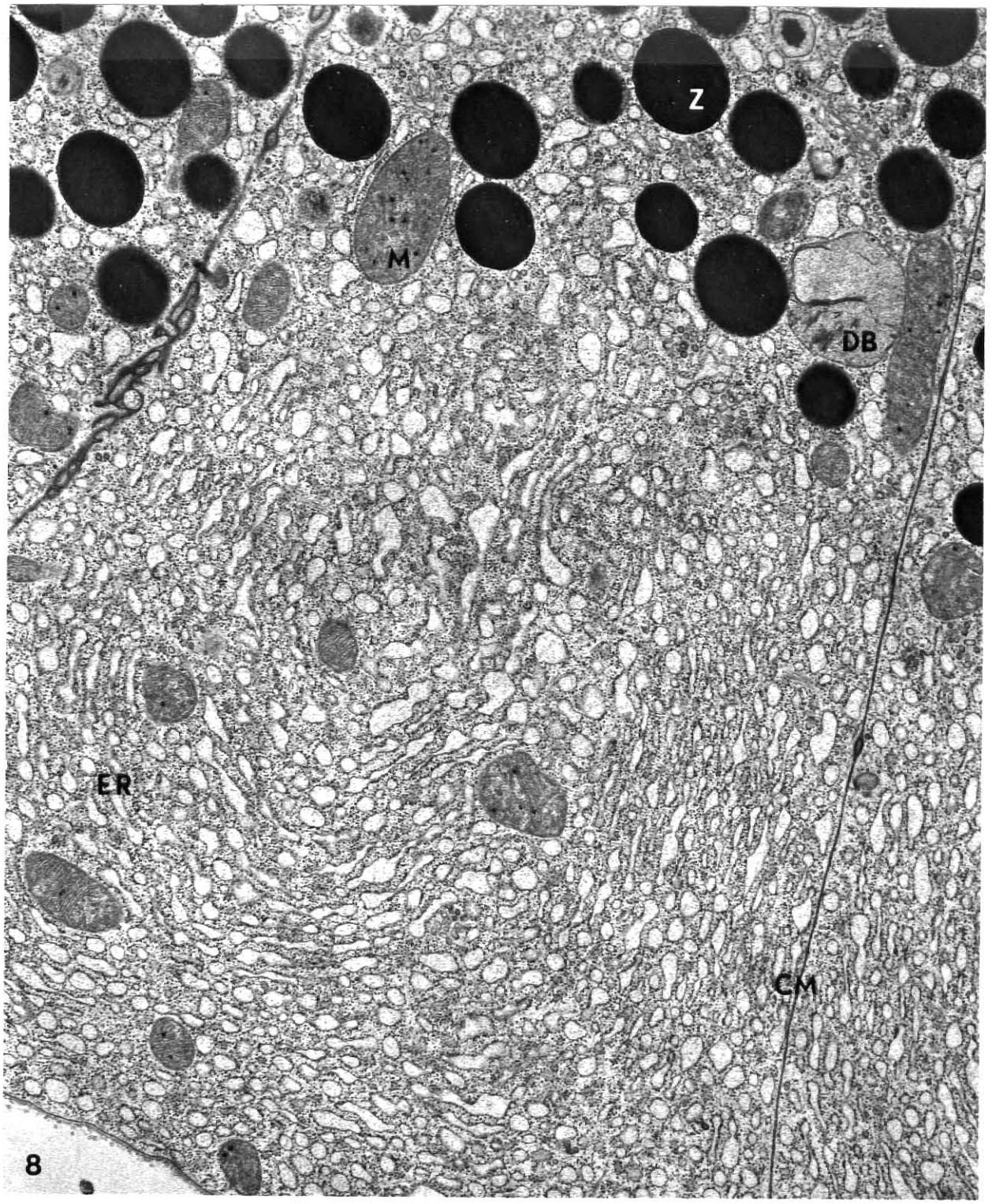

Z
M
DB
ER
CM
8

PLATE VII

Fig. 9 Basally disrupted cell membranes (arrows) and aggregations of free ribosomes (R) in a pancreatic acinar cell from test dog. Golgi apparatus (G), mitochondrion (M). X 12,150.

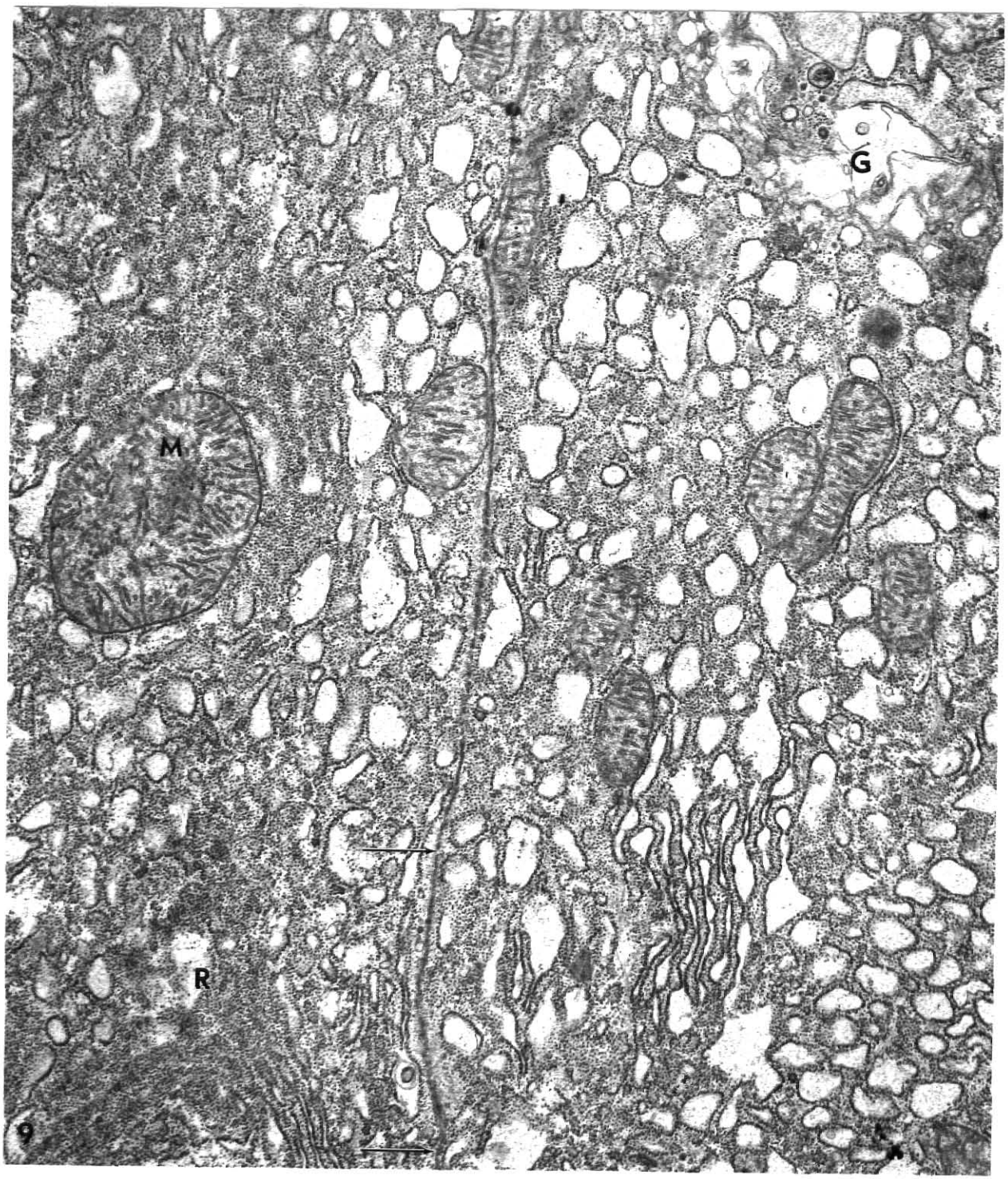

G
M
R
9

PLATE VIII

Fig. 10 Three pancreatic acinar cells from test dog illustrating disrupted cell membranes (arrows), mitochondrion surrounding ergastoplasm (M-ER), and lipid (Li). Golgi apparatus (G), lumen (L), microfilaments (F), mitochondrion (M), nucleus (N). X 12,150.

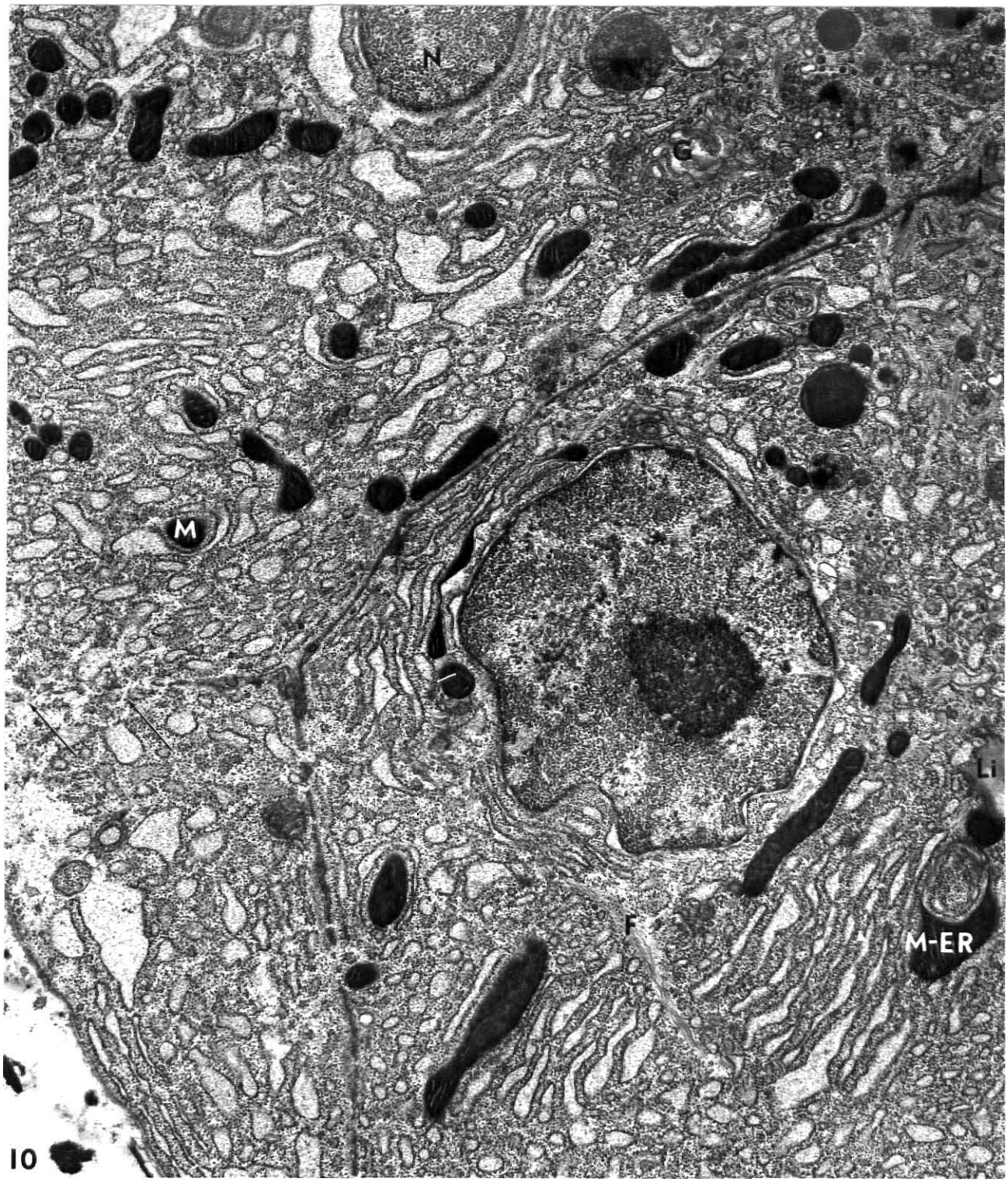

N
G
M
Li
F
M-ER
10

PLATE IX

Fig. 11 Intercellular bridge (arrow) connecting two pancreatic acinar cells from test dog. There is a large mitochondrion (M) with distinct granules adjacent to two desmosomes (D). Microfilaments (F), prozymogen granule (P), and zymogen granule (Z). X 39,550.

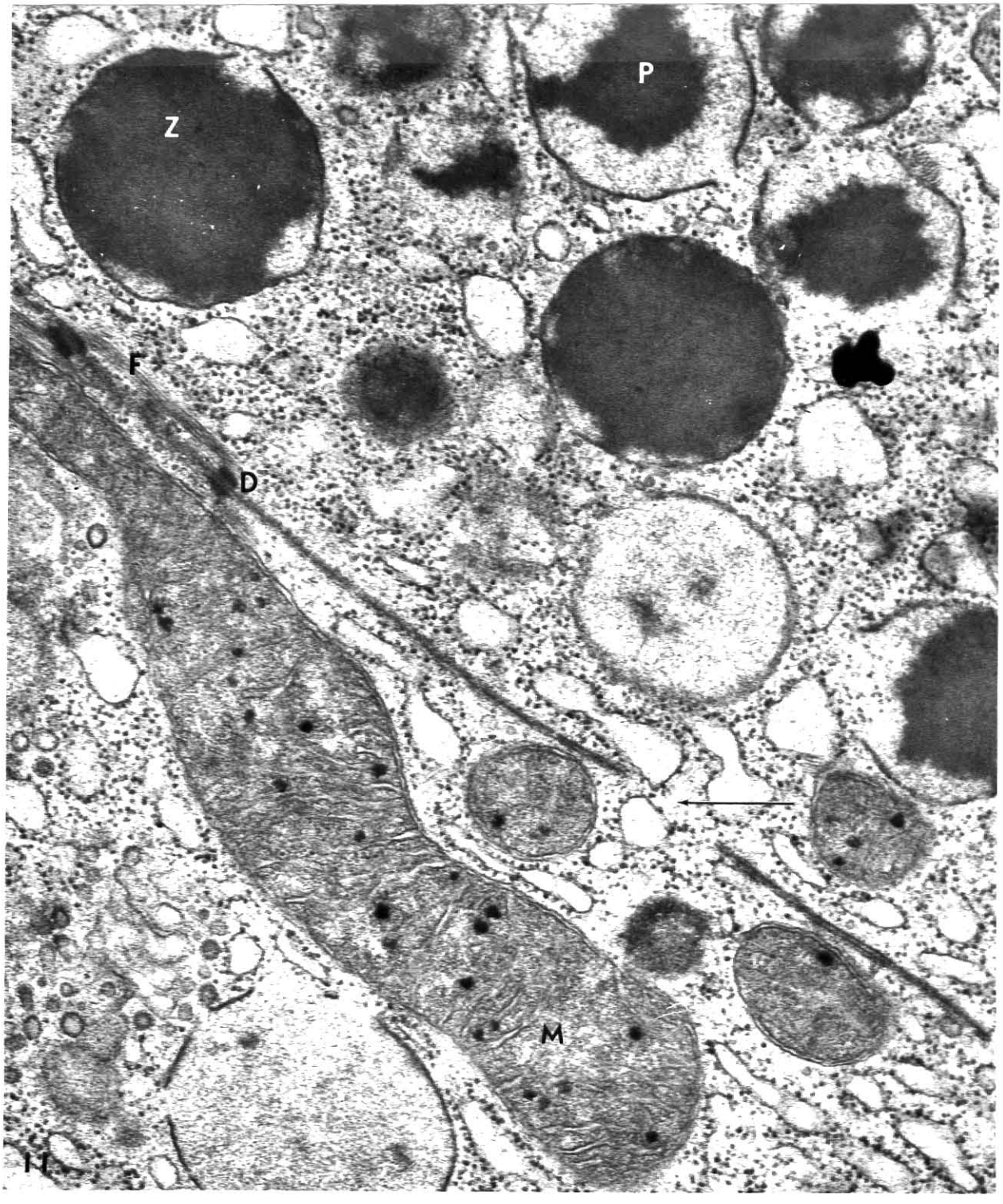

Z
P
F
D
O
M
H

PLATE X

Fig. 12 A large nucleus (N) is surrounded by vesiculated endoplasmic reticulum (ER) and swollen mitochondria (M), in a pancreatic acinar cell from test dog. Cell membranes (CM), Golgi apparatus (G), nucleopores (Np), and zymogen granule (Z). X 12,150.

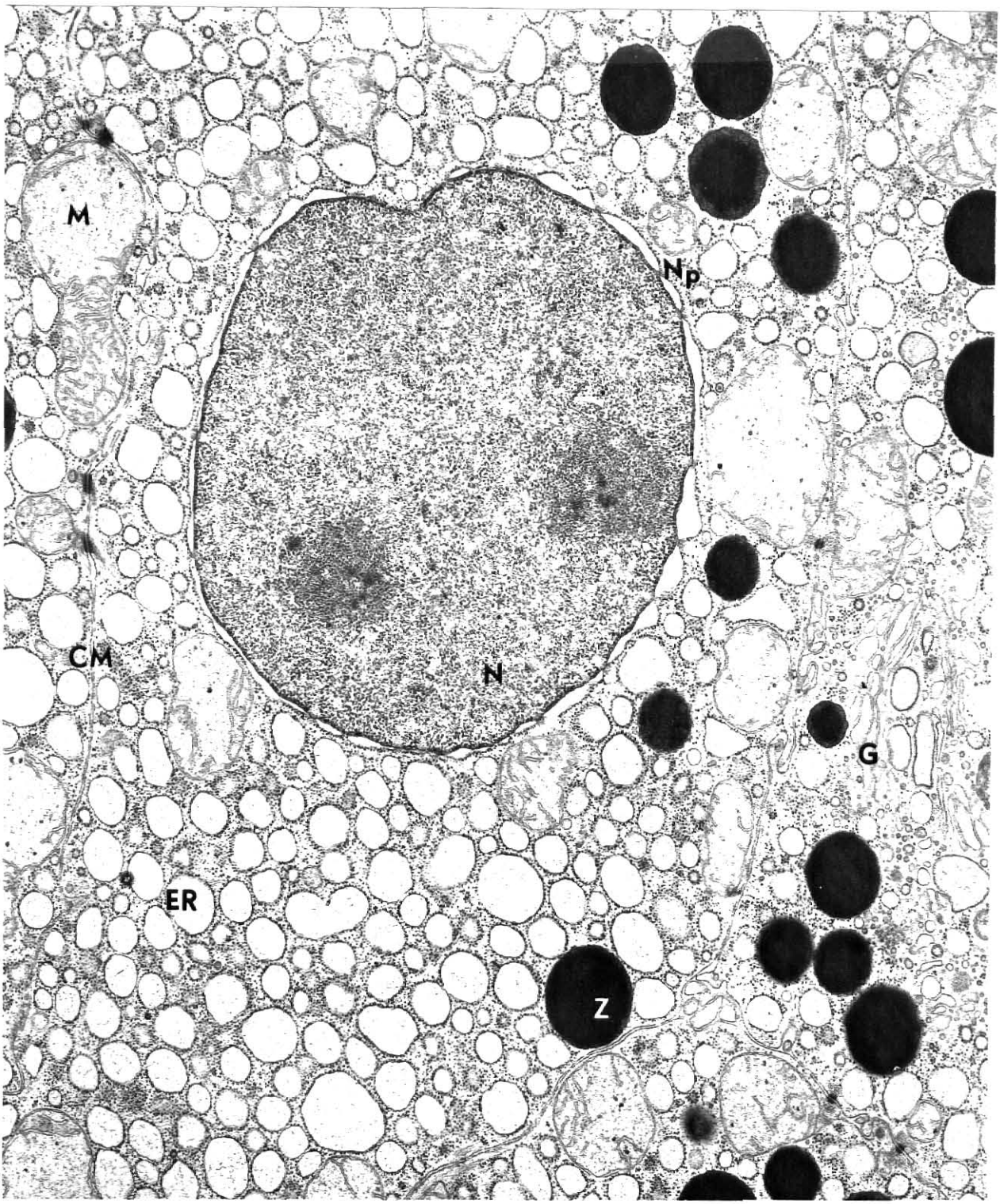

M
Np
CM
N
G
ER
Z

PLATE XI

Fig. 13 Large mitochondria (M) are surrounded by vesiculated rough endoplasmic reticulum (ER) in a pancreatic acinar cell from test dog. Cell membranes (CM). X 39,550.

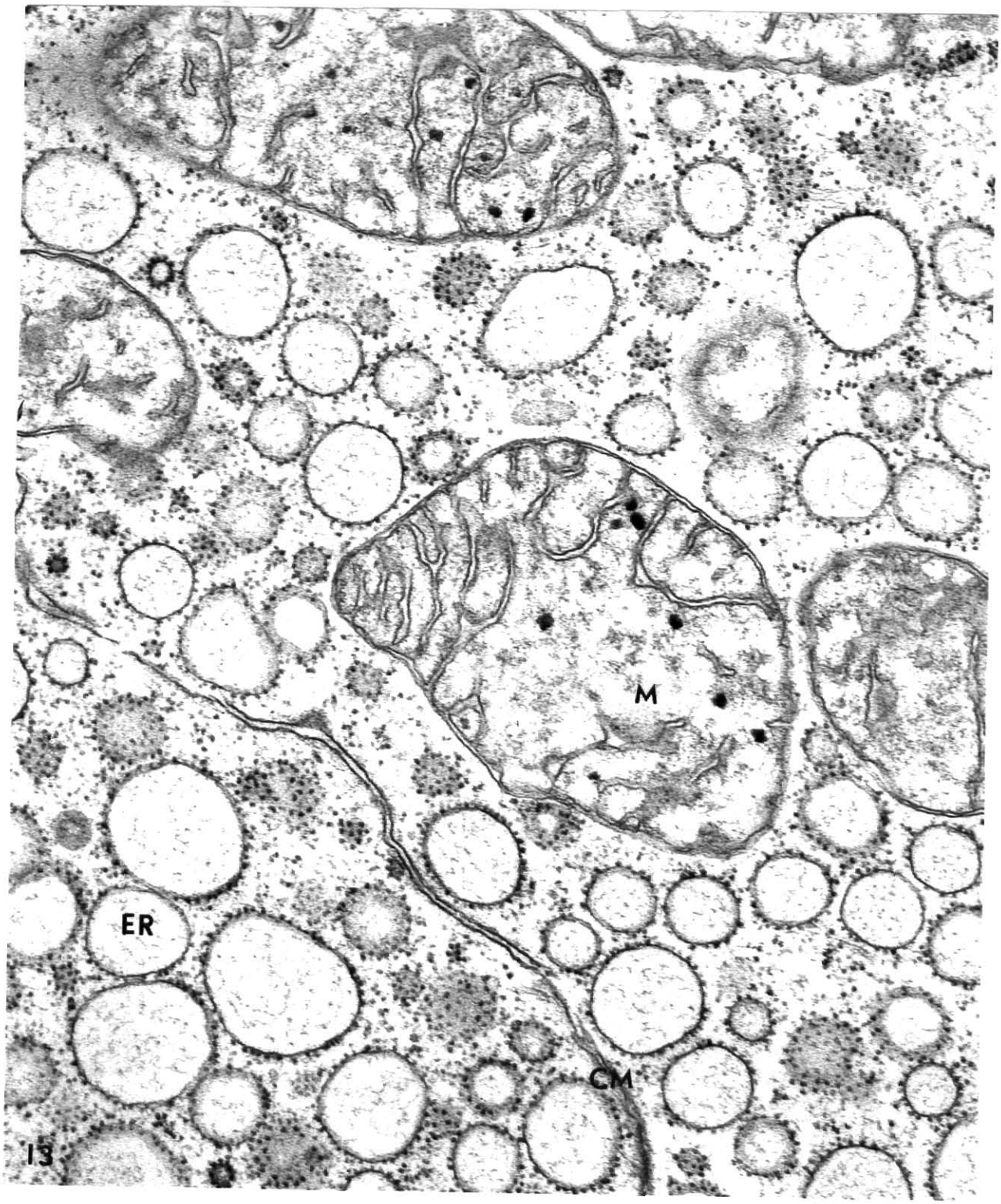

M
ER
CM
13

PLATE XII

Fig. 14 Two pancreatic acinar cells from a test dog. Note that the rough endoplasmic reticulum (ER) is arranged in parallel lamellae, the Golgi apparatus (G) is compact, and the mitochondria (M) are subnuclear. Dilated cisterna (C) of ergastoplasm, microfilaments (F), intercellular bidges (arrows). X 12,150.

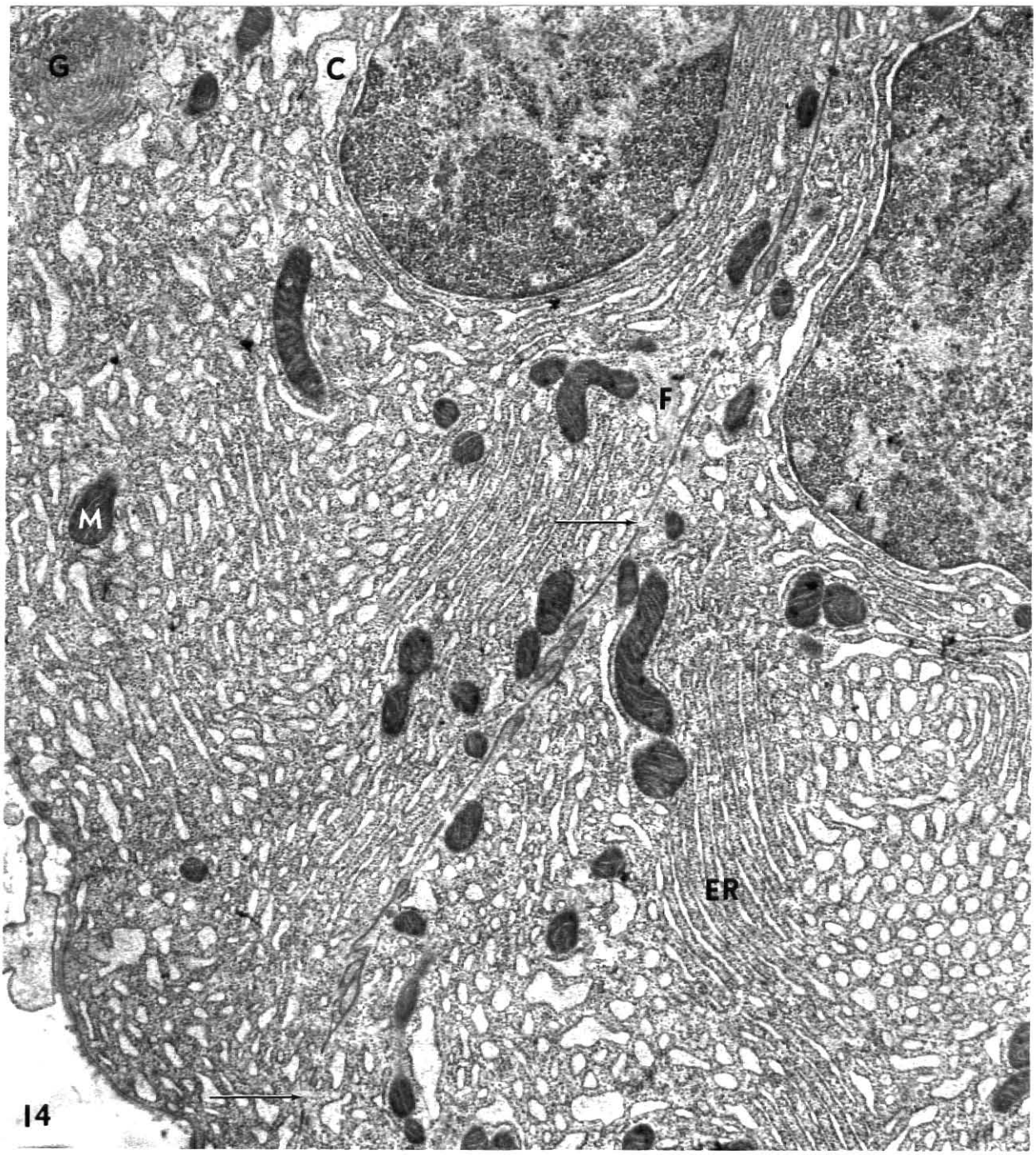

G
C
F
M
ER
14

PLATE XIII

Fig. 15 Dense complex body (DB) and Golgi apparatus (G) in a pancreatic acinar cell from test dog. Mitochondrion (M), and prozymogen granule (P). X 39,550.

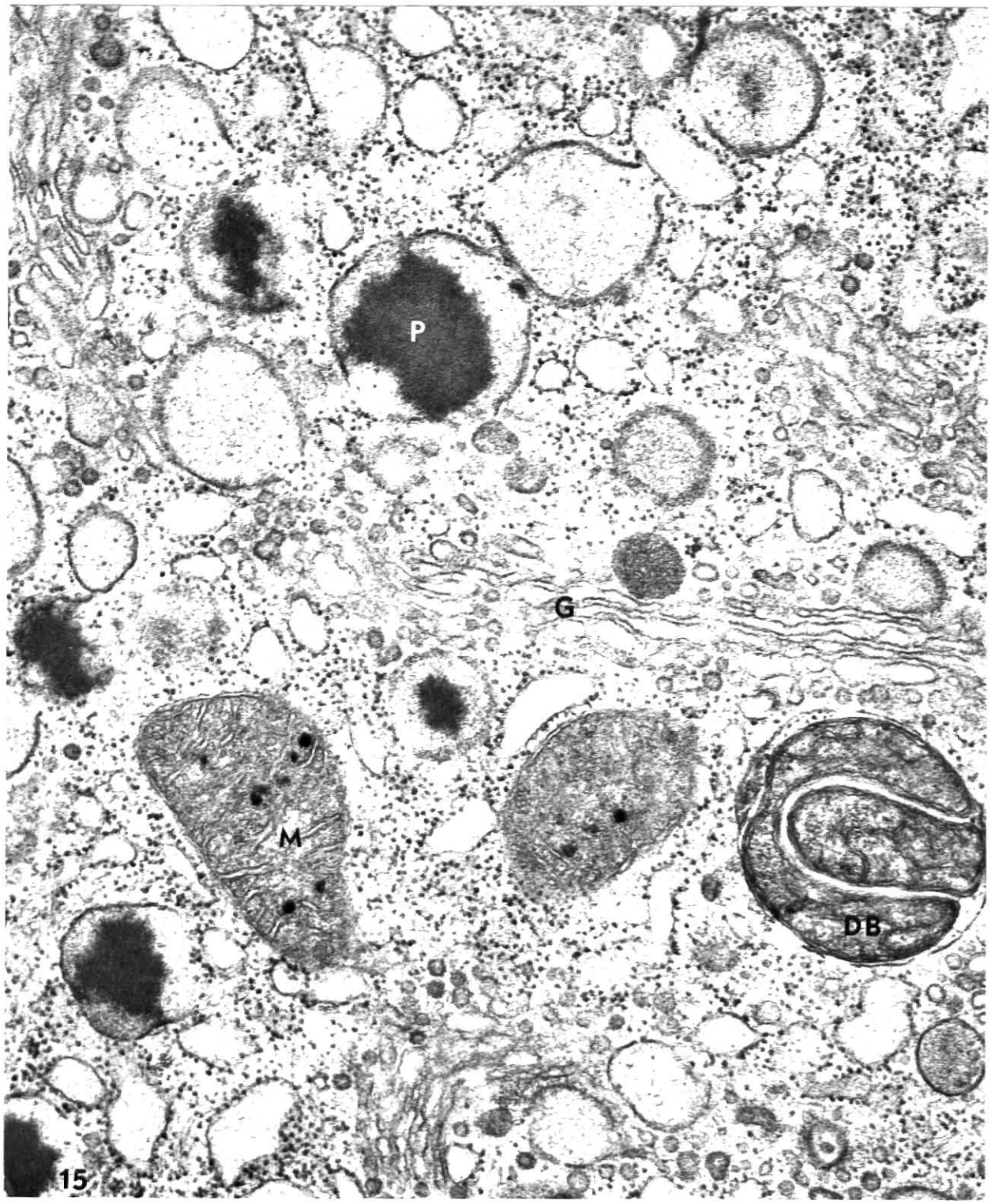
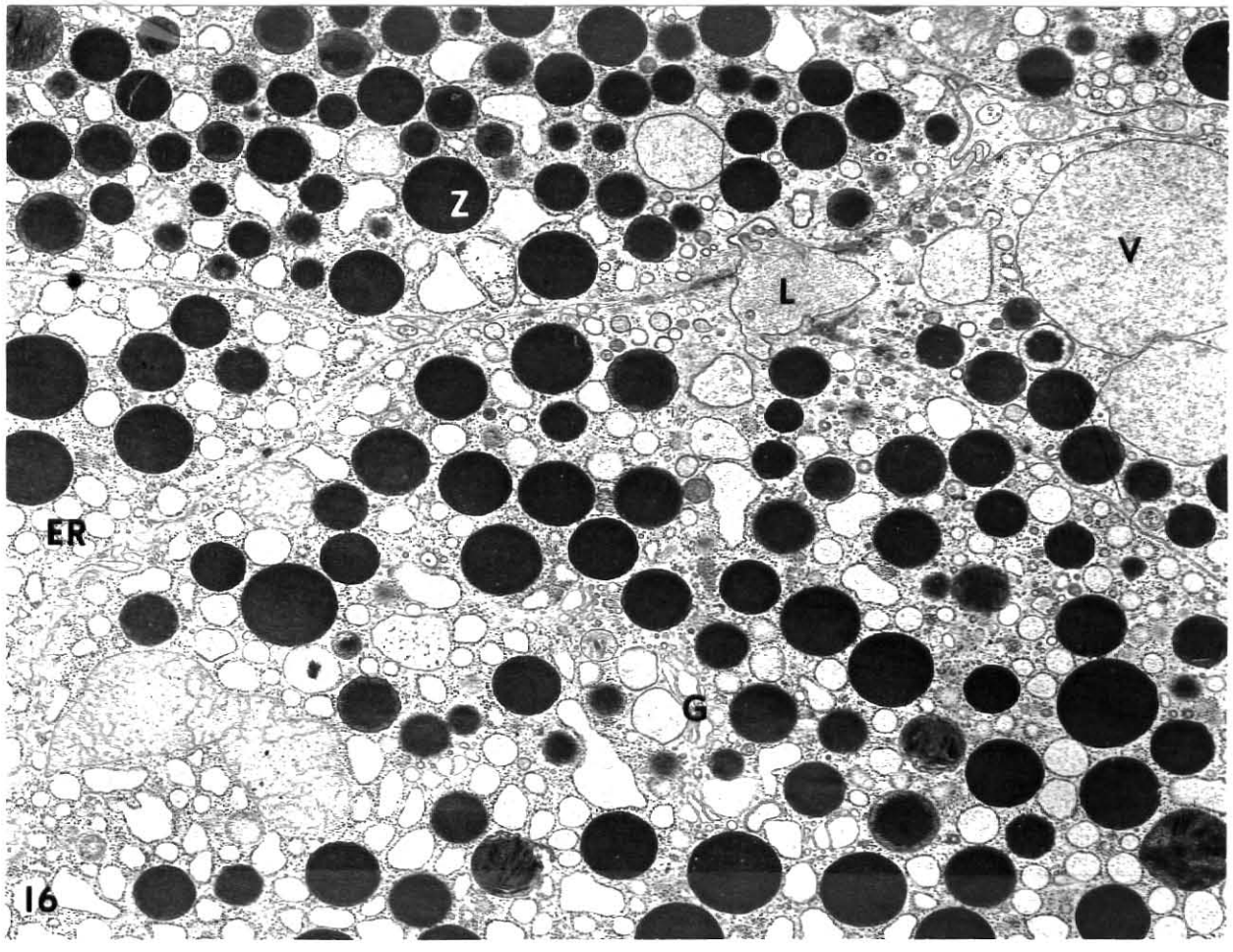

P
G
M
DB
15

PLATE XIV

Fig. 16 Three acinar cells on a common lumen (L), test dog. One cell contains two large vacuoles (V). Vesiculated endoplasmic reticulum (ER), Golgi apparatus (G), and zymogen granule (Z). X 7,250.

Fig. 17 Four dense complex bodies (DB) surrounded by vesiculated endoplasmic reticulum (ER) in pancreatic acinar cell from test dog. X 39,550.

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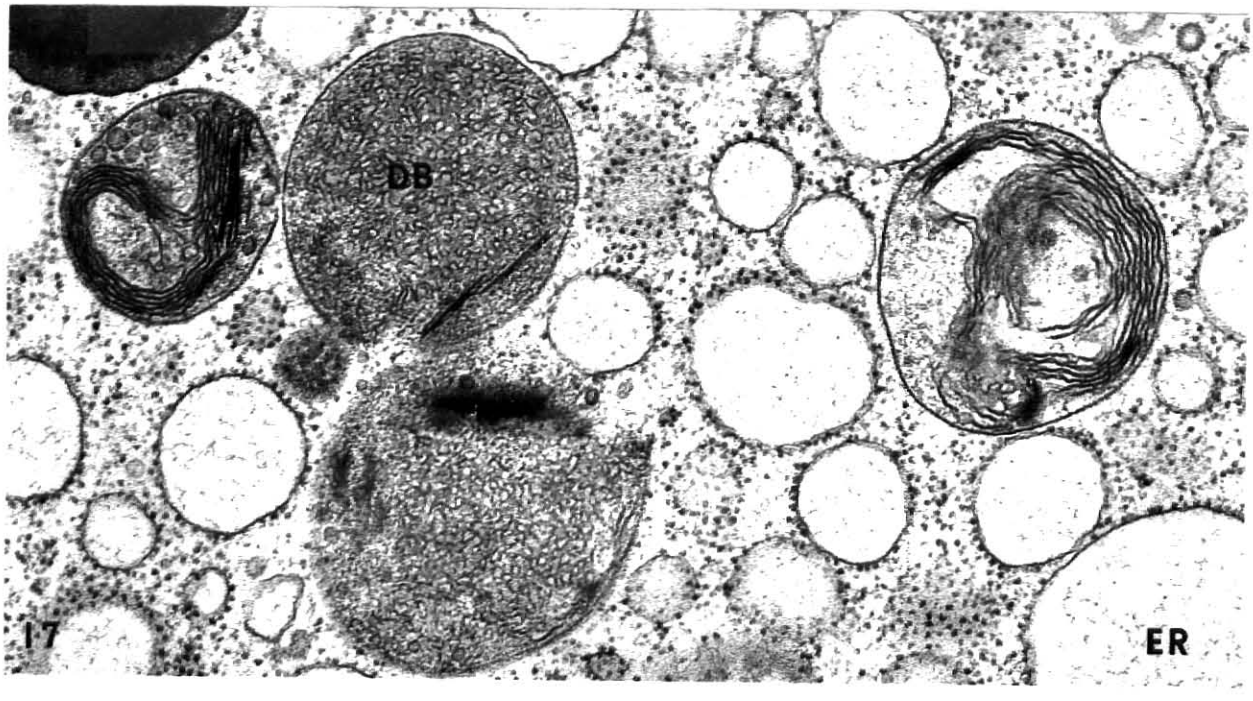


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# ULTRASTRUCTURAL CHANGES IN THE PANCREATIC ACINAR CELL PRODUCED BY STAPHYLOCOCCAL TOXIN

by

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## ABSTRACT

The electron microscope was used to study the fine structure of the pancreas of six dogs. Four test dogs were injected with 0.02 ml/kg of crude staphylococcal alpha toxin via the ventral pancreatic duct. Two surgical controls were injected, in the same manner, with sterile physiological saline.

Pancreatic acinar cells from control and test dogs have many similarities. Acinar cells from both are connected by a junctional complex and undulations in cell membranes. They are separated between junctions by a narrow intercellular space and are surrounded by a periacinar space.

Loss of parallel lamellar arrangement of endoplasmic reticulum, changes in structure of mitochondria, and changes in configuration of Golgi apparatus have been reported in the literature as pathological. This paper shows that changes in endoplasmic reticulum, mitochondria, and Golgi occur between individual cells and dogs, not between control and test animals.

There are several differences noted between the acinar cells from control and test dogs. The most interesting observation is the absence of intracisternal granules in acinar cells of test dogs and their presence in control dogs. This observation may indicate a cessation of protein synthesis.

Dense complex bodies, vacuoles, and lipid were more numerous in acinar cells of the test dogs than in the control dogs. Also seen in a test dog was a mitochondrion surrounding a cluster of ergastoplasm. Areas of free ribosomes were numerous in the acinar cells of test dogs.

Two other changes were noted: 1) disruption of the basal membranes, and 2) accumulation of degenerate organelles in the apices of the acinar cells. As a result of these observations two postulations were made:

1. Toxin leaves the duct, enters the intercellular space, moves to the periacinar space, and enters the base of the cell.
2. Toxin leaves the duct and directly enters the apices of the acinar cells.