Nagoya J. med. Sci. 28: 247-260, 1966.

ULTRASTRUCTURAL CHANGES OF PANCREATIC ACINAR CELLS FOLLOWING ANTIBIOTICS ADMINISTRATION. EXPERIMENTS OF GUINEA PIGS WITH CHLORAMPHENICOL AND TETRACYCLINE

MASAO IMAI

2nd Department of Internal Medicine, Nagoya University School of Medicine
(Director; Prof. Shingo Aoyama)

ABSTRACT

This paper deals with electron microscopic changes in the acinar cells of the pancreas after the injection of antibiotics. Chloramphenicol and tetracycline therapeutic doses 25 mg/kg body weight/day respectively were administrated to guinea pigs for 10 or 20 days and the two same overdoses 250 mg/kg body weight/day for 5 days. Pancreozymin 3 units/kg body weight was administered singly or combined with chloramphenicol therapeutic dose and overdose. The changes found in these series with special emphasis upon zymogen granules, rough-surfaced endoplasmic reticulum, mitochondria and Golgi complex were compared.

The two antibiotics therapeutic dose series show some slight but not significant changes—in relation to rough-surfaced endoplasmic reticulum—in the acinar cells.

The two overdose series reveal different serious changes—a marked decrease in number and vacuolisation of zymogen granules, and a decrease in number of intracisternal granules and vesiculation of rough-surfaced endoplasmic reticulum, large vacuoles surrounded by mitochondria or by a single mitochondrion-like structure occur. The Golgi complex is somewhat dilated.

Pancreozymin singly induced accelerated secretion of zymogen granules and increase of dense material in the Golgi complex and intracisternal granules in the rough-surfaced endoplasmic reticulum, but pancreozymin combined with over dosed chloramphenicol does not turn the rough-surfaced endoplasmic reticulum disturbed.

By comparing these changes found in the different series, the role and significance of the organellae in the acinar cells are discussed in relation to secretion of the pancreas and enzymatic protein synthesis.

INTRODUCTION

Electron microscopic studies of the acinar cells of the pancreas have been reported in different animals and also repeated experimentally as such-fasting-refeeding change following pilocarpine injection[1)2)], change injured by neutral red

今 井 昌 雄
Received for publication, June 16, 1965.

injection[3], changes stimulated by secretin[4][5], and changes induced by protein metabolism inhibitors such as ethionine[6][7][8][9] and β-3-thienylalanine[10][11], and by non-protein-feeding[12].

Inhibitory effects of antibiotics on intracellular protein synthesis have been differently discussed. Chloramphenicol does not affect intracellular glycolytic and respiratory systems, but rather intracellular protein synthesis[13]. It does not disturb metabolism of ribonucleic acid[14]. Obstructive action of chloramphenicol on protein synthesis takes place at the level of the arrangement of amino acids by inhibiting adhesion of messenger-RNA on the ribosomal surface[15][16]. Furthermore, protein synthesis not disturbed by chloramphenicol is probably the protein synthesis in which the messenger-RNA does not participate[17]. Tetracycline also inhibits mitochondrial phosphorylation, influences RNA or DNA metabolism[18][19][20] and affects ribosomes[21].

Straub *et al.*[22] found in the pigeon pancreas that the transformation of the precursor protein into amylase consists of two processes, one of which is inhibited by chloramphenicol. Newly produced enzyme proteins in acinar cells have been understood to move from their original site to zymogen granules which are the final product of intracellular secretion. From this, the abnormalities of the zymogen granules must be considered as a result of unusual changes occurring in different stages of the protein synthesis.

The present study is concerned with electron microscopic observations of the formation of zymogen granules after the treatment with chloramphenicol and tetracycline partly in combination with pancreozymin, using the pancreas of guinea pigs, to elucidate the roles of different organellae in the pancreatic exocrine secretion as the backgrounds for biochemistry.

MATERIALS AND METHODS

Sixty guinea pigs, weighing 350 to 400 g, were given semisynthetic balanced diets three times a day at 6 hour intervals. Tetracycline and chloramphenicol, together with pancreozymin, were administered according to the schedule as shown in Table 1. The animals were generally fasted overnight before sacrifice.

The animals received daily intramuscular injection of tetracycline (hostacycline, pyrrolidinmethyl tetracycline Hoechst) and chloramphenicol (chloromycetin Sankyo). The therapeutic dose means 25 mg/kg body weight/day and the overdose 250 mg/kg body weight/day. A half of the chloramphenicol animals were treated with pancreozymin 3 units/kg body weight shortly before sacrifice. Based upon the Hunt's experiment[52], they were sacrificed at 10, 20, 30 and 60 minutes after the injection at the sixth day of experiment after overnight fasting. Twelve received a single intracardiac injection of pancreozymin 3 units/kg body weight and were sacrificed at 10, 20, 30 and 60 minutes.

TABLE 1. Schedule of Experiments

Experimental group	No. of guinea pigs used	Antibiotics and their doses	Days of administration	Pancreozymin treatment	Period of experiment (days)
Tetracycline-therapeutic dose series	3 3	Tetracycline 25 mg/kg body weight/day	10 20	–	11 21
Tetracycline-overdose series	3	Tetracycline 250 mg/kg body weight/day	5	–	6
Chloramphenicol-therapeutic dose series	3 3	Chloramphenicol 25 mg/kg body weight/day	10 20	–	11 21
Chloramphenicol-over dose series	3	Chloramphenicol 250 mg/kg body weight/day	5	–	6
Chloramphenicol-therapeutic dose pancreozymin series	12	Chloramphenicol 25 mg/kg body weight	20	–	21
Chloramphenicol-overdose pancreozymin series	12	Chloramphenicol 250 mg/kg body weight/day	5	+	6
Pancreozymin series	12		–	+	6
Control series	6		–	+	6

Pancreatic tissues for electron microscopy were removed immediately after sacrifice, cut into small pieces and immersed in chilled 1% osmium tetroxide solution buffered with veronal acetate to pH 7.2. After fixation for two hours at 4°C, the specimens were dehydrated with ethanol within one hour and were embedded in n-butyl-metacrylate and styrene with 2% benzoyl peroxide. Sections were cut on a microtome JUM (Nippon Densi Co.) with glass knives and stained with 1% solution of uranyl acetate in 50% ethanol. The stained sections were examined with an electron microscope JEM 5 G. (Nippon Densi Co.). Electron micrographs were taken at original magnifications of 3,000-50,000 and subsequently enlarged photographically. Tissues for light microscopy were fixed in 10% phosphate-buffered formalin solution and embedded in paraffin. The sections prepared were stained with hematoxylin and eosin, and partly with PAS technique.

OBSERVATIONS

Control series

Acinar cavities were filled with homogeneous substances similar in density to zymogen granules. Some were empty (Fig. 1).

Acinar cells: The apical surfaces formed a varying number of finger-like microvilli which were on an average 0.10 μ in length and 0.5 μ in maximum (Fig. 2). The basal surfaces ran fairly straightly. Immediately below them, there were moderately dense basement membranes which were 150 to 400 Å. The lateral surface was relatively smooth. Interdigitations and terminal bars

were observed here.

Zymogen granules were accumulated in the apical zone and some were seen immediately beneath the apical plasma membrane. The zymogen granules were homogeneous dense round granules and were 0.3 to 1.0 μ in diameter. They had a distinct single membrane.

The endoplasmic reticulums were found in the basal zone and consisted of many cisternae, lamellae and tubes. They were associated with numerous RNP particles which have been believed to be exceedingly abundant in ribonucleic acid[35] (Fig. 3). The membranes of reticulum had almost the same thickness (60 to 80 Å) as that of the plasma membrane and nuclear membrane. Large dense granules which corresponded to the intracisternal granules of Palade[23] were found in the cisternae (Fig. 4).

The mitochondria were randomly distributed. Their double membranes were 5 to 8 mμ in thickness. The inner and outer layers were respectively about 70 Å. Their matrix contained sometimes granules which were denser than the surrounding cytoplasmic matrix. The cristae mitochondriales were in parallel and rectangular to the long axis of each mitochondrion.

The Golgi complexes were found in the supranuclear zone. Their lamellae were composed of paired membranes, each of them being 70 Å in thickness and the interspaces were also 70 Å. The vesicles had different sizes, forms and density. They were 40 Å in diameter and almost as large as zymogen granules. The vacuoles were more dilated sacs, some of which contained clear or slightly flocculent materials (Fig. 5).

The nuclei were found in the basal zone and usually round. The nuclear envelopes were a double membrane of 250 Å thickness. The inner layer was thicker than the outer. The outer layer sometimes projected towards the cytoplasm

Experimental series

Chloramphenicol- and tetracycline-therapeutic dose series:

No significant changes took place in these series. The rough-surfaced endoplasmic reticulums remained almost unchanged, but segmental dilatation of the cisternae and slight scattering away of RNP particles from the reticulum surface occurred. Intracisternal granules remained almost unchanged but were slightly reduced in number and size (Fig. 6).

Mature zymogen granules were seen in the apex and immature zymogen granules near the nucleus. Most of the mitochondria were of normal size and appearance, but some were swollen, some of the cristae mitochondriales lost their original parallel arrangement (Fig. 7). The Golgi complex remained almost normal and intact. The nuclei were almost intact. Centroacinar cells did not show any change (Fig. 8).

Chloramphenicol- and tetracycline-overdose series:

Different significant changes occurred in some organellae. The rough-surfaced endoplasmic reticulums were decreased in extent and were in fragmentation and vesiculation. The distribution of RNP particles upon the reticulum surface became sparse. The RNP particles scattered away were often found in the neighborhood of the reticulums (Fig. 9). Intracisternal granules were markedly decreased in number and size (Fig. 10). Zymogen granules also decreased in number and size in the apical zone. Some were vacuolated. Others were almost as large as those of the control but inhomogeneous. Still others contained slightly flocculent materials at their centers (Fig. 11). The Golgi complexes showed no significant changes but became somewhat dilated (Fig. 12). The mitochondria did not show any conspicuous changes but some of them indicated an interesting picture. A number of the mitochondria surrounded a large vacuole completely or incompletely (Fig. 13 and 14). Further, a small number of them appeared to fuse with each other and form a single structure which encircled completely the large vacuole. In the structure, many long cristae were found arranged radially around the central vacuole and connected with each other forming a meshwork.

The central vacuole was round but sometimes irregularly serrated in its outline and was sometimes empty and sometimes contained slightly flocculent material or amorphous osmiophilic substance. The central vacuole was feebly stained with PAS technique.

Strongly dense crystal-like bodies were seen near the nuclei (Figs. 15 and 16).

Pancreozymin series:

Ten minutes after the injection, movement from the apical zone to the acinar cavity of zymogen granules was observed. The acinar cavity began to be filled with high electron dense material. The zymogen granules appeared homogeneous and of uniform size. Twenty and thirty minutes after the injection, a large number of zymogen granules disappeared in the apical zone while the acinar cavity was filled with dense material which corresponded to mature zymogen granules (Fig. 17). The limiting membrane of zymogen granules was often in contact with the apical plasma membrane. In the Golgi vacuoles, amorphous material which was somewhat less dense than zymogen granules was often observed (Fig. 18). Sixty minutes after the injection, a few zymogen granules only were seen in the apical zone. Amorphous dense materials were often seen in the growing Golgi vacuoles. The rough-surfaced endoplasmic reticulum became dilated and came to accumulate dense homogeneous spherical bodies—intracisternal granules—of different sizes (Fig. 19). The mitochondria showed very slight changes in their shape, size, distribution, density and internal cristae. The nuclei did not show any significant change.

Chloramphenicol-therapeutic dose pancreozymin series:

A marked discharge of zymogen granules was found at 10 to 20 minutes after the injection. At the same time, the acinar cavities were filled with materials which were slightly less dense than the zymogen granules found in the control series. Microvilli remained as many as and as large as those in the control series.

Chloramphenicol-overdose pancreozymin series:

A marked discharge of zymogen granules was not found at 10 to 20 minutes after the injection and even later (Fig. 20). The central large vacuoles surrounded by a number of mitochondria completely or incompletely or those surrouded by a single mitochondrion-like structure occurred in the same manner as that found in the two antibiotics overdose series (Fig. 21). Amorphous osmiophilic substance was also found here but not changed by pancreozymin.

DISCUSSION

By cell fractionation and C^{14} labelled leucine, Palade and Siekewitz[27)28)] postulated that digestive enzymes were synthesized by attached ribosomes in endoplasmic reticulum, transferred into its cisternae and finally packed and stored in the form of zymogen granules. According to Zamecrick *et al.*[29)30)], enzymatic and structural materials are carried on the surface of the ribosomes, to which messenger-RNA adheres in order to guide the arrangement of amino acids. The materials are released from the ribosomes and transferred across the membrane of the endoplasmic reticulum into the cisternal spaces. This may correspond to Palade's first stage[31)]. Subsequent intracellular transport and storage can be observed at the level of electron microscopy, and electron micrographs must be reflected merely as secondary, tertiary or quartery structures of primary amino acid configuration. The effect of antibiotics upon the acinar cells must be observed within the limit of this level.

The attack site of chloramphenicol has been explained as follows: chloramphenicol inhibits the transfer of amino acids from soluble RNA to ribosomal protein (Nathans *et al.*[25)]), or disturbs the formation of the inducible enzymes and indirectly alters their regulation mechanism (Sypherd[26)]). The synthesis of all proteins can be inhibited by chloramphenicol of high concentration (Aronson *et al.*[16)17)]). Chlortetracycline has been observed to depress phosphorylation in mitochondrial preparation of mammalian tissues (Loomis[19)]) or to inhibit oxidation (Van Meter *et al.*[20)]). Further, chlortetracycline has been reported to affect the RNA metabolism and oxytetracycline the DNA metabolism.

The ribosomes are the morphological units of protein synthesis, which link into the molecular biology, while the role of the endoplasmic reticulum membrane has been not definititely elucidated[29)31)]. Some authors postulated that

the endoplasmic cisternae were a dynamic circulatory system serving as an initial site of enzymatie protein synthesis and storage of secretory products[1)32)]. However, some protein precursors must be incorporated through another way into the protein skeleton of the endoplasmic reticulum itself. From the observations of its high susceptibility in ethionine intoxication, Ekholm[6)] explained that synthesis of structural proteins—replacement of amino acids in their peptide chains—appeared to be rapid in the endoplasmic reticulum. Slight and serious changes of the endoplasmic reticulum observed respectively in the two antibiotics therapeutic dose and overdose series may support his explantation (Fig. 9).

Palade *et al.*[41)] demonstrated biochemically that dense granules of the endoplasmic reticulum—intracisternal granules—contained amylase, trypsin and ribonuclease in a high concentration comparable with that in zymogen granules and concluded that the intracisternal granules meant rapid segregation of enzymatic protein without passing through the Golgi complex. The intracisternal granules are demonstrated in all of the antibiotics series but found decreased in number and size and smaller than those reported by Palade[23)], especially observed least in the two antibiotics overdose series (Fig. 10). The intracisteranal granules decreased here may be interpreted to be in the first disturbed stage of the pancreatic secretion cycle.

Protease and ribonuclease have been observed to be abundantly distributed and of high concentration in zymogen granules (Palade *et al.*[41)42)]). This is comparable with the result examined by cell fractination of Hokin[34)] and that examined by fluorescent-labeled antibody method of Marschall[36)], and supports Heidenhain's concept that zymogen granules contain future enzymes—enzyme precursors of pancreatic juice. The comparison of chromatographies of bovine pancreatic juice[37)] and zymogen granule preaparation[38)39)40)] also supports this. Hokin *et al.*[33)] postulated two different possibilities of secretion following ribosomal protein synthesis in fasted animals, one to pass through zymogen granules and another in a soluble form invisible by electron microscopy. Indeed, 60% of chymotrypsinogen in whole homogenate is contained in zymogen granule fraction and only a negligible portion in the final supernatant[41)]. The marked decrease of zymogen granules found in the two antibiotics overdose series seems to depend upon the change of the equilibrium between the two secretory pathways or upon the decrease of digestive enzymes during the normal equilibrium. This decrease, further together with affected changes of zymogen granules is fairly consistent with the decrease of amylase and lipase in the homogenates of experimentally injured pancreatic tissue observed in our laboratory[49)]. The inhomogeneous zymogen granules observed here may be the granules of incompletely synthesized labile proteins affected by the two overdosed antibiotics and be comparable with those found in the β-3-TA pancreas[10)].

The vacuoles found in the apical cytoplasm of acinar cells has been differently explained. Many authors have considered it to be autolysis[43)44)45)46)] following rupture of zymogen granules. However, the two antibiotics overdose series did not show any rupture of zymogen granules in acinar cells. Furthermore, our laboratory[49)] revealed that zymogen granules were absent in sequestered areas and strongly resistent against homogenization.

The vacuoles, on the other hand, may be interpreted to be cast-off skins of zymogen granules which left their membrane after discharge of their content[31)]. However, electron-lucid vacuoles found deep below the apical plasma membrane remain undetermined whether they are, pathologically, incompletely formed zymogen granules or cast-off membranes.

Several investigators[3)6)7)8)10)11)] have observed the change of the Golgi complex of acinar cells exposed to protein synthesis inhibitors. Hruban[10)] found the slight hyperplasia of the Golgi materials in the β-3-TA pancreas and said that the formation of secretory protein was inhibited to a greater extent than the synthesis of lipoprotein. Others[10)11)] stated that two kinds of synthesis were simultaneously inhibited by a large amount of the inhibitors. In the ethionine pancreas, Ekholm[6)] indicated lesser susceptibility of the Golgi materials which did not participate directly in enzyme synthesis. Haguenau and Bernhard[47)] reported that the Golgi complex of tumor cells of high elevated pathological protein synthesis was not different in appearence from that of normal cells except for its slight hypertrophy. From the observations of the slight dilatation only, it is presumed that the resistance against abnormalities of protein synthesis exposed to the two overdosed antibiotics may be to exist in the Golgi complex.

The mitochondria of acinar cells have been understood to take no direct role in forming secretory substances but rather work indirectly as a supplier of energy necessary for protein synthesis and possibly for its transport. The fact that the mitochondrial fraction has an enzymatic activity and radioactive chymotrypsinogen after *in vivo* labelling[22)] are explained by contamination with microsomes and zymogen granules. Ekholm[6)] suggested in the ethionine pancreas that the disturbance of protein synthesis induced a change of permeability to alter osmotic conditions of the surface membrane of the mitochondria. On the contrary, Herman *et al.*[7)8)] observed no significant changes of the mitochondria in the ethionine pancreas and non-protein-feeding pancreas, and Hruban[10)] also in β-3-TA pancreas. On the other hand, Loomis[19)] and van Meter *et al.*[20)] reported that chlortetracycline of considerably high concentration depressed oxidative phosphorylation in the mitochondrial preparation of acinar cells. Our laboratory indicated that the mitochondria showed no significant changes in succinic dehydrogenase activity as well as in electron micrographs after experimental charges[49)]. This supports that the mitochondria is insusceptible to the two antibiotics or reveals that the concentration of these antitibiotics is not enough.

Palade[48] observed lipid droplets encircled by mitochondria in the acinar cells of starved guinea pigs. The vacuoles surrounded completely or incompletely by mitochondria or encircled by a single mitochondrion-like structures found in the two antibiotics overdos series correspond to the lipid droplets of Palade (Figs. 13 and 14). A gradual transition from the surrounding mitochondria to the single structure is well demonstrated in the chloramphenicol overdose pancreozymin series (Fig. 21). Palade explained lipid droplets of this complex to be reserved fat oxidized for energy source in connection with fat oxidative enzymes contained in the mitochondria. Similar findings have been reported in rats[12)64)]. The vacuoles observed here also contain amorphous osmiophilic material which corresponds to Palade's droplets. It may be said that the content of the vacuoles is of lipid nature and the mitochondria and mitochondrion-like structure oxidize fat in the vacuoles. Lipid droplets have been known to be produced by ethionine and other amino acids which inhibit protein synthesis[7)43)]. It is supposed that the osmiophilic material contained is lipid formed due to protein synthesis disturbed by the two antibiotics. The PAS positive staining of these vacuoles suggests a possibility of the presence of mucosaccharides, which may be also related to the disturbed protein synthesis.

Dalay *et al.*[50] reported that DNA or RNA content and total protein of the nuclei of acinar cells remained unchanged during secretion. By radioautography, Palade *et al.*[31)63)] suggested that the direct participation of the nuclei in producing digestive enzymes was of little importance. All of the antibiotics experimental series confirmed this and revealed that nuclear material of acinar cells was not affected by the two antibiotics.

Pancreozymin brings about considerable reduction of zymogen granules in the apical zone and accumulation of dense material in the acinar cavity. This means accelerated secretion of acinar cells. The chromatographic similarity of zymogen granules and pancreatic juice provides a biochemical background for this finding[38)39)]. Furthermore, this reduction of zymogen granules is consistent with the decrease of enzymatic activity (trypsin, amylase and lipase) in the homogenate of pancreatic tissue after the pancreozymin injection[49)].

Pancreozymin has been believed to participate in transferring digestive enzymes across the acinar cell membrane but not directly in enzymatic synthesis[54)]. In fact, the pancreozymin series does not induce any significant changes of the rough-surfaced endoplasmic reticulum and nuclei, but the increased release of zymogen granules to stimulate protein synthesis indirectly. Watanabe[2)] reported that the protein synthesis began again 4 hours after the release of zymogen granules. Pancreozymin has been found to induce a transient vasodilation[55)] and an increased blood flow in the pancreas[56)]. This is not observed within 60 minutes in the pancreozymin series. The frequent occurrence of modified zymogen granules and large vesicles communicating with the acinar cavity (Aermodsson[53)]) is not observed in the pancreozymin series.

On the other hand, the pancreozymin series showed the increase in number and size of vacuoles containing amorphous osmiophilic material in the Golgi complex. Generally, the secretory process of the acinar cells, although active participation of the Golgi complex remainsundetermined, has been understood as follows: Secretory proteins formed in the rough-surfaced endoplasmic reticulum, losing RNP particles, move to the Golgi region and make an initial stage of zymogen granules here[57)58)59)]. Radioautographic studies showed that the rough-surfaced endoplasmic reticulum segregated and condensed secretory material only in hindered transport from here to the Golgi complex[31)60)61)63)], while the observations of hypertrophy of Golgi materials in acinar cells stimulated with secretin (Suzuki[4)]) suggested a possibility that digestive enzymes might be synthesized not only in the rough-surfaced endoplasmic reticulum but also in the Golgi region in the abnormal conditions. The change observed in the pancreozymin series may support the active participation of the Golgi complex. The chloramphenicol overdose pancreozymin series showed no marked release of zymogen granules into the acinar cavity. This may suggest that pancreozymin can not repair the rough-surfaced endoplasmic reticulum of enzymatic protein synthesis disturbed by overdosed chloramphenicol and takes an indirect role in this kind of synthesis.

ACKNOWLEDGEMENT

The author is deeply grateful to Professor Dr. Shingo Aoyama, Professor Dr. Shooichi Sugiyama, Dr. T. Suchi and Dr. H. Kato for their guidance throughout the study and for their criticism in the preparation of the manuscript.

REFERENCES

1. Palay, S. L. The morphology of secretion. in "Frontier in Cytology". Editor S. L. Palay., New Haven, Yale Univ. Press. 305, 1958.
2. Watanabe, Y. and Arakawa, K. Secretion and endoplasmic reticulum. *Symp. Cellular Chem.* **13**: 287, 1963 (in Japanese).
3. Weiss, J. W. Intracellular changes due to neutral red as revealed in the pancreas and kindeny of the mouse as revealed by the electron-microscopy. *J. Exp. Med.* **101**: 213, 1955.
4. Suzuki, I. Ultrastructure of pancreas acinar cell. *Jap. J. Gastroent.* **56**: 85, 1959 (in Japanese).
5. Kurosumi, K. Electron microscopic analysis of secretion mechanism. *Int. Rev. Cytol.* **11**: 1, 1961.
6. Ekholm, R., Edlund, Y. and Zelander, T. The ultrastructure of the exocrine pancreas after brief ethionine exposure. *J. Ultrastruct. Res.* **7**: 102, 1962.
7. Herman, L. and Fitzgerald, P. J. The degenerative changes in pancreas acinar cells caused by dl-ethionine. *J. Cell Biol.* **12**: 277, 1962.
8. Herman, L. and Fitzgerald, P. J. Restitution of pancreatic acinar cells following ethionine. *ibid.* **12**: 297, 1962.
9. Watanabe, Y. Electron microscopical observation of rat pancreas acinar cell following dl-ethionine. *J. Electro.* 8: 150, 1959.

10. Hruban, Z., Swift, H. and Wissler, R. W. Effect or β-3-thienylalanine on the formation of zymogen granule of pancreas. *J. Ultrastruct. Res.* **7**: 359, 1962.
11. Hruban, Z., Swift, H. and Wissler, R. W. Analog-induced incubation in pancreas acinar cells. *ibid.* **7**: 273, 1962.
12. Weisblum, B., Herman, L. and Fitzgerald, P. J. Changes in pancreatic acinar cells during protein deviation. *J. Cell. Biol.* **12**: 313, 1962.
13. Hopps, H. E., Wisseman, C. L., Hahn, F. E., Hayes, C. L. and Smadel, J. E. Mode of action of chloramphenicol. 6. Relation between structure and activity in chloramphenicol series. *Antibiot. Chemother.* **6**: 531, 1956.
14. Gale, E. F., and Folkes, J. P. The assimilation of amino-acids by bacteria. 15. Actions of antibiotics on nucleic acid and protein synthesis in staphylococcus aureus. *J. Biochem.* **53**: 493, 1953.
15. Tanaka, N. Ribonucleic acids and antibiotics. *Sogo-igaku.* **20**: 867, 1963 (in Japanese).
16. Aronson, A. I. and Spiegelman, S. Protein and ribonucleic acid synthesis in a chloramphenicol-inhibited system. *Biochim. Biophys. Acta.* **35**: 70, 1961.
17. Aronson, A. I. and Spiegelman, S. On the nature of the ribonucleic acid synthesised in the presence of chloramphenicol. *ibid.* **53**: 84, 1961.
18. Miura, Y. Attack points of antibiotics. *Naika* **11**: 819, 1963 (in Japanese).
19. Loomis, W. F. On the mechanism of action of aureomycin. *Science* **111**: 474, 1950.
20. Van Meter, S. C., Spector, A., Olson, J. J. and Williams, J. H. Efect of aureomycin on the respiration of normal liver homogenates. *Proc. Soc. Exp. Biol. Med.* **81**: 215, 1952.
21. Miura, Y., Nakamura, Y., Yoshizawa, Y. and Matsudaira, H. Comparative studies on the phosPhorus metabolism of staphylococcus in the presence of chlortetracycline and oxyteracycline. *Antibiot. Chemother.* **3**: 822, 1953.
22. Straub, F. B., Ulmann, A. and Venetinaner, P. Ribonucleic acid and the formation of amylase in cell free preparation from pigeon pancreas. *Biochim. Biophys. Acta.* **43**: 152, 1960.
23. Palade, G. E. Intracisternal granules in the exocrine pancreas cells. *J. Biophys. Biochem. Cytol.* **1**: 59, 1955.
24. Palade, G. F. A small particulate component of the cytoplasma. *ibid.* **1**: 59, 1955.
25. Nathans, D. and Lipmann, F. Amino-acid transfer from aminoacyl ribonucleic acids to protein on ribosomes of escherichia coli. *Proc. Nat. Acad. Sci. U.S.A.* **47**: 497, 1961.
26. Sypherd, P. S., Strauss, N. and Treffer, H. P. The prefferential inhibition by chloramphenicol of induced enzyme synthesis. *Biocem. Biophys. Res. Commun.* **7**: 477, 1962.
27. Palade, G. E. and Siekewitz, P. A cytochemical study on the pancreas of the guinea pig. 3. *In vivo* incorporation of leucine-1-C^{14} in to the proteins of cell fraction. *J. Biophys. Biochem. Cytol.* **4**: 557, 1958.
28. Palade, G. E. and Siekewitz, P. Microsomal ribonucleoprotein particles as a site of protein synthesis. *Fed. Proc.* **18**: 324, 1958.
29. Zamecnik, P. C. Historical and current aspects of protein synthesis. *Harvey Lect.* **54**: 250, 1960.
30. Hoagland, M. B. The relationship of nucleic acid and protein synthesis as revealed by studies in cell-free systems. *in* The nucleic acids. Vol. 3, 349, 1960.
31. Palade, G. E., Siekewitz, F. and Caro, L. G. Structure, chemistry and function of the pancreatic exocrine cells. in "Ciba Found. Symp. Exocrine Pancreas", 23, 1962.
32. Policard, A. La RNA cytoplasmique, les ribosomes et la synthèse des proteins. in "Cellules vivantes et populations cellulaires" 92, Masson, Paris, 1964.
33. Hokin, L. E. and Hokin, M. R. The synthesis and secretion of digestive enzymes by pancreas as tissue *in vitro*. in "Ciba. Found. Symp. Exocrine Pancreas". 186, 1962.
34. Hokin, L. E. Isolation of the zymogen granules of dog pancreas and their properties. *Biochim. Biophys. Acta.* **18**: 379, 1955.

35. Marshall, J. M. Distribution of chymotrypsinogen, procarboxypeptidase, desoxyribonuclease and ribonuclease in bovine pancreas. *Exp. Cell. Res.* **6**: 240, 1954.
36. Heidenhain, R. Beiträge zur Kentniss der Pancreas. *Arch. ges. Phisiol.* **10**: 557, 1875.
37. Keller, P. J., Cohen, E. and Neurath, H. Proteins of bovine pancreatic juice. Rates of synthesis of the cathionic proteins. *J. Biol. Chem.* **234**: 311, 1959.
38. Greene, L. J., Hirs, C. H. W. and Palade, G. E. On the protein composition of bovine pancreatic zymogen granules. *ibid.* **238**: 2054, 1963.
39. Keller, P. J., Cohen, E. and Neurath, H. Enzymic composition of some cell fractions of bovine pancreas. *ibid.* **236**: 1404, 1961.
40. Neurath, H. Proteolytic enzymes of pancreas. in "Ciba Found. Symp. Exocrine Pancreas". 67, 1962.
41. Palade, G. E. and Siekewitz, P. A cytochemical study on the pancreas of the guinea pig. 1. Isolation and enzyme activities of cell fractions. *J. Biophys. Biochem. Cytol.* **4**: 203, 1953.
42. Palade, G. E. and Siekewitz, P. A cytochemical study on the pancreas of the guinea pig. 2. Functional variations in the enzymatic activity of microsomes. *ibid.* **4**: 309, 1953.
43. Rendi, R. Intracellular modifications in the pancreas during secretion. *Nature* **179**: 1196, 1957.
44. Leblond, C. P. and Sergeyeva, M. A. Vacuolisation of the acinar cells in the pancreas of the rat after treatment with thiroxine or acetylcholine. *Anat. Rec.* **90**: 235, 1944.
45. Van Holtzer, R. L. and Lanker, J. L. Tissue fractination studies on mouse pancreas. Intracellular distribution of nitorogen, desoxyribonucleic acid, ribonucleic acid, amylase, acid phosphatase, desoxyribonuclease and cytochrome oxidase. *J. Biol. Chem.* **234**: 2359, 1959.
46. Takagi, F., Suzuki, T. and Yasuda, H. Electron microscopic cytohistopathology. 1. Studies of autolysis changes of ultracell structures. *Jikeikai Med. J.* **2**: 21, 1955.
47. Haguenau, F. and Bernhard, W. J. L'appareil de Golgi dans les cellules normales et cancéreuses de vertébres. *Arch. Anat. Micromorph. Exp.* **44**: 27, 1955.
48. Palade, G. E. in "Subcellular particles" (T. Hayashi, Editor), 64, Ronald Press. New York, 1958.
49. M. Fujimoto and Y. Goto. The medication of the experimental pancreatitis. *Jap. J. Castoent.* **61**: 411, 1964 (in Japanese).
50. Dalay, M. M. and Mirsky, A. E. Formation of protein in the pancreas. *J. Gen. Physiol.* **36**: 243, 1953.
51. Harper, A. A., Blair, E. L. and Scratcherd, T. The distribution and physiological properties of pancreozymin. Ciba Found. Symp. Exocrine Pancreas. 168, 1962.
52. Hunt. Cited from "Ciba Found. Symp. Exocrine Pancreas". 182, 1962.
53. Aermodson, L. H. L'effet de secretin et pancreozymin dans la infrastructure de pancreas éxocrine. *J. Microscopie.* **1**: 68, 1963.
54. Hokin, L. E. and Hokin, M. R. Studies of pancreatic tissue *in vitro*. *Gastronterology* **36**: 368, 1959.
55. Holton, P. and Jones, M. Changes in blood content of cat pancreas during activity. *J. Physiol.* **150**: 479, 1960.
56. Jones, M. Cited from "Ciba Found. Symp. Exocrine Pancreas". 68, 1962.
57. Farquhar, M. G. and Welling, S. R. Electronmicroscopic evidence suggesting secretory granules formation within Golgi apparatus. *J. Biophys. Biochem. Cytol.* **3**: 319, 1957.
58. Bowen, R. H. The cytology of grandular secretion. *Quart. Rev. Biol.* **4**: 299, **4**: 484, 1929.
59. Sjöstrand, F. S. and Hanzon, V. Function of the Golgi apparatus in the exocrine pancreas cell. *Science* **134**: 1434, 1961.
60. Caro, L. G. Electron microscopic radioautography of thin section. The Golgi zone as

a site of protein concentration in pancreatic acinar cells. *J. Biophys. Biochem. Cytol.* **10**: 37, 1961.
61. Warshawsky, H., Leblond, C. P. and Droz, B. Synthesis and migration of proteins in the cells of the exocrine pancreas as revealed by specific activity determination from radioautographs. *J. Cell Biol.* **16**: 1, 1963.
62. Kurosumi, K. Golgi appratus and its role in secretory activity of gland cells. *Symp. Cellular Chem.* **13**: 309, 1963 (in Japanese).
63. Caro, L. G. and Palade, G. E. Protein synthesis, storage and discharge in the exocrine cell. An radioautographic study. *J. Cell. Biol.* **20**: 473, 1964.
64. Herman, L., Sato, T. and Fitzgerald, P. J. Pancreas. in "Electron Microscopic Anatomy", Academic Press Inc. New York, 59, 1964.

EXPLANTATION OF FIGURES

FIG. 1. Survey picture of exocrine pancreas cell of control guinea pig. ×8000

FIG. 2. The apical region of pancreatic acinar cells. The apical plasma membrane shows microvilli (MV) projecting toward the cavity. Zymogen granule (ZG). Control. ×28000

FIG. 3. The basal region of acinar cells. Cell boundaries (CB) between the exocrine pancreas acinar cells. At lower right, mitochondria (Mt) are seen. Control ×18000

FIG. 4. Large dense intracisternal granules (Ic) are seen within distended cisternae of the rough-surfaced endoplasmic reticulum. The granules are not bounded by a proper membrane, but appear as a homogenous structure which has a relatively thick outer shell. Control. ×36000

FIG. 5. The Golgi complex. Golgi vesicles (GVes) are seen. Control. ×25000

FIG. 6. Intracisternal granules (Ic) in the 20-day treatment with chloramphenicol therapeutic dose (25 mg/kg/day). Their size and distribution are slightly diminished. N (nucleus). ×17000

FIG. 7. In the 20-day treatment with chloramphenicol (25 mg/kg/day), mitochondria (Mt) are fairly rounded. The cristae lose the paralell disposition and are shorter and sparser than in control group. Their branching is also seen. Intracisternal granules (Ic) are seen surrounded by cisternae of rough-surfaced endoplasmic reticulum. ×33000.

FIG. 8. Survey picture of the apical zones of 4 acinar cells (AC) and a cetroacinar cell (CAC). In the centroacinar cell, endoplasmic reticulum are sparse and the mitochondria (Mt) are comparatively few and small. Significant changes are not seen. Same condition. ×15000.

FIG. 9. Survey picture of acinar lumen (L) and acinar cells. In the apical zones, zymogen granules (ZG) are diminished in number and vesiculation of the rough-surfaced endoplasmic reticulum are markedly seen throughout the cell body. 5-day treatment with chloramphenicol (250 mg/kg/day). ×11000.

FIG. 10. Intracisternal granules (Ic) are decreased in number and size, and are rarely seen within distended sacs of rough-surfaced endoplasmic reticulum (ER). Mt (mitochondria) 5-day treatement with chloramphenicol (250 mg/kg/day). ×13600

FIG. 11. The electron-lucent zymogen granules (arrow) are seen in the apical portion just beneath the microvilli (MV). These granules are limited by the single smooth membrane and contain electron-dense core and electron-lucent zone. 5-day treatment with chloramphenicol (250 mg/kg/day). ×22000.

FIG. 12. Survey picture of Golgi complex in acinar cell in the 5-day treatment of chloramphenicol (250 mg/kg/day). Wide extension of Golgi membrane. ×36000.

FIG. 13. A large vacuole (V) is completely encircled by a mitochondr on-like structure (Mtn). The cristae are arranged radially around the central vacuole and often branching connecting with each other. Its long and numerous cristae are posed in fusion partially and form the bifurcations but not gather in cluster. On the whole, the matrix is not vacuolated. A central vacuole is limited by a dense zig-zag membrane. CB (cellular boundary) 5-day treatment with chloramphenicol (250 mg/kg/day). ×55000.

FIG. 14. A large vacuole (V) is incompletely surrounded by a few mitochondria (Mt). The vacuole contains amorphous, osmiophilic materials and is not limited by a zig-zag membrane. The inner membrane of the mitochondria is slightly depressed and irregularly arranged. CB (cellular boundary) 5 day treatment of chloramphenicol (250 mg/kg/day). ×46000.

FIG. 15. Five dense bodies in plaque (arrow) are seen near the nucleus (N). The nuclear envelope shows a double membrane. Its interspace is partially enlarged and several nuclear pores are seen. 5-day treatment with chloramphenicol (250 mg/kg/day). ×18000.

FIG. 16. The endoplasmic reticulums (ER) with diminished RNP particles appear vesiculated within which lie lipid droplets (arrow) with an empty periphery. Same condition. ×26000.

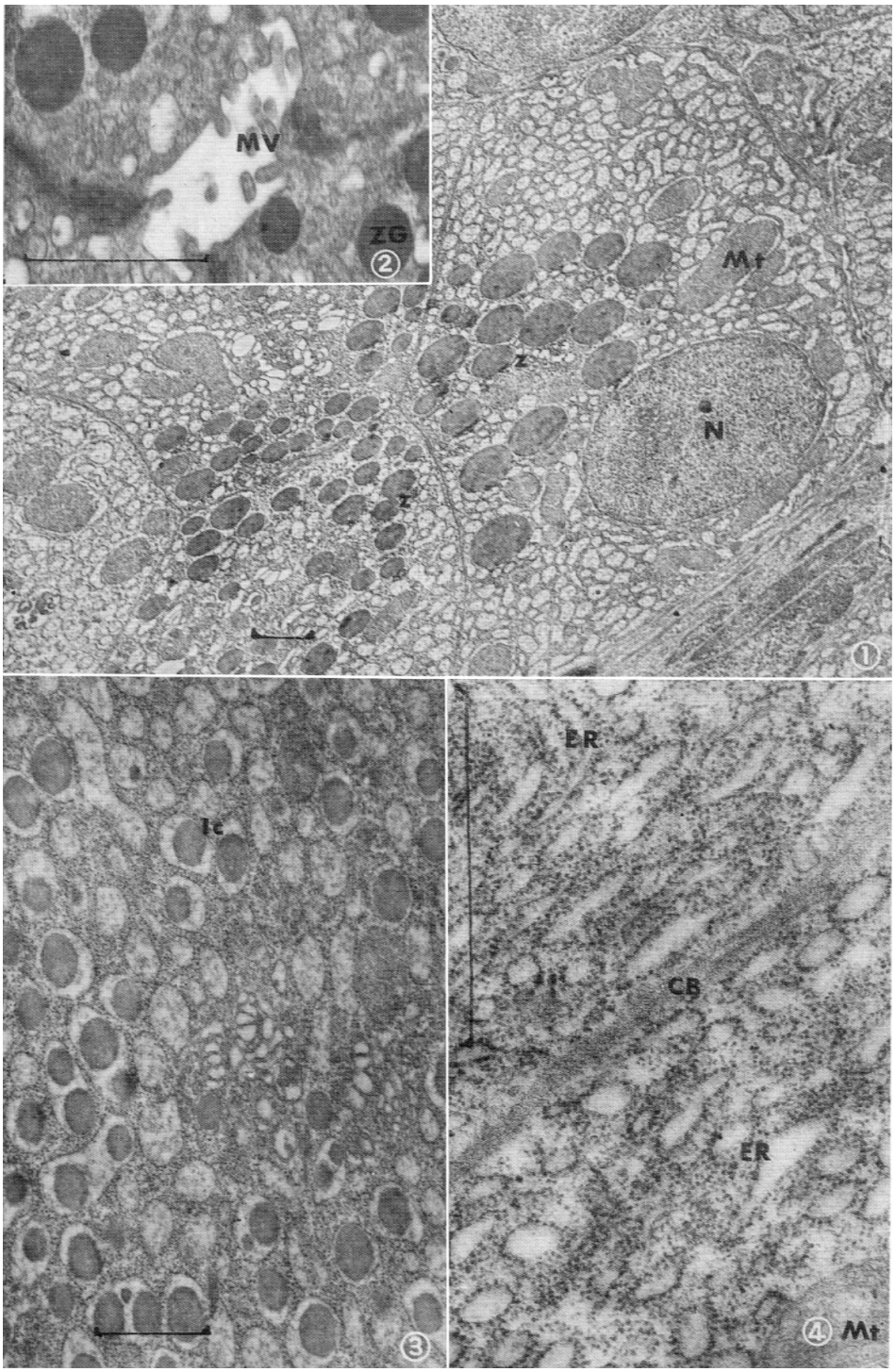
FIG. 17. An acinar lumen (L) is filled with dense material which is comparable with mature zymogen granules (ZG) and is continuous with the apical zone. 5-day feeding with balanced diet, 20 minutes after pancreozymin injection. ×10000.

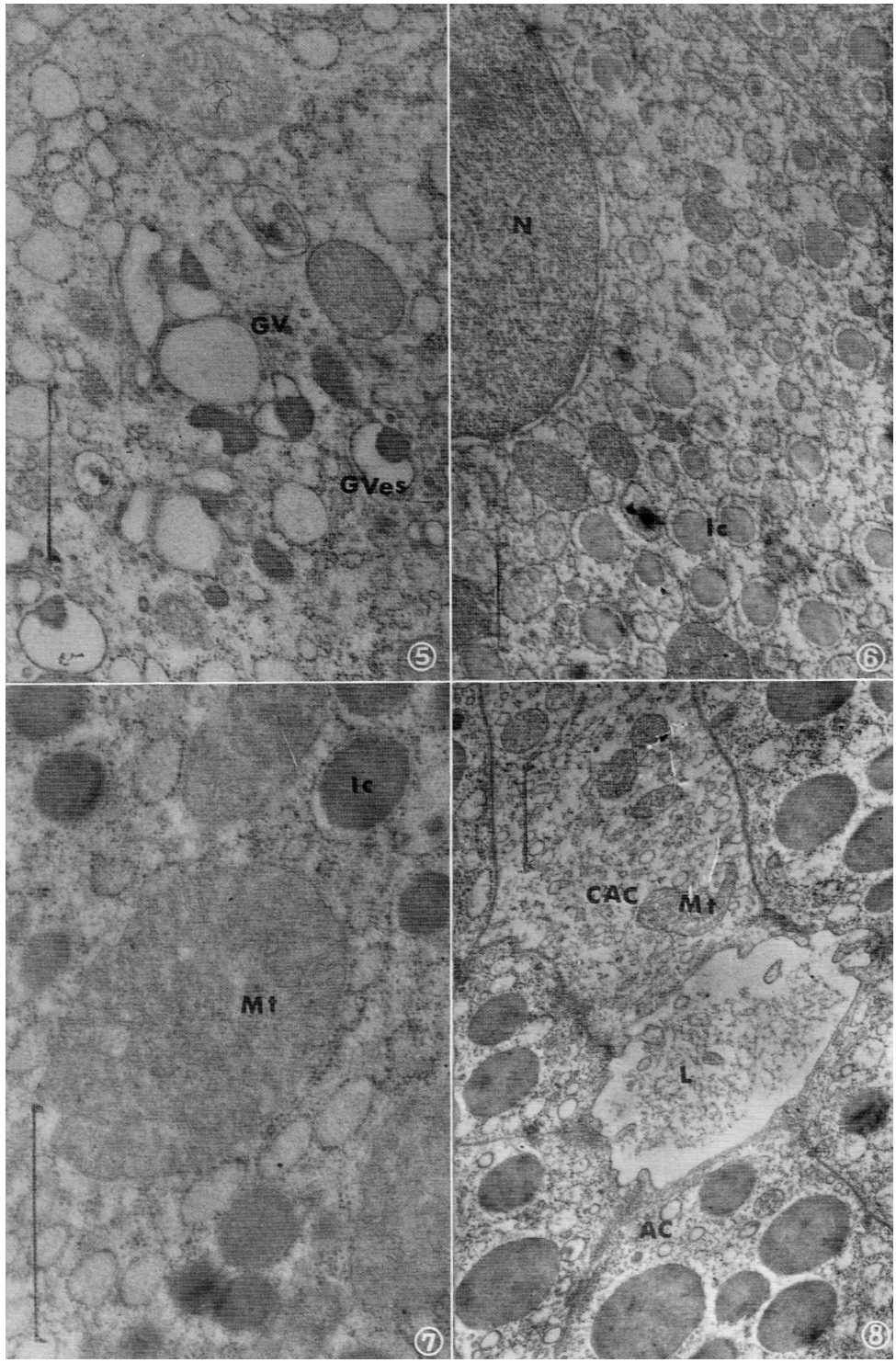
FIG. 18. The vacuole of Golgi complex are markedly dilated and contains amorphous materials (arrow). There are large mature dense zymogen granules (ZG) near the Golgi complex. 5-day feeding with balanced diet, 60 minutes after pancreozymin injection. ×26000.

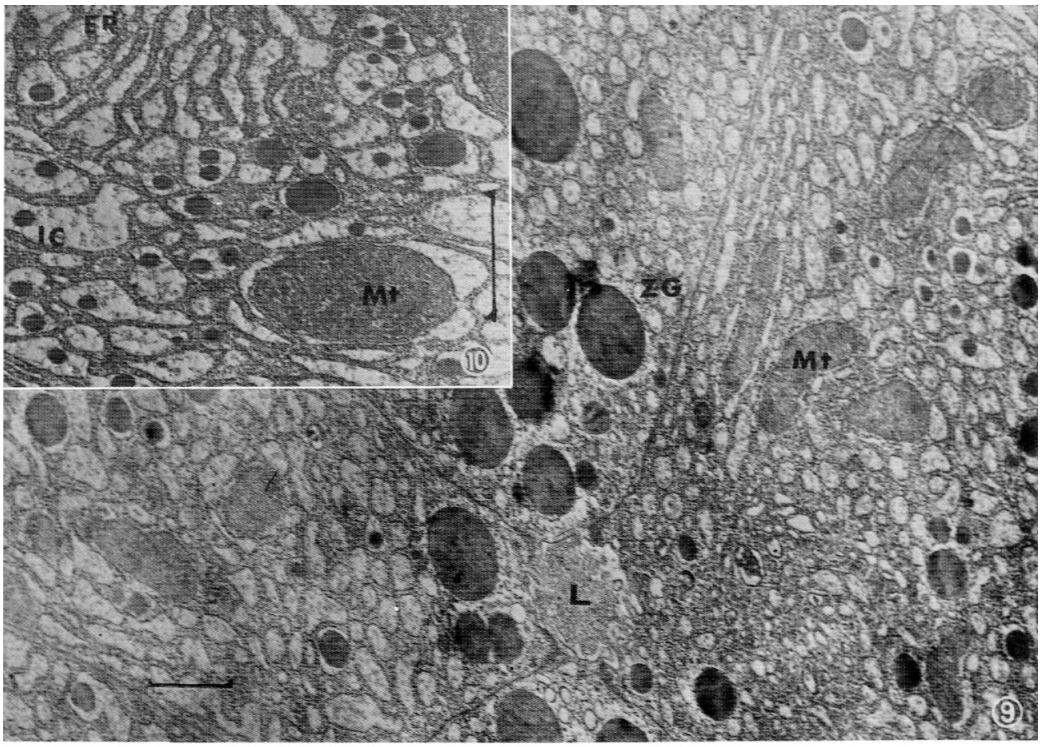
FIG. 19. Intracisternal granules (Ic) are seen in few cisternae of the rough-surfaced endoplasmic reticulum. Same administration. ×25000.

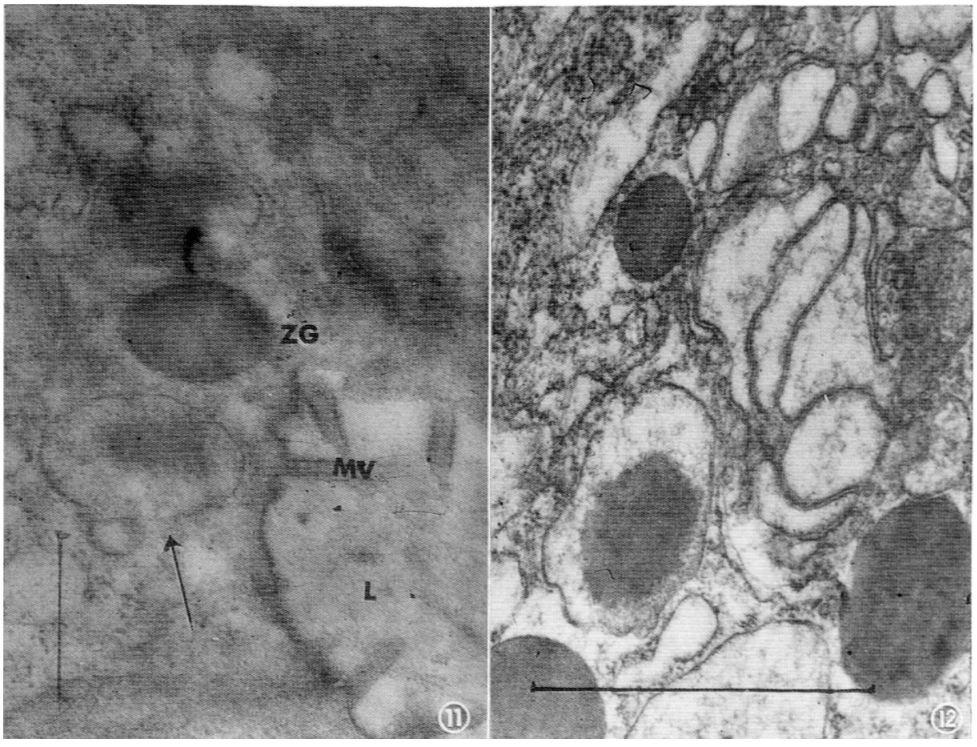
FIG. 20. The acinar lumen (L) is not filled with dense materials. 5-day treatment with chloramphenicol (250 mg/kg/day). 20 minutes after pancreozymin injection. ×14000.

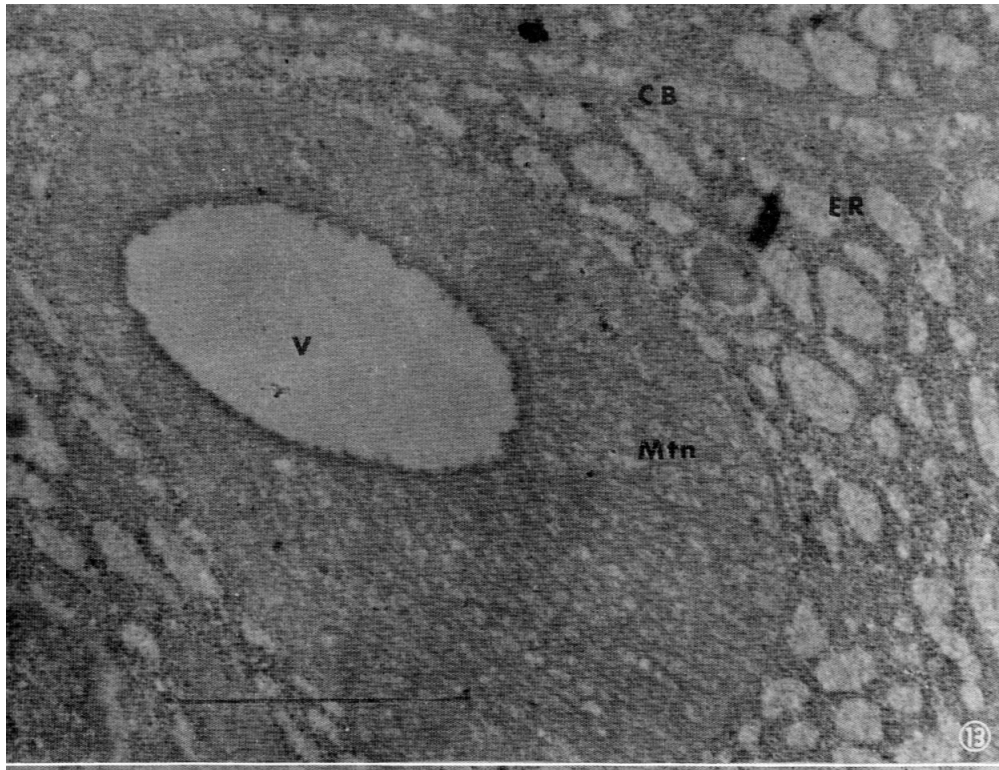
FIG. 21. Vacuole (V) encircled by mitochondrion-like structure (Mtn) do not disappear and show no significant changes. Other mitochondria (Mt) are observed. Same administration. ×30000.


MV
ZG
②
Mt
Z
N
Z
①
Ic
③
ER
CB
ER
④ Mt

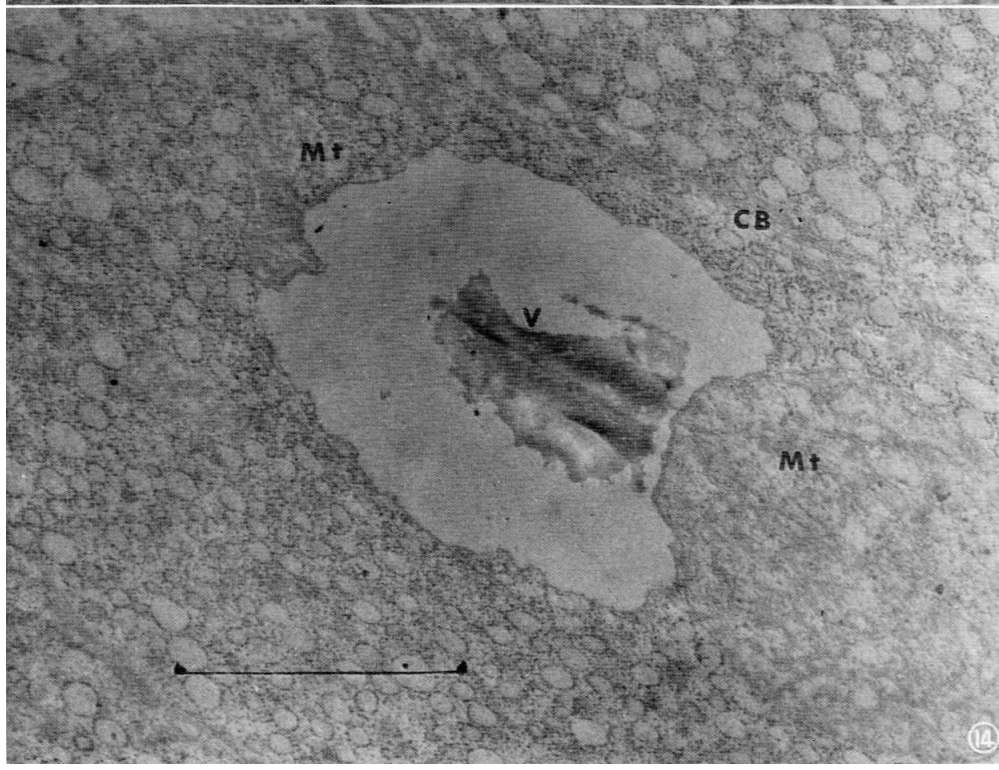

GV
GVes
⑤
N
lc
⑥
lc
Mt
⑦
CAC
Mt
L
AC
⑧


ER
IC
Mt
ZG
Mt
L
10
9

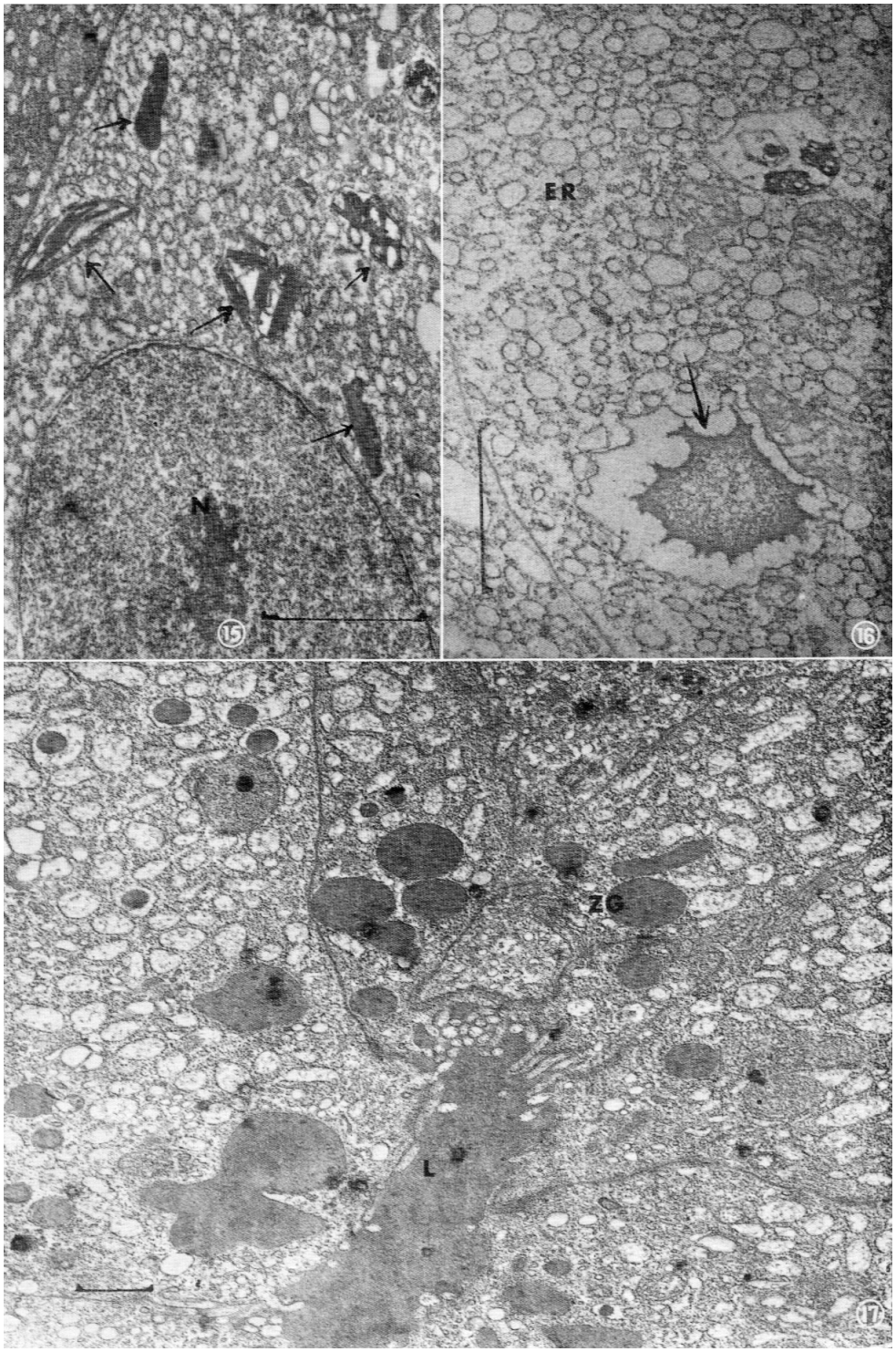

ZG
MV
L
11
12

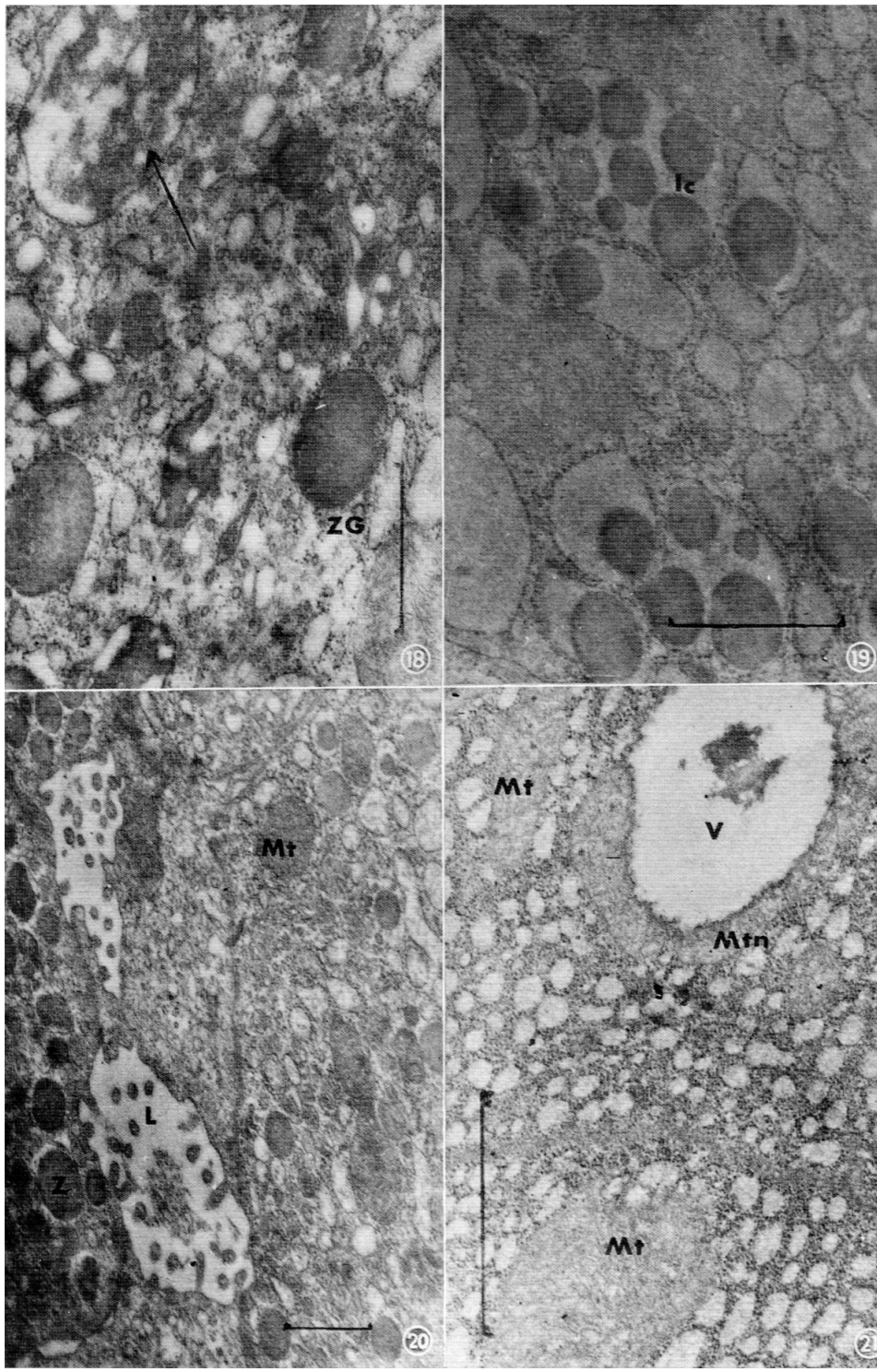


⑬



⑭


N
15
ER
16
ZG
L
17


ZG
18
lc
19
Mt
L
Z
20
Mt
V
Mtn
Mt
21