DYNAMIC CHANGES IN THE ULTRASTRUCTURE

OF THE ACINAR CELL OF THE RAT PAROTID

GLAND DURING THE SECRETORY CYCLE

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ABSTRACT

Synchronization of the secretory cycle in vivo was obtained by injecting isoprenaline as an inducer of secretion. A quantitative correlation between enzyme release, its subsequent reaccumulation, and the sequence of ultrastructural changes was found. At the ultrastructural level secretion was paralleled by depletion of zymogen granules through fusion of the granule membrane with the lumen membrane and discharge of the content. Each zymogen granule membrane, once connected with the lumen, acted as a lumen membrane. Fusion was thus sequential and resulted in a dramatic enlargement of the lumen space. During the entire process the passage between the lumen and the intercellular space remained blocked by the tight junctions, as shown by their impenetrability to ferritin. Reduction of the lumen size following enzyme discharge seemed to be achieved by withdrawal of lumen membrane in the form of small smooth vesicles which appeared mostly in the apical part of the cell. At the same time, the cell retracted towards the lumen, the whole process being completed within 2 hr from onset of secretion. Disappearance of the smooth vesicle followed, concomitant with formation of many condensing vacuoles and appearance of mature zymogen granules. The fate of the zymogen granule membrane, including its fusion with the lumen membrane, resorption in the form of small smooth vesicles, and its eventual reutilization mediated by the Golgi system, is discussed.

INTRODUCTION

It is well known that the exportable proteins in the exocrine gland cell are accumulated within membrane-bounded zymogen granules. Palade (1), Ekholm et al. (2), and Ichikawa (3), studying the pancreas in different animals by electron microscopy, observed fusion of zymogen granules with the cell membrane bordering on the lumen of the acinus. Similar findings were obtained by Parks studying the rat and mouse parotid glands (4). However, it has apparently been difficult to demonstrate a quantitative correlation between the fusion of the zymogen granule membrane with the cell membrane and the secretory activity of the gland. In order to obtain optimal conditions for testing such a correlation, it is necessary to use a system in which stimulation of secretion would result in a synchronous process in the majority of the cells. This requirement could not be met previously mainly because of lack of a suitable stimulant.

It has been shown that rat parotid slices secrete about 50% of the total exportable proteins in 40–60 min when induced by epinephrine, while there is essentially no secretion in absence of the hor-

mone (5). Evidence has also been discussed which indicates that epinephrine or norepinephrine is probably the physiological inducer of secretion acting upon release from adrenergic nerve fibers (5, 6). Byrt (7) recently showed that another catecholamine, isoprenaline, caused within 2 hr the secretion of more than 99% of the amylase which had been accumulated in the parotid gland of the rat. Synthesis of exportable protein was apparently unaffected, so that 16 hr later the glands again reached the original high level of accumulated amylase.

Because of these advantageous properties, isoprenaline was chosen as the inducer of secretion in the present work. It was expected that synchronizing the gland cells by initiating secretion with isoprenaline would also facilitate the study of subsequent stages of the secretory cycle after secretion has been terminated. There has been no definite information in morphological or biochemical terms about the fate and turnover of the zymogen granule membrane during the secretion-accumulation cycle in the gland. Before the undertaking of a detailed investigation of this problem by labeling and isolating the membranes and by electron microscopic radioautography, it seemed useful to carry out a morphological study of the whole sequence of events in vivo, starting with the onset of secretion and ending with maximal reaccumulation of exportable proteins. It was expected that such an investigation would establish the major changes in the gland cell structures and the time period during which they took place. Indeed, there was still doubt even about the first step of the secretion process in the rat parotid gland, since Rutberg (8), Scott and Pease (9), and Byrt (7) indicated that the content of the zymogen granules is discharged into the cell cytoplasm before secretion into saliva. The present work shows that when secretion is induced by isoprenaline in vivo, the lumen of the acinus becomes dramatically enlarged by progressive fusion of the cell membrane with the zymogen granule membrane. The rapid return of the lumen to its original size, the appearance of smooth vesicles, the subsequent stages of zymogen granule reformation, and the timing of these events are also described.

MATERIALS AND METHODS

Enzyme Secretion

Albino rats (160 ± 10 g) fasted overnight were used. Secretion was initiated by intraperitoneal injection of 0.5 ml of a freshly prepared 1% solution of isoprenaline (isopropylarterenol hydrochloride, Mann Research Laboratories, New York), containing also 0.15 M NaCl. Reaccumulation of amylase was allowed to proceed under continued starvation. Animals were sacrificed at different times after injection (20 min to 20 hr) by heart incision after ether anesthesia.

For the quantitative determination of amylase, special care was taken to ensure complete removal of the parotid gland. At each time point the glands from three animals were pooled and total amylase was estimated in homogenates prepared in cold potassium phosphate buffer, 20 mM, pH 6.9, containing 7 mM sodium chloride (10).

Fixation and Embedding

Tissue was fixed for electron and light microscopy with neutralized 2% glutaraldehyde (Fluka) in Ringer's solution (115.5 mM NaCl; 2 mM KCl 1.8 mM $CaCl_2$ and 2 mM sodium phosphate buffer pH 7.2). The parotid tissue was injected in situ with the fixative solution at 25°C. The gland quickly hardened, assuming a characteristic pale brown color easily distinguishable from the color of the surrounding connective tissue. The glands were carefully removed and collected in the same fixative solution. Fixation was then continued at 4°C for 2–4 hr. The aldehyde-fixed tissue was washed with several changes of Ringer's solution and again fixed at 4°C for 2–3 hr in 2% OsO_4 in Ringer's solution without $CaCl_2$ but containing 30 mM phosphate buffer.

The tissue was dehydrated by transfers to increasing concentrations of ethanol and was embedded in Epon, as described by Luft (11).

Experiments with Ferritin

Cadmium-free ferritin was prepared by centrifugation for 2 hr at 2 × 10⁵ g of an aqueous solution of 10% ferritin obtained from Pentex, Inc. Kankakee, Illinois. The pellet was suspended in 3 ml of Ringer's solution and dialyzed against two changes of 200 vol of the same solution for 22 hr at 4°C. The final concentration of ferritin was about 35% (w/v).

Ferritin penetration into the tissue was attempted both before and after fixation: (a) The glands were removed before fixation, cut into 1 mm³ pieces, shaken gently at 37°C in the ferritin solution for 20 min, transferred into the glutaraldehyde fixative at 25°C, and further processed as above. (b) The glands were fixed in glutaraldehyde at 25°C for 20 min, washed 4 times in Ringer's solutions by decantation, transferred to the ferritin solution, and shaken gently for 45 min at 37°C. The tissue was again transferred into glutaraldehyde at 25°C and further processed as already described.

Controls and stimulated glands were treated with ferritin by both procedures.

Light Microscopy and Statistical Measurements

Sections 1-2 μ thick were cut from the Epon-embedded tissue, mounted on glass slides, and photographed by phase contrast microscopy.

Measurements of different cell parameters were done on prints of phase-contrast micrographs enlarged $\times$ 1680. Only cell sections showing the nucleus and bordering on the lumen were chosen. At least 20 cells were used for measurements at each time point. Interacinar distances, measured as the minimal interval between the periphery of adjacent acini, and lumen perimeters were determined only on acini showing one or more cells sectioned through the nucleus. The perimeter of the lumen was measured with a cartographic miles counter. Cell height was measured in the radial direction from the lumen. All data are given as the mean together with the 95% confidence interval.

Electron Microscopy

Thin sections were cut on a Porter-Blum Sorvall MT-2 ultramicrotome. They were stained with uranyl acetate and lead citrate, as described by Reynolds (12). Electron micrographs were obtained with an RCA EMU 3G or a JEM-7A electron microscope.

RESULTS

Injection of isoprenaline caused within 1 hr the secretion of 96% of the amylase which had been accumulated in the gland (Fig. 1). Subsequent reaccumulation of exportable enzyme was first detected 6 hr after onset of secretion and reached 76% of the initial level after 20 hr. Amylase content in control animals after an identical fasting period (40 hr in total) was 60% of the initial value, probably due to hunger secretion (13). The pattern of amylase content after isoprenaline injection is similar to that previously reported by
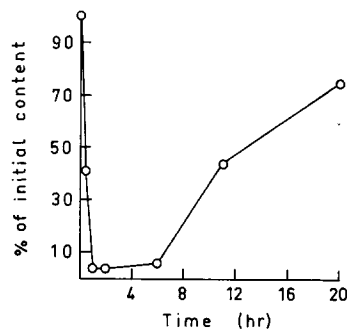

FIGURE 1 Amylase secretion from the parotid gland following injection of isoprenaline. Experimental conditions, as described in Materials and Methods.

Byrt (7). The morphological changes during the secretory cycle, as observed by phase-contrast microscopy, are shown in Fig. 2 a-f. At zero time typical acini, homogenously loaded with zymogen granules, are seen. The acinus consists of about 5 cells radially disposed around a narrow lumen which has a diameter one to two times that of a zymogen granule. The nucleus is found in the basal part of the cell. The interstitial space between acini is narrow and thus resolved only in part (Fig. 2 a). At 20 min after onset of secretion the lumen is dramatically dilated, suggesting its enlargement at the expense of the disappearing zymogen granules. The cells appear slightly enlarged, causing a further reduction in the interacinar space (Figs. 2 b and 3). At 50 min the lumen is still large and only a few zymogen granules subsist. A significant reduction in cell size has occurred, resulting in a pronounced increase of the interacinar space (Figs. 2 c and 3). At 2 hr, the size of the lumen is drastically reduced, returning almost to that found at zero time. The cells which were completely devoid of zymogen granules at this time attained their minimum size (Figs. 2 d and 3). During the next 2 hr little change was observed. Zymogen granule reaccumulation, which was detected at about 6 hr (Fig. 3 b), became evident at 11 hr (Fig. 2 e) and seemed completed at 20 hr (Figs. 2 e, f and 3 b). Throughout the process no significant changes in the nuclei were observed. The changes in average size of the cells, interacinar spaces, lumens, and number of zymogen granules during the secretory cycle are shown in Fig. 3 a-d. There is good agreement between amylase content and the number of zymogen granules (Figs. 1 and 3 b). It is also evident that there is an inverse relationship between the size of the cells and the size of the interacinar spaces (Figs. 3 c and d). The average diameter of zymogen granules increases by 20% from zero time to 50 min secretion (0.9 $\pm$ 0.1 μ to 1.1 $\pm$ 0.1 μ). Since the smaller zymogen granules were usually located closer to the lumen, they were first to be discharged, thus accounting for the apparent increase in average size of the remaining granules during secretion. At 20 hr the average diameter of the granules again approached that measured at zero time (0.8 $\pm$ 0.1 μ).

Changes in Ultrastructure

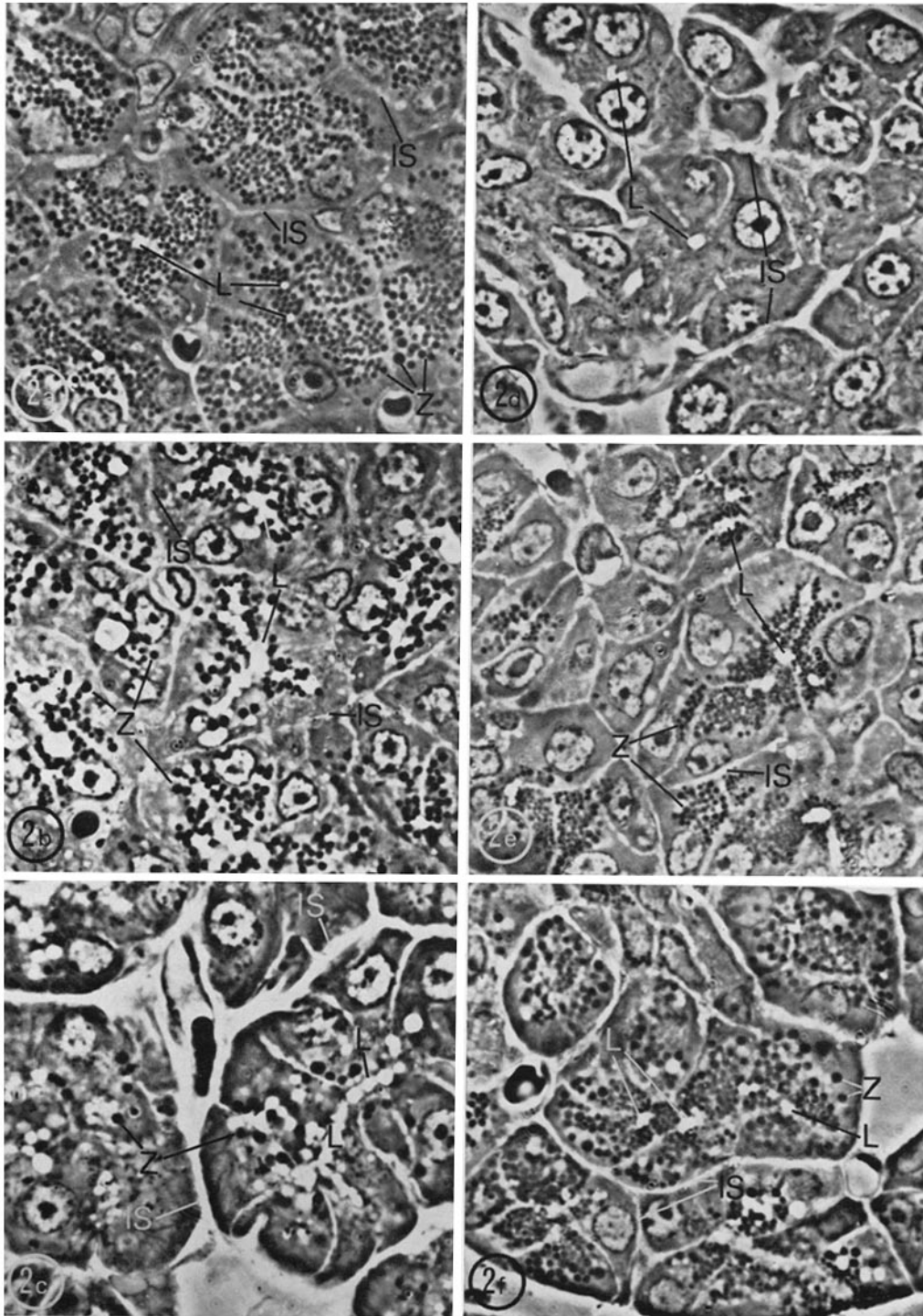The fine structure of all cellular components in the control gland was well preserved, showing the

FIGURE 2 Morphological changes in parotid acini during the secretory cycle as observed by phase-contrast microscopy. 2 a. Control glands. 2 b–f. Glands after isoprenaline injection: 20 min (b); 50 min (c); 2 hr (d); 11 hr (e); and 20 hr (f). Note the following changes: 1) a drastic increase in the size of the lumen (L) at 20 min and its complete retraction at 2 hr; 2) progressive disappearance of the zymogen granules (Z) until complete depletion at 2 hr and their reappearance and accumulation at 11 and 20 hr; 3) progressive changes in the size of the acinar cells and interacinar spaces (IS). Sections were from the same preparations used for electron microscopy. × 1280.

FIGURE 3 3 a–d. Changes occurring during the secretory cycle. 3 a. Average perimeter of the lumen. 3 b. Number of zymogen granules per cell section. 3 c. Height of the acinar cells. 3 d. Interacinar distances. The measurements were done as described in Materials and Methods.

typical appearance of the parotid exocrine cells as already described (4, 8, 9, 14–17) (Fig. 4). The lumen has an empty appearance, and the plasma membrane bordering on the lumen presents numerous microvilli (Figs. 4, 5). In ferritin-treated tissue, the intercellular clefts are loaded with ferritin molecules which are prevented from reaching the lumen by the tight junctions. Oc-

casionally, microtubules are seen to end in the fibrillar coating found under the plasma membrane at the level of the desmosomes (Fig. 5). Eventually, zymogen granule membranes were observed to be in apposition with each other or with the plasma membrane of the lumen, but no fusion occurred (Fig. 5). Injection of isoprenaline did not induce any observable changes in the

The following abbreviations are used in the explanation of the figures in the text:

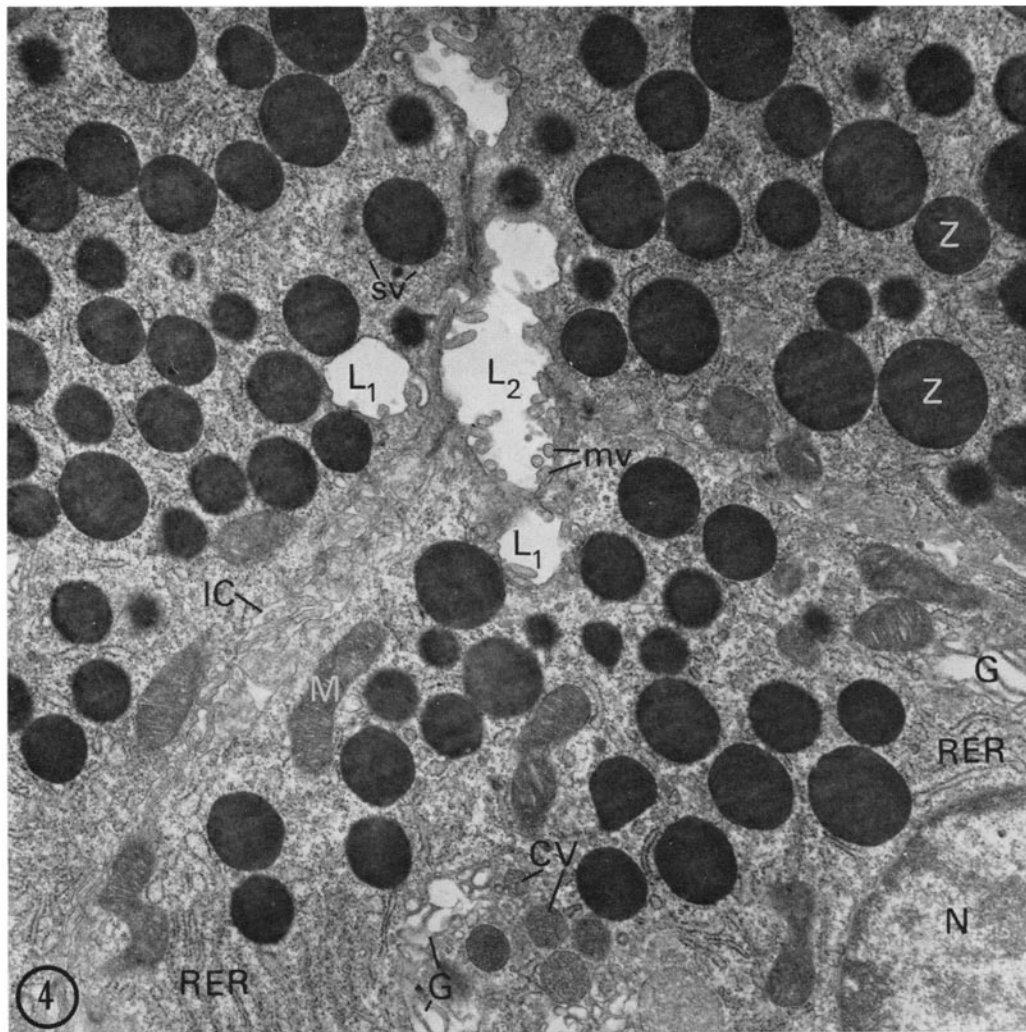|				
C	centriole		L	lumen
CV	condensing vacuole		M	mitochondrion
D	desmosome		mv	microvilli
F	ferritin		N	nucleus
G	Golgi complex		p	papillar infoldings of plasma membrane
g	ghost of zymogen granule membrane or myelin figures formed during aldehyde fixation		sv	small smooth vesicles
			rb	round body
gm	granular material		RER	rough endoplasmic reticulum
IC	intercellular cleft		T	tight junction
IS	interacinar space		Z	zymogen granule

FIGURE 4 Parotid acinar cell of a fasted rat before injection of isoprenaline. Three cells radially disposed around the lumen are loaded with zymogen granules (Z) more densely packed between nucleus (N) and the lumen (L). The contorted lumen cut transversely at L_1 and obliquely at L_2 is small and has an empty appearance. The intercellular clefts (IC) are narrow. The Golgi complex (G) with small condensing vacuoles (CV) is clearly delineated. Mitochondria (M) and rough endoplasmic reticulum (RER) are more concentrated at the basal pole of the cell. × 14,500.

structure of nuclei, mitochondria, and the rough endoplasmic reticulum. All structural changes which occurred during secretion appeared to be confined to membranes of the smooth type.

Onset of secretion was accompanied by extensive fusion of zymogen granules with the lumen, accounting for its enlarged festooned borders. Progressive stages of the fusion process can often be observed in the same cell section (Figs. 6, 8 a).

Microvilli appeared to remain confined mostly to the area of the original lumen, which can be identified by its tight junctions and demosomal barriers (Figs. 6, 7, 8 a). As compared with the empty lumen in the control gland (Figs. 4, 5), the lumen in the secreting gland contained variable amounts of a granular material, presumably secreted protein (Figs. 6, 7, 8 a). Occasionally, bits of partially damaged membranes were present in
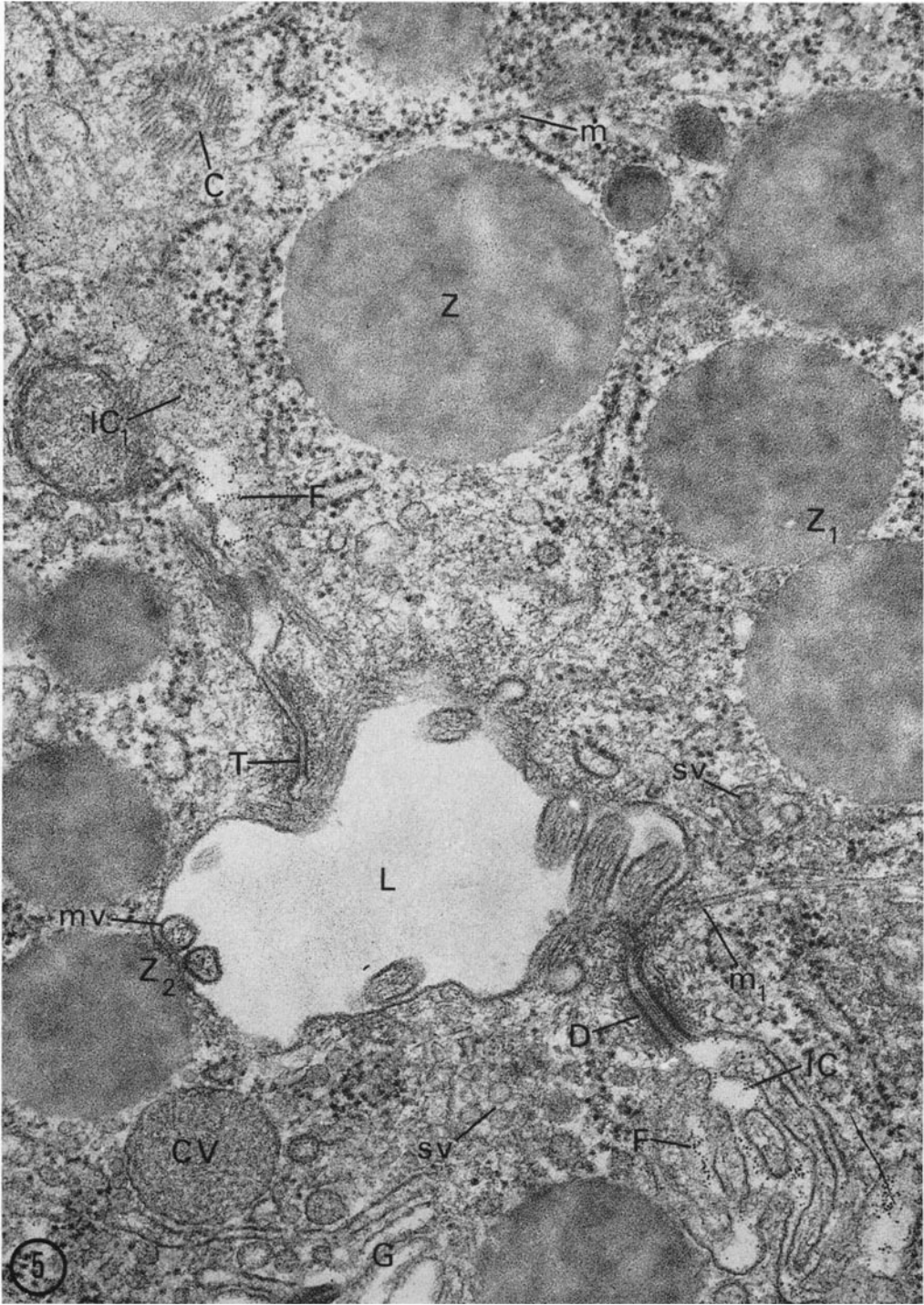
FIGURE 5 Same as in Fig. 4. The intercellular cleft (*IC*) containing ferritin molecules (*F*) is sectioned tangentially at (*IC*$_1$) and closed toward the lumen (*L*) by desmosomes (*D*) and tight junctions (*T*). Ferritin does not penetrate these barriers. The empty lumen contain numerous microvilli (*mv*). Microtubules (*m*) appear to end in the terminal web found under the plasma membrane at the level of the desmosome (*m*$_1$). A centriole is seen at (*C*). A small condensing vacuole (*CV*) and a few smooth membrane vesicles around the lumen are seen (*sv*). Membranes of zymogen granules touch each other or the lumen (*Z*$_1$, *Z*$_2$) but do not fuse. × 45,500.
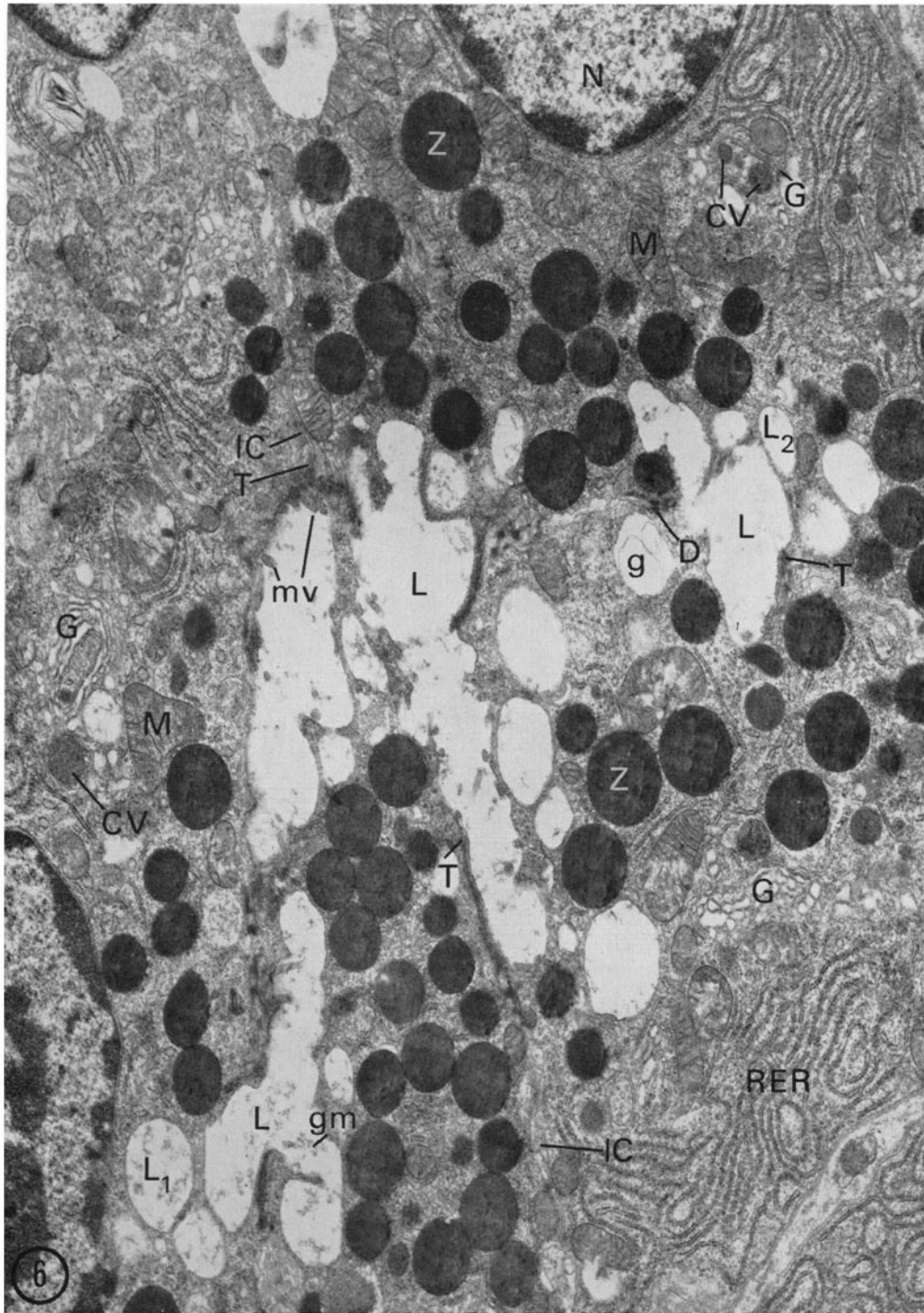
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FIGURE 6 Discharge of zymogen granule content through fusion with the lumen 20 min after isoprenaline injection. The lumen (L) has increased in size, its irregular borders indicating places of fusion with zymogen granules. The original site of the lumen can be identified by the presence of desmosomes (D) and tight junctions (T). Unsecreted zymogen granules (Z) preserve their original density. Microvilli (mv) lining the plasma membrane are still present in the vicinity of the tight junctions (T). The lumen contains various quantities of a granular material (gm). Discharged granules without apparent connection to the lumen might also be seen (L_1, L_2). The intercellular cleft (IC) is very narrow. The other cellular organelles are unchanged. × 10,000.
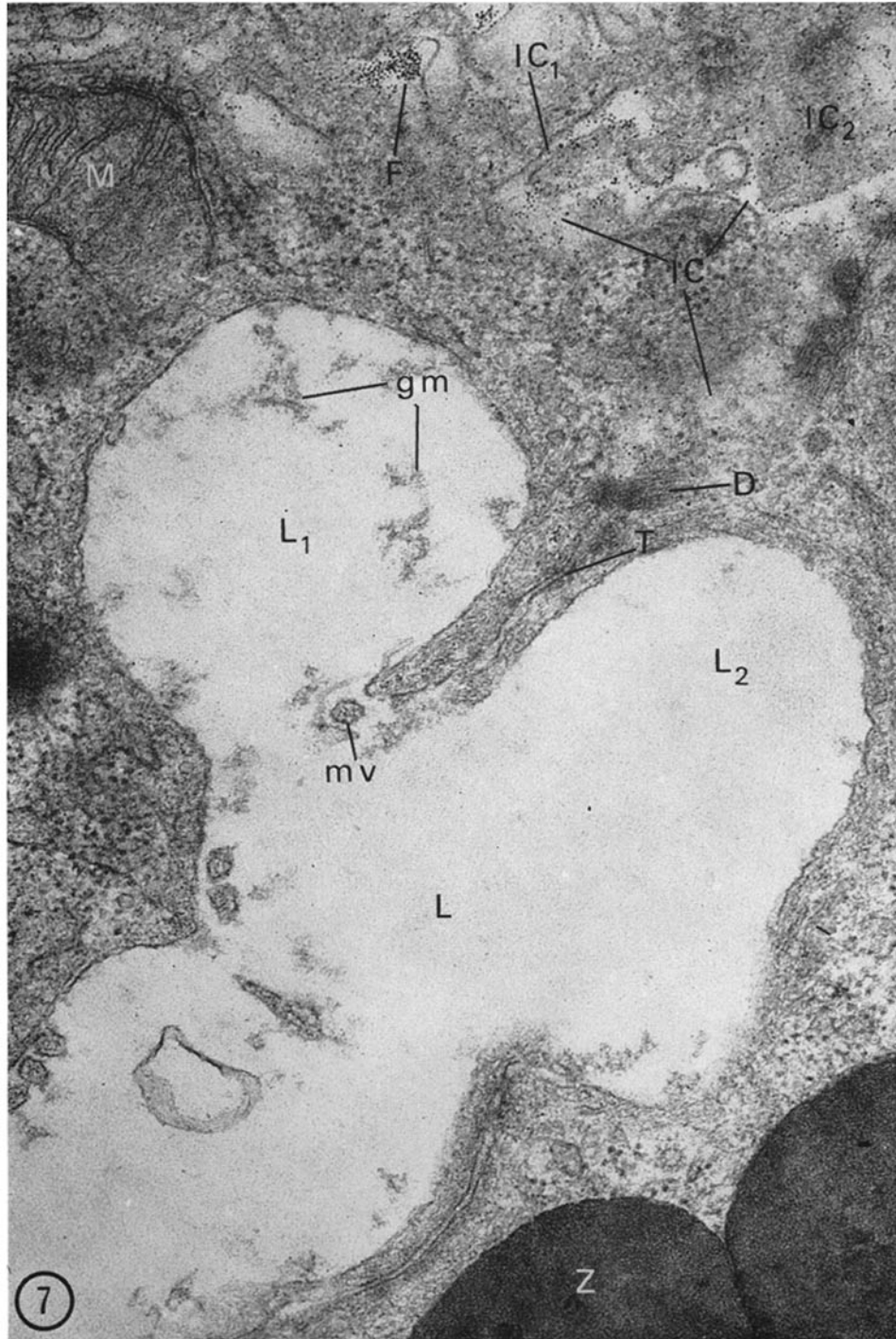
FIGURE 7   Same as in Fig. 6, at higher magnification. The lumen (L) containing small quantities of granular material (gm) is not accessible to the ferritin molecules (F) filling up the intercellular cleft (IC). The plasma membrane is sectioned normally at IC_1 and tangentially at IC_2. In shape and size the lumen invaginations are still reminiscent of the zymogen granules from which they have originated (L_1, L_2). Ferritin was added after fixation. $\times$ 43,000.
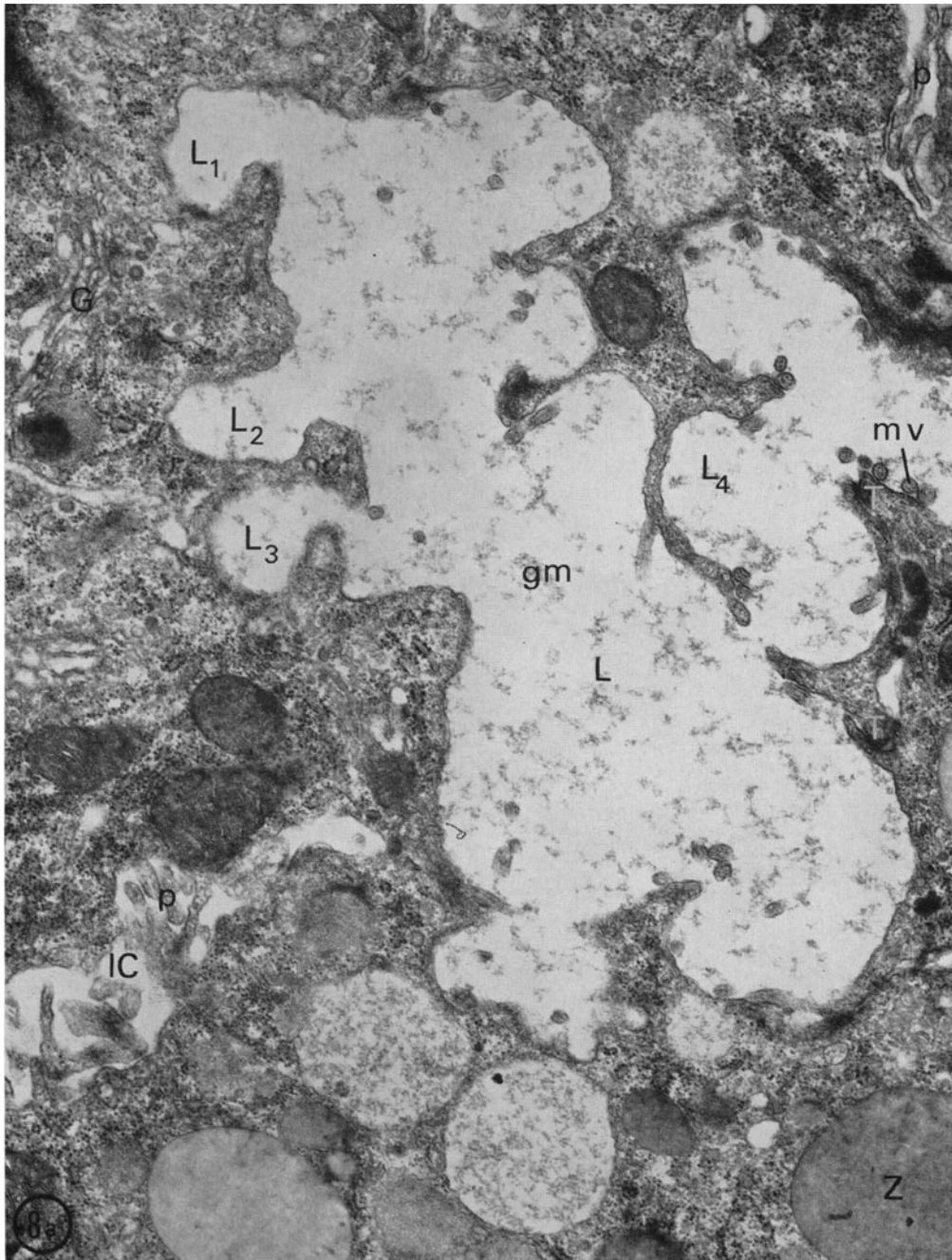
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FIGURE 8 Discharge of zymogen granules during secretion 50 min after isoprenaline injection. The discharge is now almost complete, only few zymogen granules being still present (Z). The intercellular clefts (IC) are enlarged and show many long papillar infoldings of the plasma membrane (p). Lumen retraction can be observed at this time, the lumina being heterogeneous in shape and size, as indicated also in Fig. 3 a.

Fig. 8 a   shows a large lumen with traces of its recent fusion with the zymogen granules (L₁–L₄) and containing granular material (gm). × 19,500.
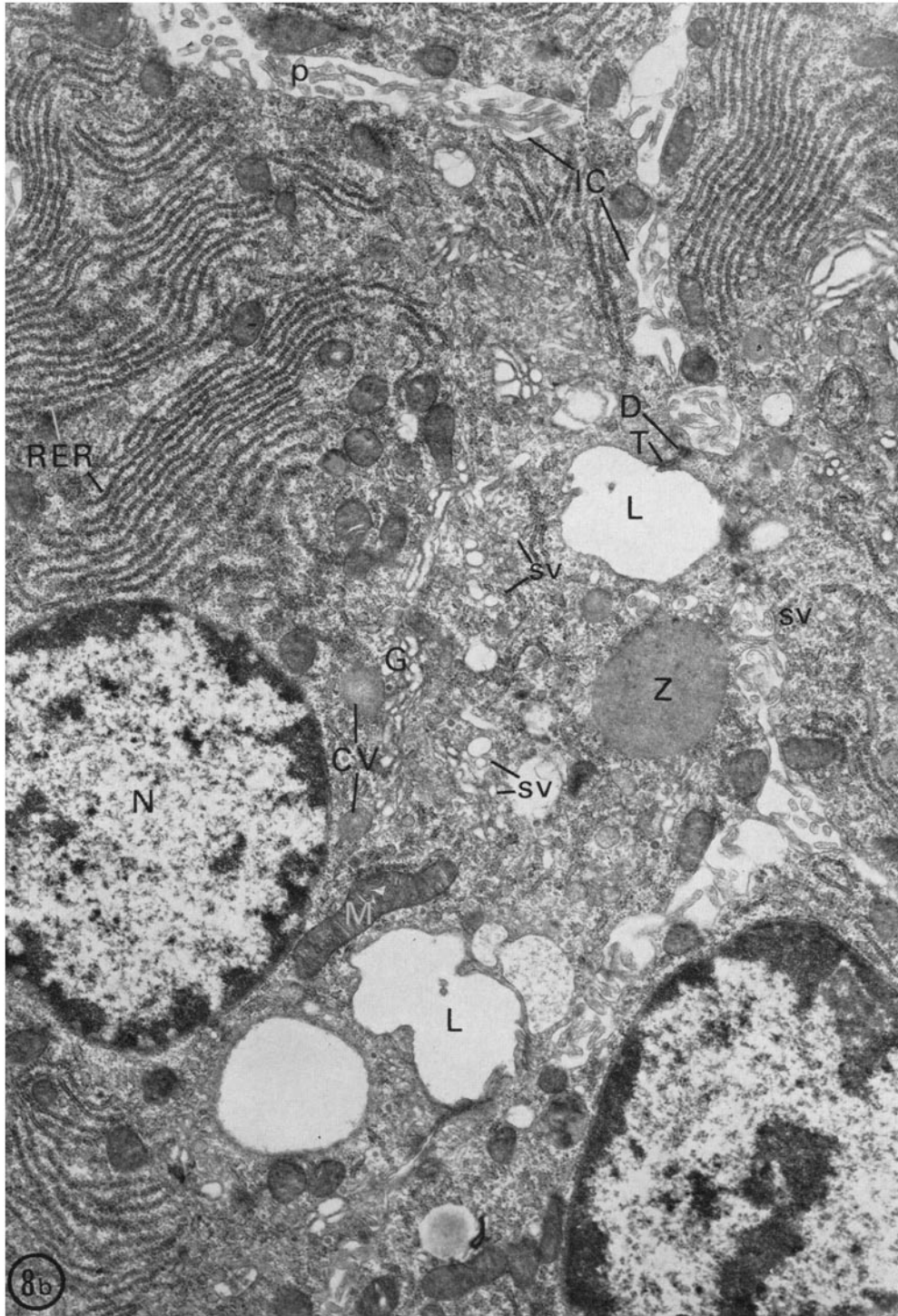
Fig. 8 b shows a lumen which has already been reduced almost to the initial size and is empty. Small smooth vesicles (sv) are present around the lumen and extend toward the Golgi complex (G). × 11,000.
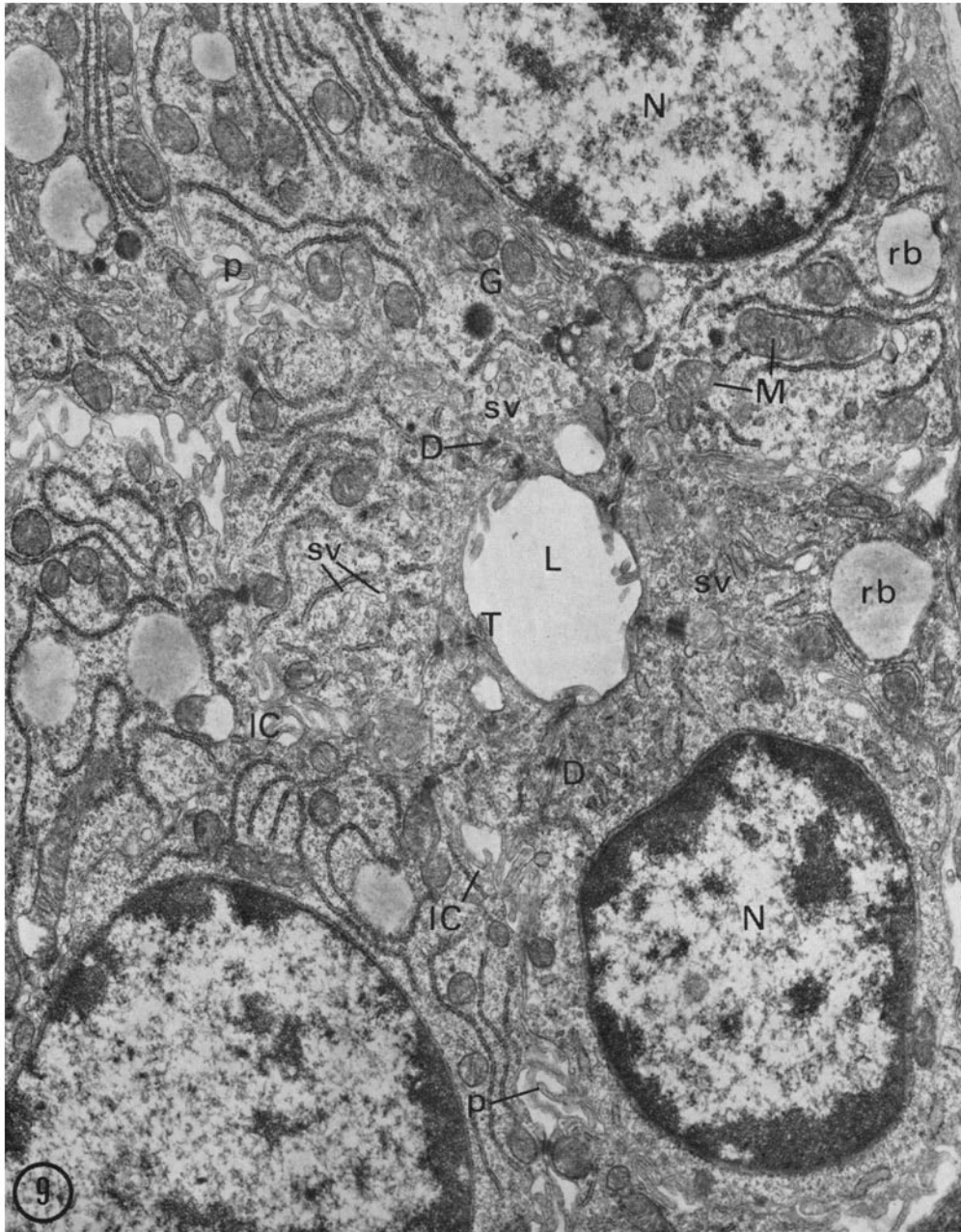
FIGURE 9   Acinar cells 2 hr after isoprenaline injection. The process of lumen reduction is now complete. The lumen (L) has the same size and empty appearance as in the control. Numerous small smooth vesicles (sv) are found around the lumen. No zymogen granules are seen. Round bodies (rb) of low density, probably lipid droplets, are seen located mainly in the basal part of the cell. Papillary infoldings of the plasma membrane are seen at p. × 13,500.
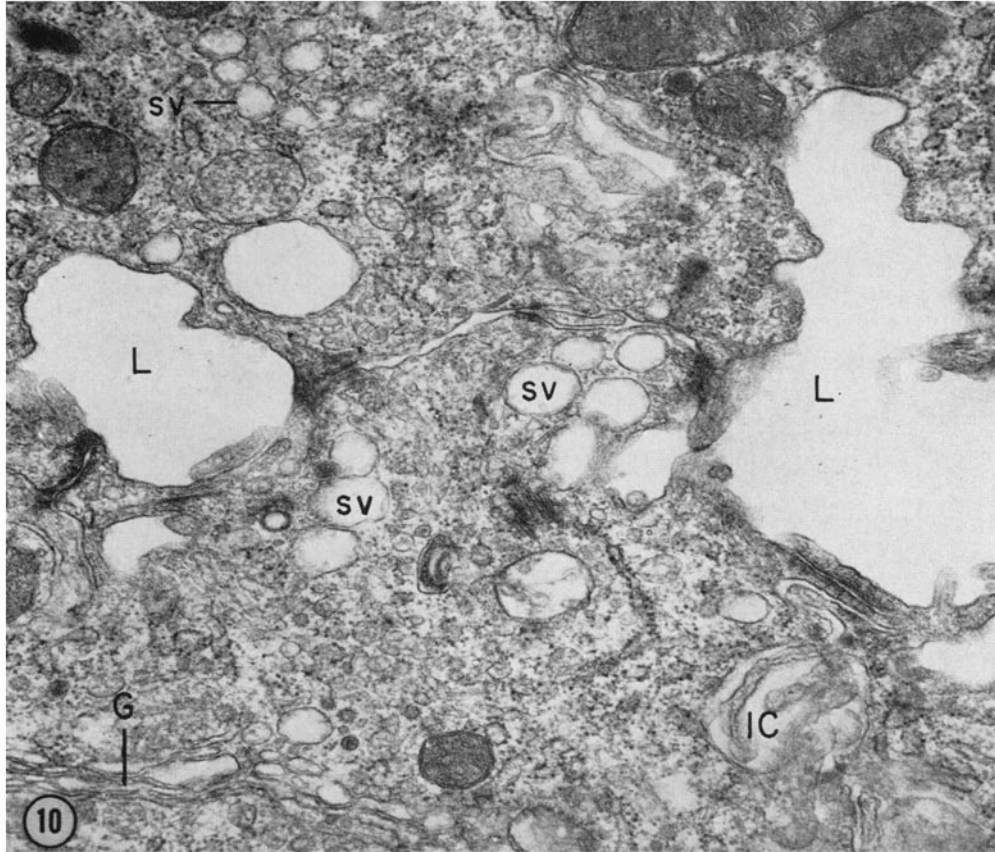
FIGURE 10   A lumen in the process of retraction, 2 hr after isoprenaline injection. Note numerous small smooth vesicles (sv) of varying size around the retracting lumens (L). × 26,000.

the lumen which may represent "blebs" or myelin figures formed upon aldehyde fixation. It is possible that this material is derived from zymogen granule membrane discharged directly in the lumen. Even so, this material seems to represent only an insignificant part of the total zymogen granule membranes (Figs. 6, 7). The presence of empty zymogen granules without apparent connection with the lumen were also observed (Figs. 6, 8). Although the lumen had increased in size many times, the desmosomes and the tight junctions remained intact, and the ferritin, present in the intercellular cleft, did not reach the lumen (Fig. 7). This was true whether ferritin was added to the secreting gland before or after fixation. The only difference between the treatments was in the amount of ferritin which penetrated the tissue.

The process of lumen reduction as seen in the light microscope was found to be accompanied by

formation of numerous small smooth vesicles varying in size from 0.1 to 0.8 μ, the small ones prevailing (Figs. 8 b, 9–12). These vesicles were still present at 6 hr (Fig. 12), but only a few remained at 11 hrs when formation of condensing vacuoles[1] and zymogen granules appeared most intense.

The lumen after 2 hr resumed its original size and empty appearance (Figs. 9, 10). During lumen reduction after secretion, the cells retracted toward the center of the acinus, as indicated also by phase-contrast microscopy. This process was accompanied by an increase in the length and number of the villus-like processes or papillar infoldings of

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[1] Smooth vesicles, about the size of zymogen granules found in the vicinity of the Golgi system and containing granular material of medium density, are referred to as condensing vacuoles (18, 19).
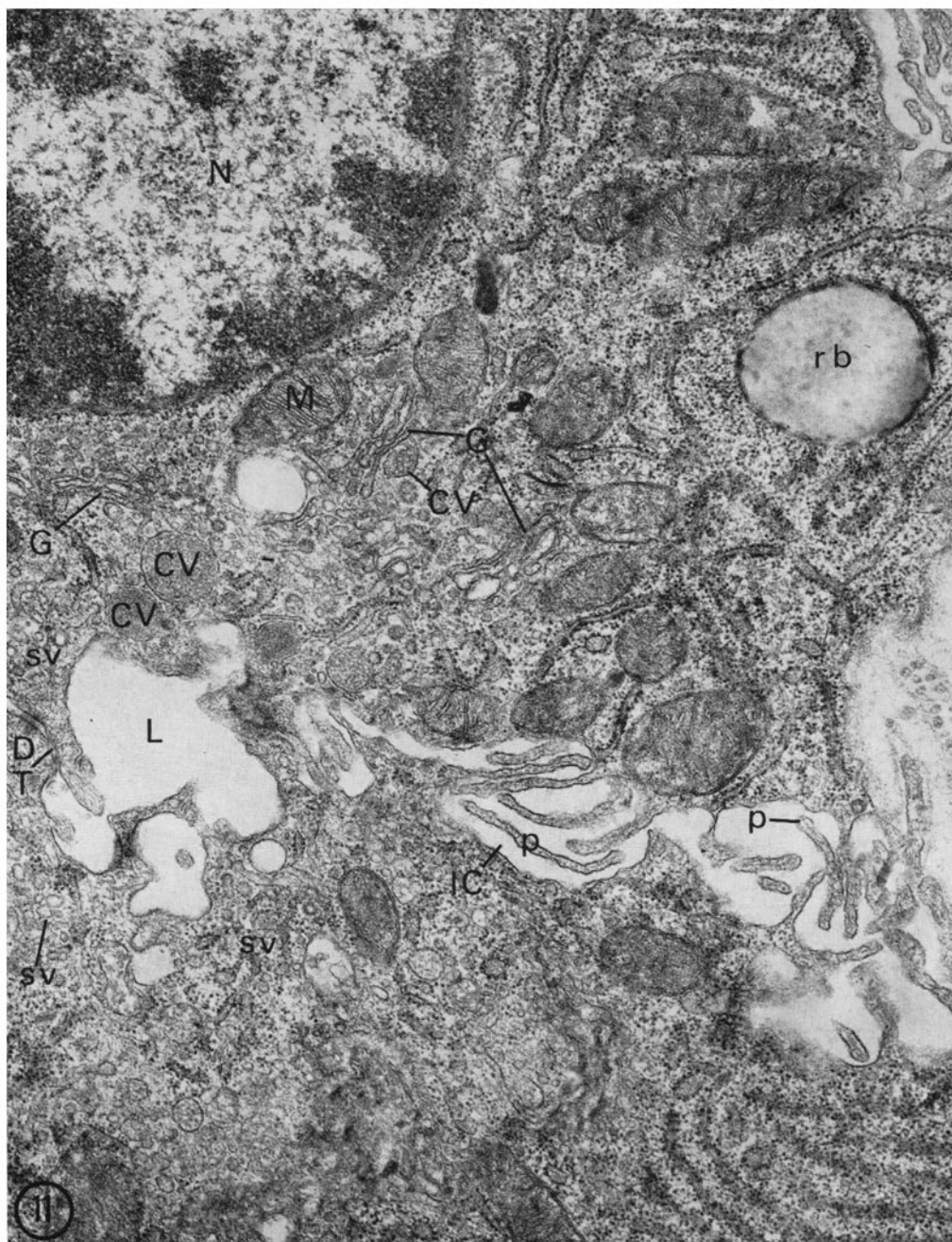
FIGURE 11   The acinar cell, 4 hr after isoprenaline injection. Many small condensing vacuoles (CV) are seen close to the Golgi complex (G) and in the vicinity of the empty lumen (L). Long and narrow papillary infoldings of the plasma membrane (p) are seen in the intercellular cleft (IC). Small smooth vesicles (sv) are still present, but difficult to distinguish from the Golgi complex. × 27,000.
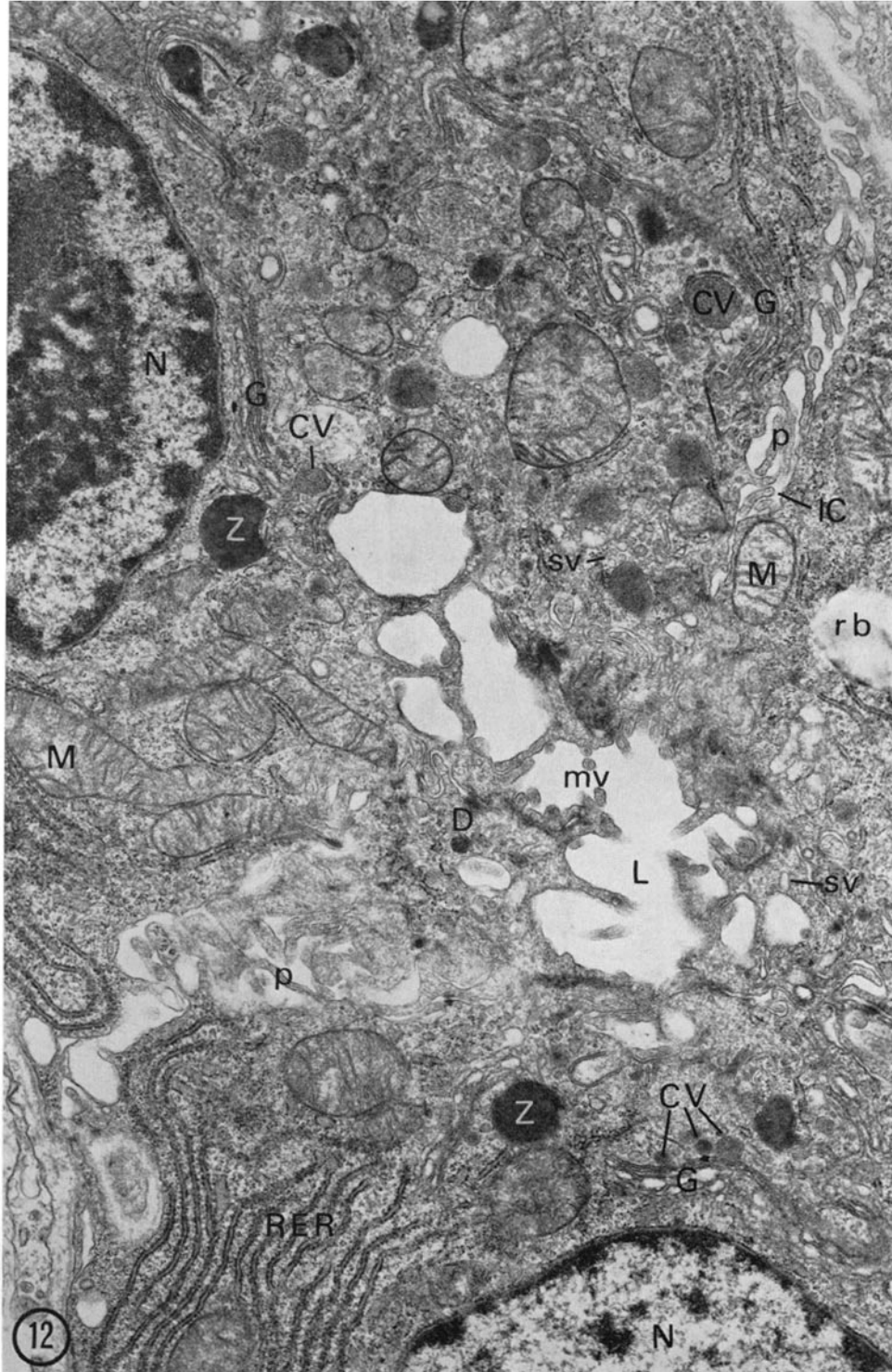
FIGURE 12   Reaccumulation of secretion granules during the secretory cycle 6 hr after isoprenaline injection. Note the numerous condensing vacuoles (*CV*) of varying sizes surrounded by Golgi vesicles (*G*) and the presence of few zymogen granules (*Z*). Also a few small smooth vesicles (*sv*) can still be seen. No further changes from the previous time points (Fig. 11) can be observed in the other cellular organelles. × 13,500.

the plasma membrane in the intercellular clefts (Figs. 8 b–12).

Further changes in smooth membranes were observed in correlation with the stage of reaccumulation of exportable enzymes. Condensing vacuoles were first seen 4 hr after onset of secretion (about 3 hr after termination of secretion [Fig. 11]). These structures appeared more frequently at 6 hr and were at all times located close to, or surrounded by, an array of Golgi vesicles (Figs. 11–13). The latter often appeared as a concentric system of flattened sacs (Fig. 13). Connections between condensing vacuoles and small vesicles of similar electron density within the Golgi system have occasionally been observed (Fig. 13). At 20 hr, when the number of zymogen granules was back to about 80% of the initial amount found before isoprenaline injection, many large condensing vacuoles were still present (Fig. 14).

Often, round bodies of medium density located in the basal part of the cell were observed (Figs. 9, 11). These bodies were not clearly membrane bounded, but often stained more intensively at their periphery (Fig. 11). Although they seemed to be more abundant 2–6 hr after the onset of secretion (Fig. 9), their occurrence is as yet not clearly correlated with the secretory cycle.

DISCUSSION

It is shown in the present work that injection of isoprenaline initiated a sequence of structural modifications in the acinar cells of the rat parotid gland (see also reference 20). These structural modifications occurred synchronously in most of the cells and showed a quantitative correlation with the exportable enzyme content of the gland. There is, therefore, a high degree of confidence that the changes in structure observed by phase-contrast and electron microscopy indeed represent the various phases of the secretory cycle. The study indicates the following sequence of events which is also illustrated in a scheme in Fig. 15. Zymogen granules fuse with the cell membrane bordering on the lumen, and thus direct discharge of the granule content into the lumen takes place. The zymogen granule membrane, by the fusion process, acquires cell membrane properties which enable it to fuse subsequently with additional zymogen granules located deeper inside the cell. Through this process of sequential fusions (cf. also reference 3) the lumen penetrates deeper into the cell until finally all the zymogen granules have been reached. The en-

larged lumen is drastically reduced through formation of small vesicles. Excess membrane material thus removed is perhaps reutilized in the formation of condensing vacuoles and their maturation to form new zymogen granules. Major points of evidence in support of the above scheme are: (a) kinetic correlation between zymogen granule depletion, membrane fusion lumen enlargement, and enzyme secretion; (b) repeated and consistent observation of sequential fusion of zymogen granules; (c) absence of fusion between zymogen granules and those parts of the plasma membrane which are not bordering on the lumen; (d) separation by the tight junctions of the lumen space from the intercellular space as shown by the impenetrability to ferritin during secretion; (e) reduction of the enlarged lumen to its original size concomitant with the appearance of small smooth vesicles and reduction of the cell size; (f) disappearance of the smooth vesicles concomitant with formation of condensing vacuoles followed by reaccumulation of mature zymogen granules and restoration of the original cell size.

Some of these points require further consideration. The concept of sequential fusion of zymogen granules with the lumen as the means of enzyme secretion derives its main support from observations by phase-contrast microscopy. The lumen is seen to extend radially into the cells, forming beaded invaginations which suggest formation by fusion of zymogen granules. Furthermore, any low-contrast, vacuole-like structure appeared to be continuous with the lumen upon proper focusing. The small changes in the diameter of the zymogen granules throughout the secretion process (0.9 ± 0.1 μ to 1.1 ± 0.1 μ) as measured in phase-contrast micrographs indicate that zymogen granules do not fuse with each other prior to fusion with the lumen. Because of the fact that sections examined by electron microscopy are much thinner than a single zymogen granule (600 A as compared to 1 μ), it is difficult to trace the continuity of the membrane of the discharging granules with the lumen by electron microscopy. However, the electron microscopic observations certainly support, on a qualitative basis, the observations on the fusion process made by light microscopy.

The finding that a zymogen granule membrane apparently fuses with additional zymogen granules only after it becomes part of the lumen membrane surely implies a highly specific biochemical alteration. The possibility that cyclic 3'5'-adenosine
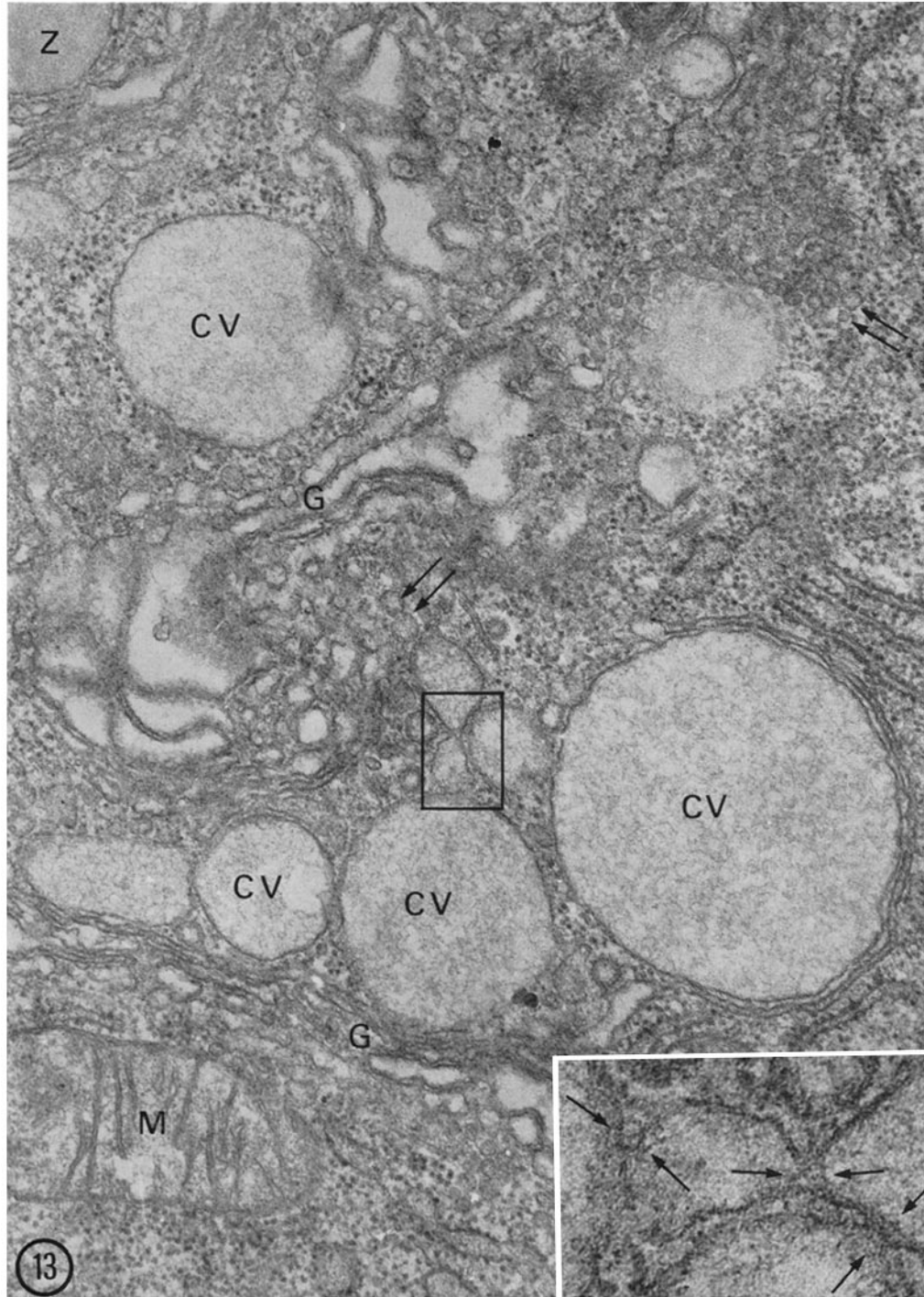
FIGURE 13 Intensive formation of condensing vacuoles during reaccumulation of amylase, 11 hr after iso-prenaline injection. Note the numerous condensing vacuoles (CV) of varying size surrounded by the Golgi complex (G). Tiny round vesicles containing varying quantities of a dense material are often seen in clusters (double arrows). Occasionally, similar vesicles are seen to fuse with large condensing vacuoles (single arrows in insert). × 33,000; insert × 120,000.
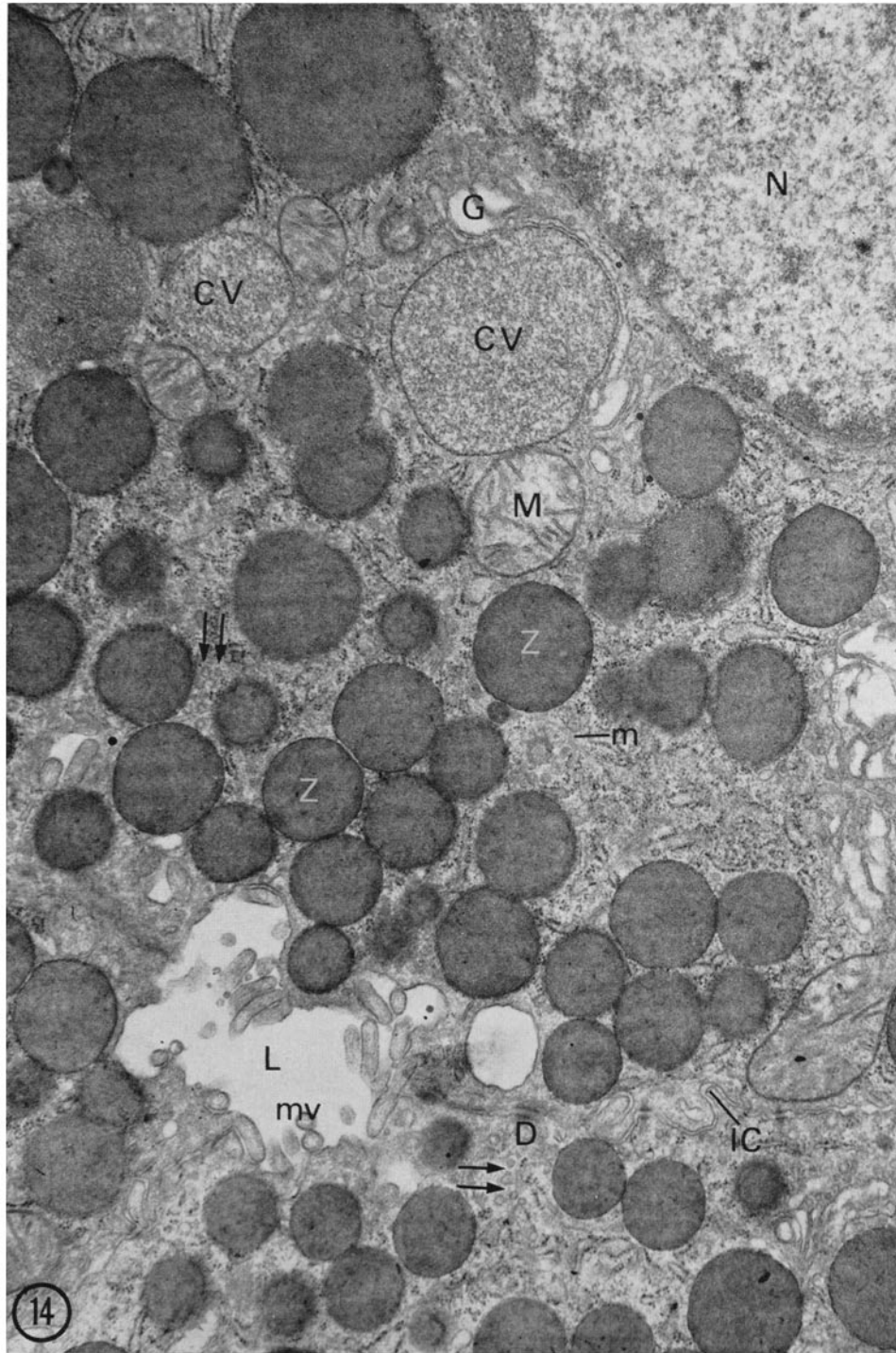
FIGURE 14 Accumulation of zymogen granules, 20 hr after isoprenaline injection. Note the numerous dense zymogen granules (Z) surrounding the narrow lumen (L). The intercellular cleft (IC) is almost devoid of papillary infoldings and is very narrow. The size of the zymogen granules varies. Large condensing vacuoles (CV) of varying density are also found close to the nucleus. The complexity of the Golgi system (G) is reduced (see for comparison, Fig. 13). Very few small smooth vesicles can still be seen around zymogen granules and close to the lumen (double arrows). A microtubule can be seen at (m). × 17,000.
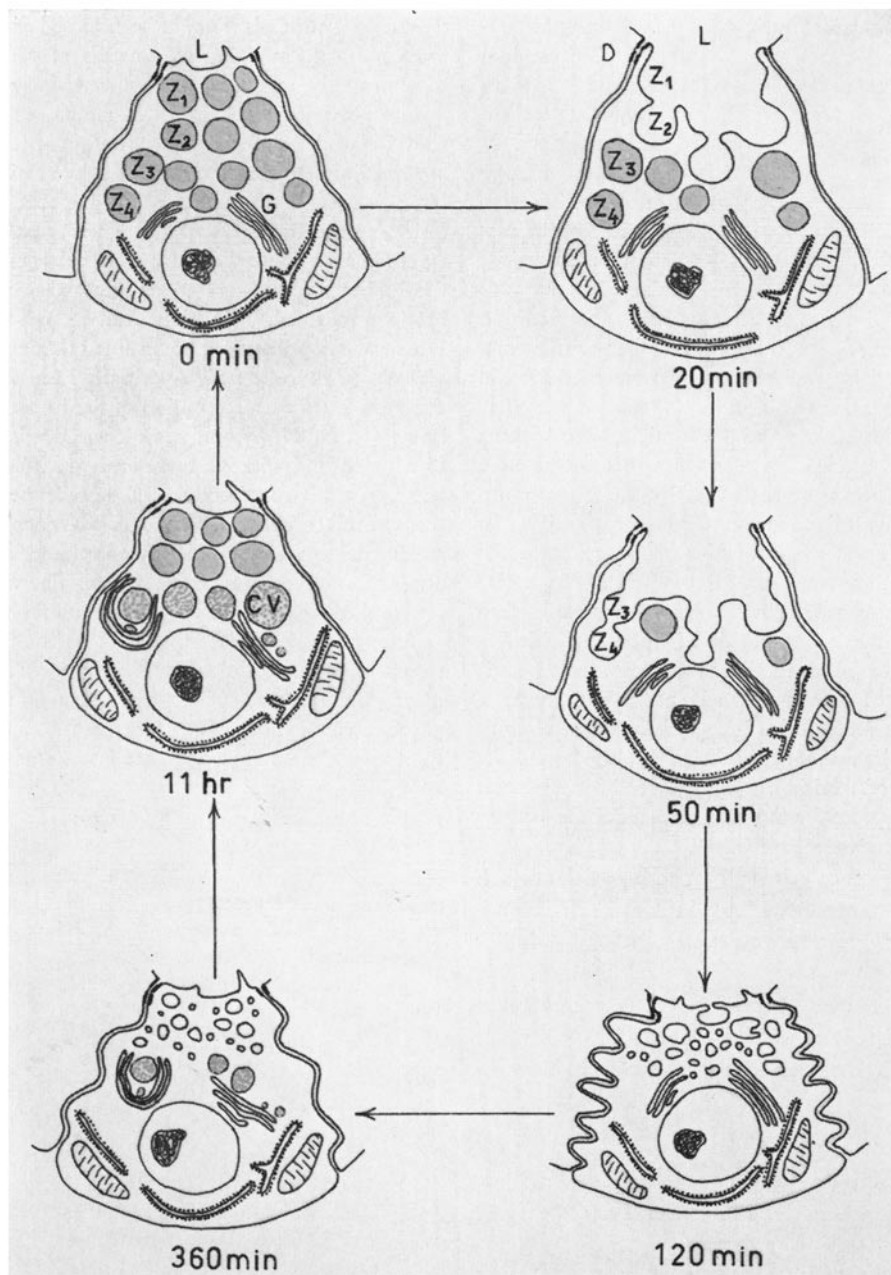
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FIGURE 15 Schematic representation of the ultrastructural changes during the secretory cycle in the rat parotid gland. Z_1–Z_4 represent zymogen granules fusing in sequential order. For explanation, see Discussion.

monophosphate (AMP) is involved in this process should be considered in view of the fact that the cyclic nucleotide induces secretion in slices of parotid gland (5, 21). Cyclic 3'5'-AMP has also been shown to play a part in biochemical events resulting in membrane modification or interaction in other systems (22, 23).

The continuity of the tight junction and des-

mosomal barrier during secretion was demonstrated in the experiments with ferritin. It had already been shown that the tight junctions in various epithelia were impermeable to ferritin (24) and even smaller protein molecules (25). In the parotid gland, ferritin penetrated readily within the intercellular space of the acini both before and after fixation. However, ferritin was not detected in the lumen at any stage of secretion. There are several possible explanations for this surprising finding: in the case of ferritin addition before fixation, it is conceivable that positive pressure caused by water secretion prevented ferritin penetration. In the case of ferritin added after fixation, the lumens and ducts might have become occluded with denatured protein. It is also possible that the ducts were sealed off by the pressure applied while mincing the tissue for fixation. In addition, since many acini are connected to the same duct system, each piece of tissue of the size used (1 mm³) might have only a few openings exposed, and it is possible that incubation with ferritin was too short to allow diffusion through such a long and tortuous channel system. Whichever of the above factors was in operation, it is obvious that the absence of ferritin from the lumen and the presence of ferritin in the intercellular space is adequate proof for the effectiveness of the barrier during secretion.

The existence of small smooth vesicles which fuse with the condensing vacuoles has been observed by Jamieson and Palade (19), and similar structures were noted in the present work. The abundant vesicles observed late during secretion at the apical pole of the cell seem to be of different identity. These vesicles appeared before any ac-

cumulation of amylase could be detected and were presumably formed in the process of reduction of the vastly extended lumen membrane.

The possibility that smooth membranes which serve for packing of exportable protein in pancreas are reutilized during secretion has been discussed by Palade (1). He anticipated that continuous fusion of the zymogen granules with the lumen would bring about a "considerable enlargement of the lumen and exhaustion of intracellular membranous material." Since such an increase of the lumen was not observed, he assumed that zymogen granule membrane fusion with the lumen occurs concomitantly with resorption of the lumen membrane (1). In the present work it was possible, due to a rapid and massive secretion, to differentiate between the two directions of membrane movement, and to show that the appearance of the small smooth vesicles is clearly correlated with the absorption of the lumen membrane. The role and fate of these vesicles are being further investigated.

The present study has revealed conditions under which specific cellular structures predominate during the secretory cycle. This information should prove useful, particularly for the isolation and, thus, the biochemical characterization of the enlarged lumen membranes, the small smooth vesicles, and the condensing vacuoles.

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