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Acute Interstitial Pancreatitis in the Rat Induced by Excessive Doses of a Pancreatic Secretagogue

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Summary. Conscious rats were infused via a jugular vein catheter with 5×10^{-6} g/kg/h caerulein for periods up to 24 h. On macroscopic inspection a progressive interstitial oedema is seen to develop in the pancreas, from one hour of infusion on and is most marked at twelve hours. This oedema is largely reabsorbed after 24 h treatment, but the pancreas is considerably indurated by this time. Serum amylase levels increase consistently to reach a tenfold elevation above controls after three, six or twelve hours infusion. Premature fusion of condensing vacuoles and secretory granules leads to formation of large vacuoles in the cytoplasm of exocrine pancreatic cells. These vacuoles fuse with the lateral and basal plasma membrane and realease their content into the extracellular space. Regular discharge of zymogen granules at the cell apex into the duct system does not occur. Vacuole formation is associated with cytoplasmic destruction of the pancreatic cells. The rate of protein synthesis decreases consistently as a result of these structural alterations and this change corresponds largely to a reduction of cellular respiration. Release of amylase from isolated pancreatic lobules of caerulein infused animals shows a progressive increase of unstimulated discharge, while in vitro stimulation with 5×10^{-6} M carbamylcholine gives secretion patterns of wash-out kinetics. Stimulated discharge of labeled secretory proteins indicates a progressive reduction in the in vitro sensitivity of the pancreatic cells to secretagogues. After 24 h infusion of 5×10^{-6} g/kg/h caerulein the pancreatic lobules are totally insensitive to the in vitro effect of carbamylcholine or caerulein.

Key words: Exocrine pancreas – Pancreatitis – Secretory process – Interstitial oedema.

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Introduction

Caerulein, a synthetic decapeptide originally extracted from the skin of the Australian amphibian Hyla caerulea, has a striking stimulatory action on exocrine pancreatic secretion in a variety of mammalian species including man (Erspamer, 1972). In two previous communications (Bieger et al., 1976a, b) it was demonstrated, that continuous infusion of 2.5×10^{-7} g/kg/h of caerulein in conscious rats leads to a rapid degranulation of the exocrine pancreas. This is associated with a marked enlargement of the Golgi complexes, while the size of the zymogen granules decreases consistently. Pancreatic protein synthesis, measured by the incorporation of tritiated leucine into TCA-precipitable proteins, remains unchanged during the first six hours of in vivo stimulation, but then increases to maximal levels 230% over control after 24 h of treatment. Intracellular transport of secretory proteins from their site of synthesis at the rough endoplasmic reticulum (RER) to their site of packaging in the Golgi complex is accelerated by a factor of five to seven after 24 h caerulein infusion, as demonstrated by a radioassay for zymogen discharge and by cell fractionation. It was concluded from these experiments that pancreatic secretagogues are able to modulate intracellular steps of the complicated sequence in the secretory process.

Similar studies in the dog (Tardini et al., 1971) infusing 1.2×10^{-6} g/kg/h revealed marked cellular alterations in the exocrine pancreatic cells associated with enzyme release. These included distension of the cisternal space of the RER and formation of large vacuoles, which developed into focal cytoplasmic degeneration. It was concluded from these studies that enzyme secretion induced by caerulein leads to cellular destruction followed by regeneration.

No destructive changes were induced by caerulein in the rat up to a dose of 5×10^{-7} g/kg/h. A significant impairment of the rate of protein synthesis and of enzyme discharge was, however, found in preliminary experiments infusing 5×10^{-6} g/kg/h for 24 h (Bieger et al., 1976a). Results of a more detailed study are reported here.

Materials and Methods

Male Wistar rats weighing 200 to 240 g (S. Ivanovas, Kisslegg, Germany) were infused via a jugular vein catheter with a standard dose of 5×10^{-6} g/kg/h of caerulein (synthetic caerulein, Batch 19414, kindly provided by Dr. H. Maier, Deutsche Farmitalia, Freiburg, Germany). During infusion the animals had free access to pelleted food and drinking water; they were loosely fixed in a sling of adhesive tape attached to a long wire, which could be moved in a longitudinal direction within a regular plastic animal cage. At the end of each infusion period the animals were killed by exsanguination from the abdominal aorta under light ether anesthesia and the pancreas was quickly removed. Small pieces were immediately homogenized in 0.05 M Tris-buffer, pH 8.0, containing 0.1 M KCl, 0.02 M $CaCl_2$ and 1% Triton x-100 for determination of enzyme content in the tissue at the end of the in vivo treatment. From the remaining organ pancreatic lobules were prepared according to Scheele and Palade (1975) and incubated in vitro in a modified Krebs-Ringer-Hepes buffer pH 7.4 containing amino acids as specified by Eagle (1959) aand 14 mM glucose.

For analysis of in vitro discharge of enzymes and newly synthesized proteins from pancreatic lobules after in vivo pretreatment with 5×10^{-6} g/kg/h caerulein the system of Jamieson and Palade

(1971) was used. Pancreatic lobules were pulse labeled for 4 min with l-leucine-4.5-^{3}H, excess radioactivity was quickly removed by washing the lobules on Büchner funnels and they were further incubated at 37° C in the presence or absence of 5×10^{-6} M carbamylcholine as in vitro secretagogue. In some experiments 10^{-9} g/ml caerulein was used without any significant difference in results. 5-ml-samples from these incubations were taken after 30, 60, 90 and 120 min and replaced by new medium. Amylase and TCA-precipitable protein radioactivity were determined at each time point and referred to the total content in tissue and medium. By this procedure the kinetics of discharge of stored zymogen granules (represented by the amylase values) and of newly formed proteins can be analyzed. All values were compared to appropriate control preparations from animals infused with saline for the same period. The rate of protein synthesis in pancreatic lobules from the same animals were determined by incubation of lobules for 1, 2 and 3 h in the presence of 0.5 μCi/ml L-leucine-4,5-^{3}H. This was compared to $^{14}Co_2$-production from ^{14}C-palmitate as a measure of cellular respiration (Bieger and Kern, 1975).

Pancreatic lobules after each period of caerulein infusion were fixed for electron microscopy in a mixture of 1% formaldehyde, 3% glutaraldehyde in cacodylate buffer pH 7.3. After dehydration in a graded series of alcohols tissue blocks were embedded in Epon 812. Sections were cut with a diamond knife on a LKB ultramicrotome, stained with 5% uranylacetate and lead citrate (Reynolds, 1963) and examined in a Zeiss EM 9S electron microscope.

Assay Procedures

Proteins were determined by the method of Lowry et al. (1951) using crystalline bovine serum albumine as standard. DNA was measured according to Burton (1956) in the modification of Richards (1974). Amylase was determined according to Bernfeld (1955), lipase according to Shibabi and Bishop (1971) in a modification of Verduin et al. (1973). Chymotrypsinogen was activated with (50 μg/mg protein) trypsin and determined according to Hummel (1959) using N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate. All 3 secretory enzymes were expressed in international units (IU) using corresponding Worthington enzymes as standard.

Samples from incubation media and tissue homogenates were precipitated at 4° C with trichloroacetic acid (TCA, 10% final concentration) overnight. They were washed twice with 5% TCA, the final pellet was dissolved in 1 ml 1N NaOH and transferred to scintillation counting vials containing 10 ml Unisolve (Koch-Light Lab., Colnbrook, England). After addition of 3 ml distilled water they were counted in a Nulcear Chicago scintillation counter (Isocap 300).

Materials

All chemicals were reagent grade (E. Merck Chemicals, Darmstadt, Germany). L-leucine-4.5-^{3}H and 1-^{14}C-palmitic acid (50 mCi/mmole) were purchased from Amersham Buchler Braunschweig, Germany. The following Worthington enzymes were used as standards: procine pancreatic α-amylase (E.C.3.2.1.1.) 684 U/mg, procine pancreatic lipase (E.C.3.1.1.) 106 U/mg and bovine pancreatic chymotrypsin (E.C.3.4.4.5.) 48 U/mg. They were obtained from C. Roth Chemicals, Karlsruhe, Germany. L-amino acids, BTEE and β-Naphthyllaurate were purchased from Serva Biochemica, Heidelberg, Germany.

Results

Even on macroscopic inspection of the pancreas a marked difference could be observed between low and high doses of caerulein. After infusion of 2.5×10^{-7} g/kg/h the exocrine pancreas accquired a transparent pinkish appearance which paralleled the progressive loss of zymogen granules from the gland (Bieger et al., 1976a). The organ itself was normal in position, size and consistency. Half an hour after the infusion of 5×10^{-6} g/kg/h the pancreas appeared

squashy, after 1–3 h infusion a marked interstitial oedema developed, which by six and twelve hours led to protrusion of the gland into the abdominal cavity and a clear separation of individual lobules (Fig. 1). The oedema was colorless and had a jelly-like consistency, so that puncturing and biochemical analysis of its composition proved impossible without contamination of blood and pancreatic juice from the duct system. After 24 h infusion the interstitial oedema had been reabsorbed, the pancreas was again white and considerably indurated. Preparation of pancreatic lobules for in vitro experiments according to Scheele and Palade (1975) was possible after one to twelve hours infusion, but not after 24 h, so that pancreatic fragments had to be used for this period. Concomitant with the progressive interstitial pancreatic oedema, amylase levels in serum increased. After half an hour caerulein infusion they were double the values found in controls, by three, six and twelve hours serum amylase was elevated by a factor of twelve and by 24 h they had fallen to the levels found after half or one hour of infusion (Fig. 2). Lipase was not measurable in the serum of control animals but was demonstrated after 5×10^{-6} g/kg/h caerulein with a consistent increase up to twelve hours infusion (data not shown). Comparative experiments infusing 2.5×10^{-7} g/kg/h up to 24 h failed to show any increase in serum amylase or lipase compared to saline infused control.

It was concluded, therefore that only excessive doses of the pancreatic secretagogue led to cellular alteration, severe pancreatic oedema and increased serum

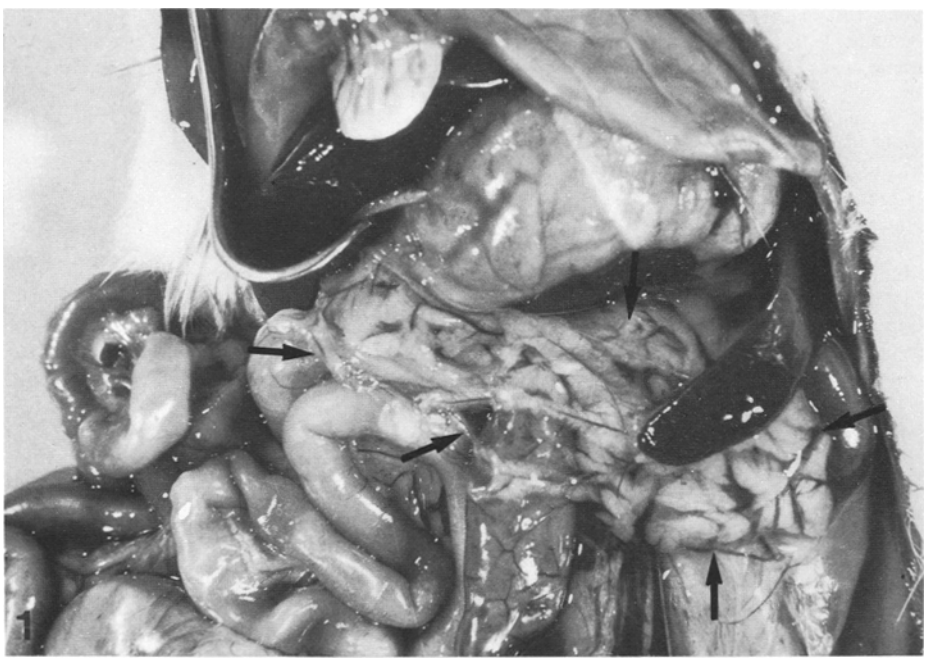

Fig. 1. Macroscopic appearance of pancreas after 3 h in vivo infusion of 5×10^{-6} g/kg/h caerulein. The gland protrudes into the abdominal cavity and displaces the spleen. The interstitial oedema pronounces the lobular structure of the organ (arrows)

enzyme levels. The structural basis for these alterations was then analyzed by electron microscopy.

Figure 3a shows the fine structural appearance of exocrine pancreatic cells from saline infused controls. Infusion of saline up to 24 h did not change the concentration of zymogen granules or the fine structure of cellular organelles involved in synthesis and packaging of digestive enzymes. Profiles of the RER were arranged in parallel with no significant distension of the cisternal space. The Golgi complexes consisted of the regular stacks of lamellae, numerous small vesicles (transporting vesicles) and a number of condensing vacuoles, which mature into zymogen granules (Fig. 3a).

Marked changes were observed after only 15–30 min of caerulein infusion: at low magnification numerous large vacuoles were observed, predominantly in the region of the Golgi complex (Fig. 3b). Closer analysis of the Golgi fine structure indicated that the vacuoles may form by distension of one Golgi saccule and by fusion of condensing vacuoles to the saccule and to each other (Fig. 4a, b). The large vacuoles in general contained a flocculent material reminescent of the content of condensing vacuoles. In some cases fragments of endoplasmic reticulum and small vesicles were observed inside the limiting membrane, indicating the initiation of an autophagic process in the exocrine cell. Definite degenerative changes were also observed in the mitochondria as early as 30 min after the treatment. In most mitochondria small vesicles had formed

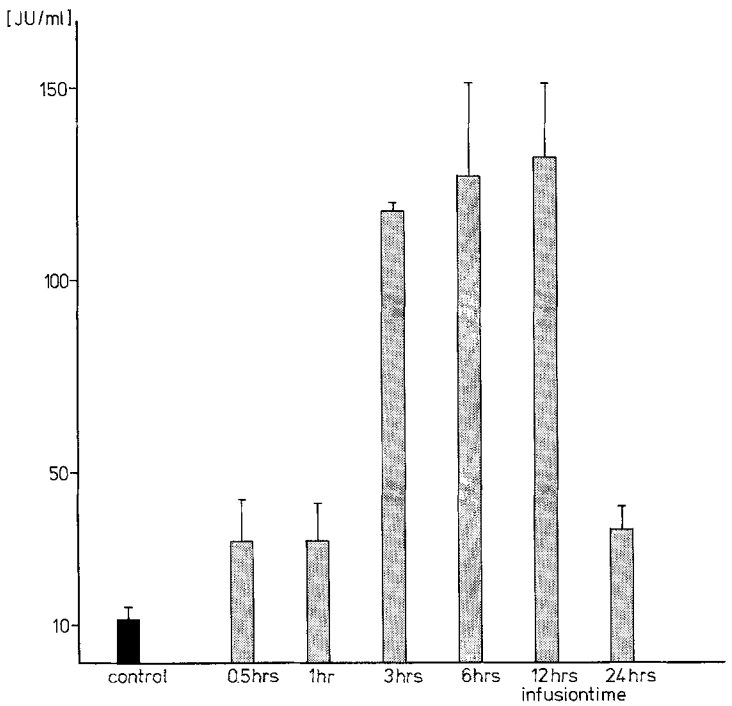

Fig. 2. Serum amylase levels in rats after caerulein infusion for various periods. The values are expressed as IU/ml serum ±SD, control values amount to 11.55±2.75 for the same infusion periods with saline

between the cristae and in the mitochondrial matrix, some were swollen with rests of the cristae preserved. They came in close contact with the vacuoles derived from the Golgi complex (Fig. 4b). Mature zymogen granules were generally crowded around the acinar lumen, there was no structural indication of a release of granules as a result of the caerulein infusion. Two to four large vacuoles were found in the Golgi complex zone (Fig. 4a, b), they might fuse to each other and come into continuity with the lateral and basal plasma membrane (Fig. 5a, b). This fusion of the vacuole limiting membrane with the plasma membrane is the structural basis for the formation of marked interstitial oedema starting 1 h after the caerulein infusion commenced.

After 3 and 6 h infusion further progression of the process of intracellular fusion of granules and vacuoles was observed, leading the formation of large "lakes" of digestive enzymes bounded by a single limiting membrane (Fig. 6a). The content of such vast vacuoles showed differing electron density and sometimes long needle-like inclusions, which were embedded in a homogenous matrix of the same electron opacity (Fig. 6a, b). The vacuoles usually occupied the whole of the apical or juxtanuclear cytoplasms and reached close to the acinar lumen, which did not show any signs of granule release (Fig. 6b).

After three hours of caerulein infusion numerous free cells were observed in the interstitial oedema, which itself contained small fibrills without any special fine structural characteristics (Fig. 7). The free cells included activated fibroblasts with elaborate RER profiles and Golgi complexes (Fig. 7) and also mononuclear and polymorphonuclear cells. Most of the mononuclear cells were motile lymphocytes with long slender processes and containing a moderate number of membrane-bounded granules (Fig. 7). In the neighborhood of blood capillaries especially mononuclear phagocytes were observed which had taken up parts of degenerated exocrine cells and sometimes zymogen granules. The largest vacuoles were found after 12 h of caerulein infusion, vast parts of the cell were occupied by one or two vacuoles, sometimes leaving only a small rim of cytoplasm (Fig. 8a). It is remarkable that the remaining cellular organelles had largely preserved their fine structure. After 24 h infusion most of the large vacuoles had disappeared from the cytoplasm. The acinar lumen was greatly distended and lined by flattened exocrine pancreatic cells, which measured 6–8 μ in thickness (Fig. 8b). In some cases the acinar lumen reached close to the basal part of the cells and was surrounded by a number of typical zymogen granules (Fig. 9a). At higher magnification numerous irregular shaped electron dense bodies were observed in the cytoplasm. They may represent the residue of collapsed vacuoles from preceeding stages or residual bodies from previous autophagic vacuoles (Fig. 9b).

The functional implication of these structural alterations for the different steps in the secretory process of the exocrine pancreas was then further analyzed biochemically. Table 1 summarizes the content of amylase in the pancreas (and the interstitial oedema fluid) for each time point of in vivo infusion. While infusion of 2.5×10^{-7} g/kg/h caerulein leads to a decrease of enzyme content already at 30 min (Bieger et al., 1976), the excessive caerulein dose did not activate the regular discharge of zymogen granules. It has to be concluded that during the first three to six hours of in vivo treatment,

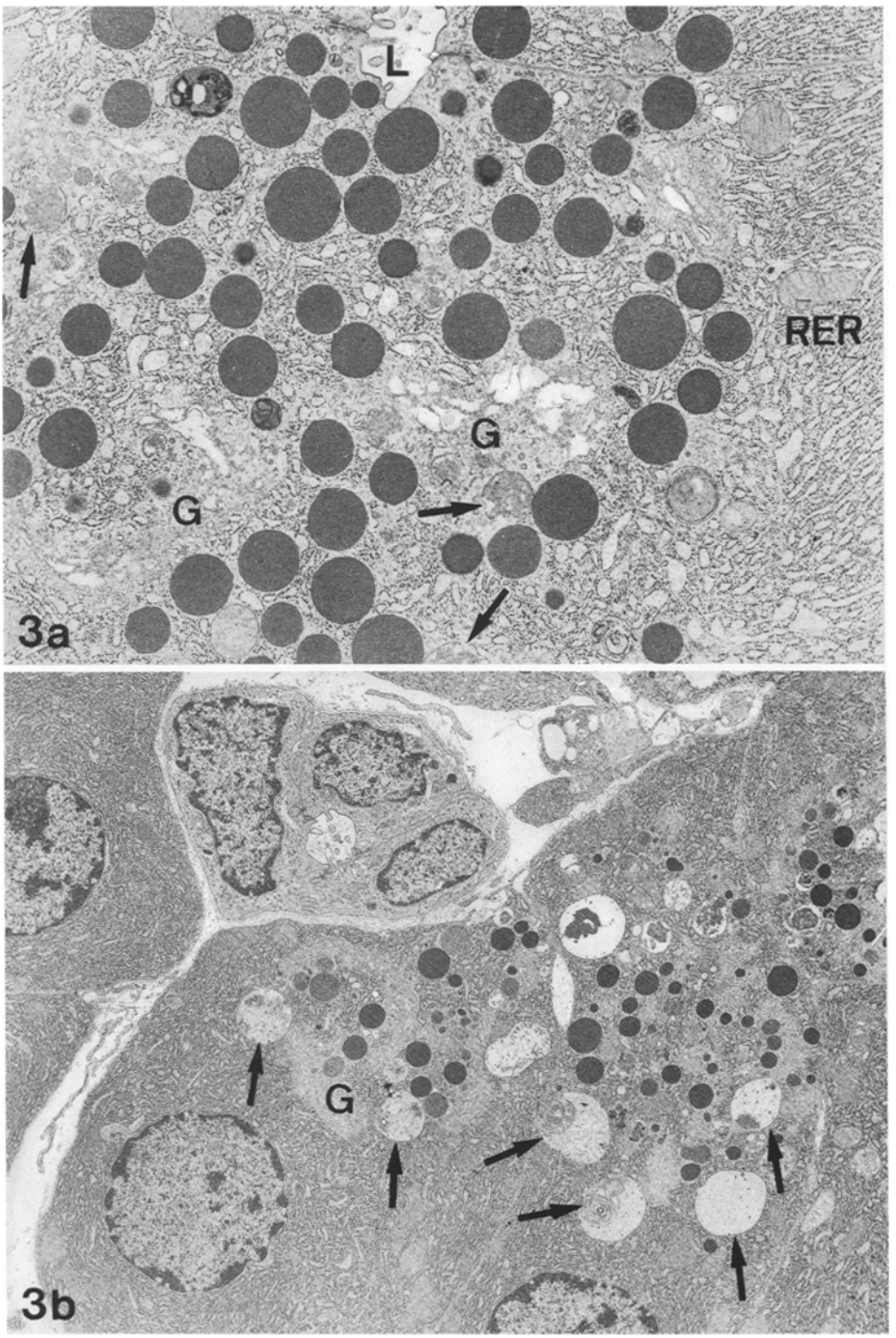

Fig. 3a and b. Comparison of fine structure of exocrine pancreas from control animal (**a**) and after 30 min caerulein infusion (**b**). **a** Shows cellular organelles involved in synthesis and packaging of digestive enzymes. *RER* rough endoplasmic reticulum, *G* Golgi complex, *L* acinar lumen, arrows indicate condensing vacuoles. Magnification × 19,200. **b** Exocrine acinus after 5×10^{-6} g/kg/h caerulein. Note numerous large vacuoles in Golgi area (arrows). Magnification × 4500

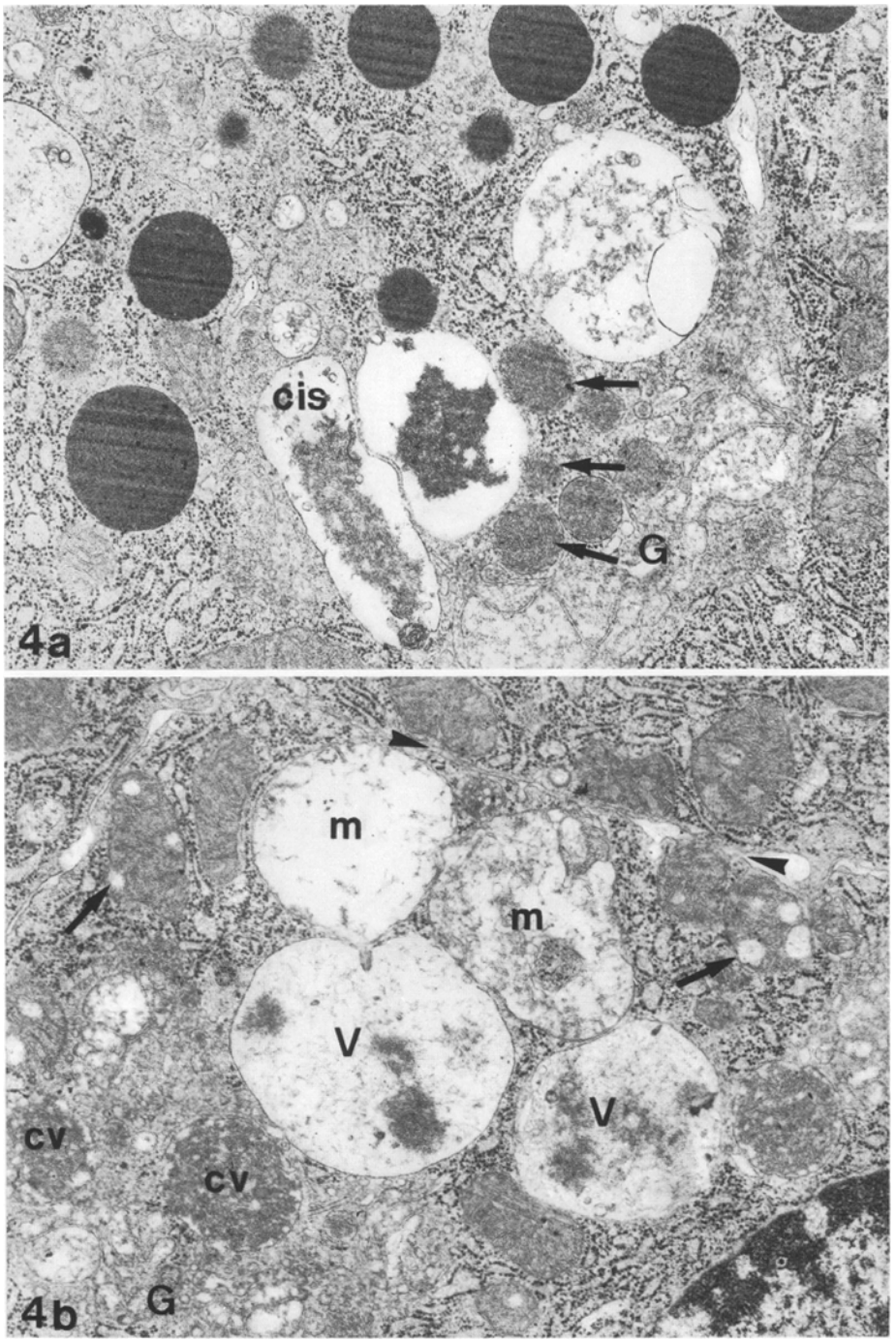

Fig. 4a and b. Details of vacuole formation after 30 min caerulein infusion. Magnification × 19200. **a** Distension of Golgi cisternae (*cis*) and fusion of condensing vacuoles (arrows) to a larger vacuole containing flocculent material. *G* Golgi complex. **b** Close contact of large vacuoles (*V*) and swollen mitochondria (*m*), which contain parts of cristae. Other mitochondria in the same cell develop vesicles between their cristae (arrows). The arrow head indicates extracellular space. *G* Golgi complex, *cv* condensing vacuole

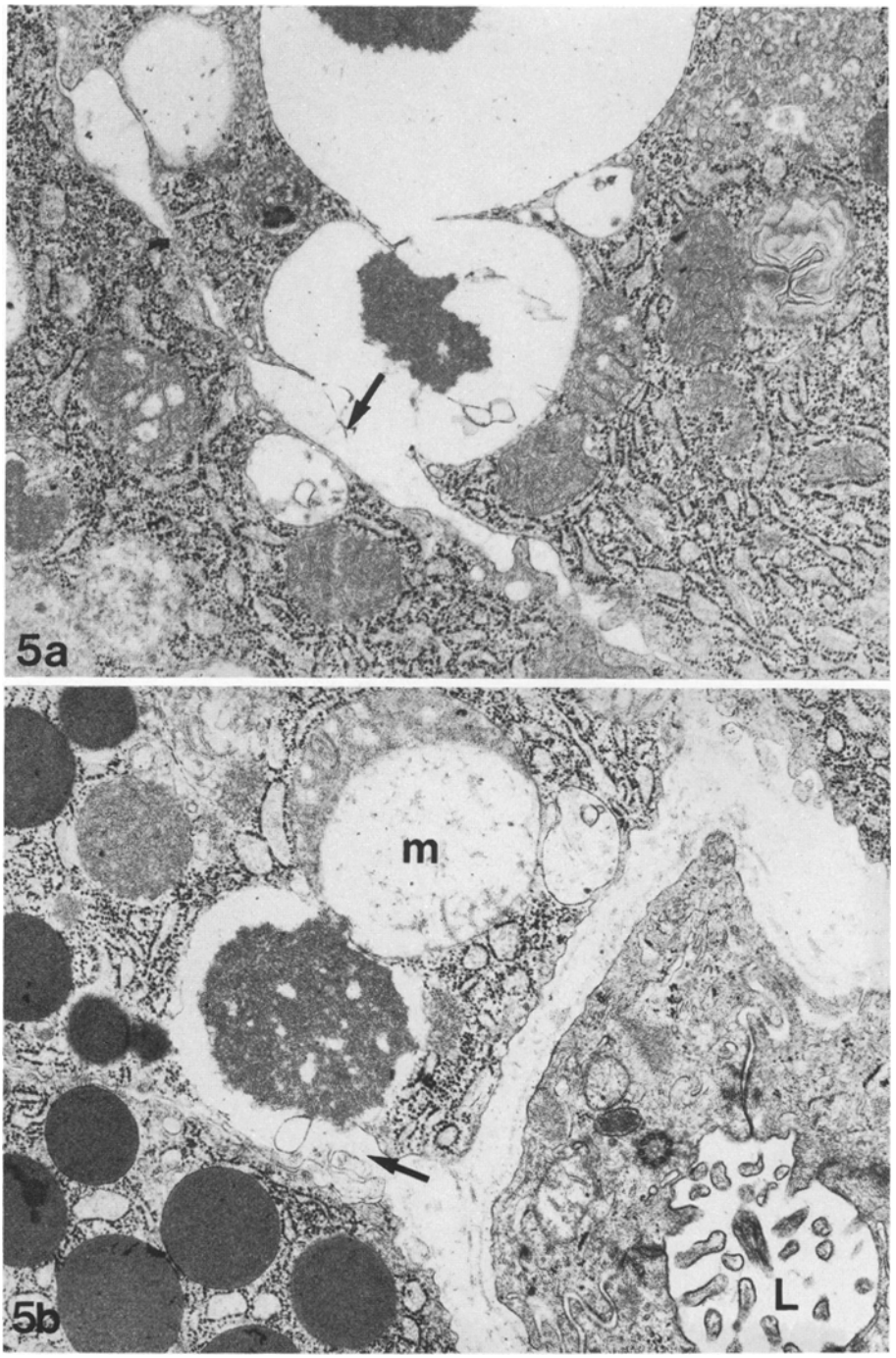

Fig. 5a and b. Fusion of large vacuoles with the lateral (**a**) and basal plasma membrane (**b**) as a structural basis for the development of the interstitial oedema after 1 h caerulein infusion. **a** Two large vacuoles have fused and are in free communication with the extracellular space (arrow). **b** A large vacuole opening at the basal plasma membrane (arrow). Note the swollen mitochondria (*m*) and the regular fine structure of an intercalated duct with cross sections of microvilli and a cilium in the luminal space (*L*). Magnification × 19,200

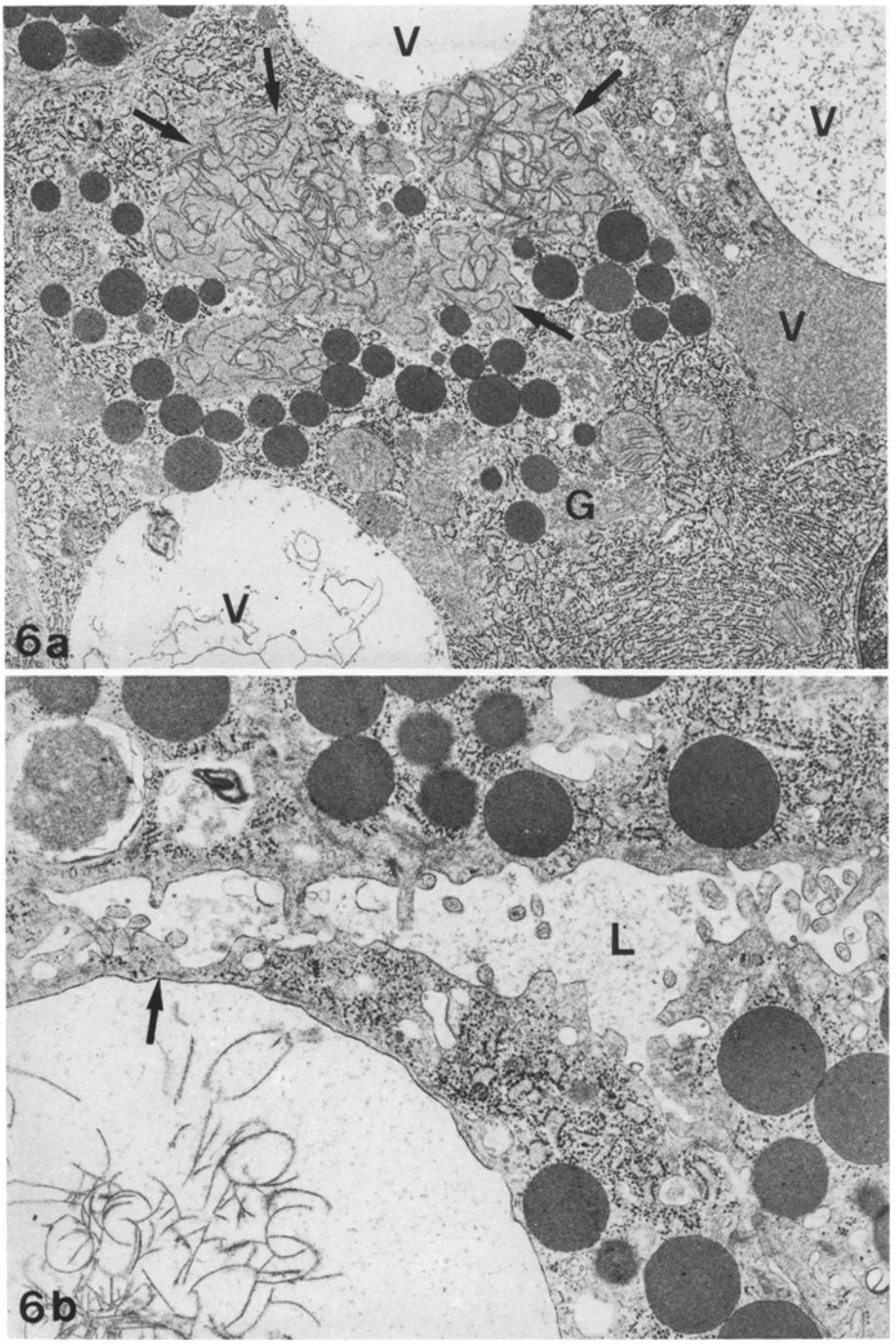

Fig. 6. **a** After 3 h caerulein infusion large "lakes" of digestive enzymes (arrows) have formed besides the vacuoles (*V*). They contain numerous needle-like inclusions in a material of moderate electron density. Magnification ×10,800. **b** zymogen granules are concentrated around the acinar lumen (*L*), which may be seperated from the large vacuoles only by a small rim of cytoplasm (arrow). Magnification 20,000×

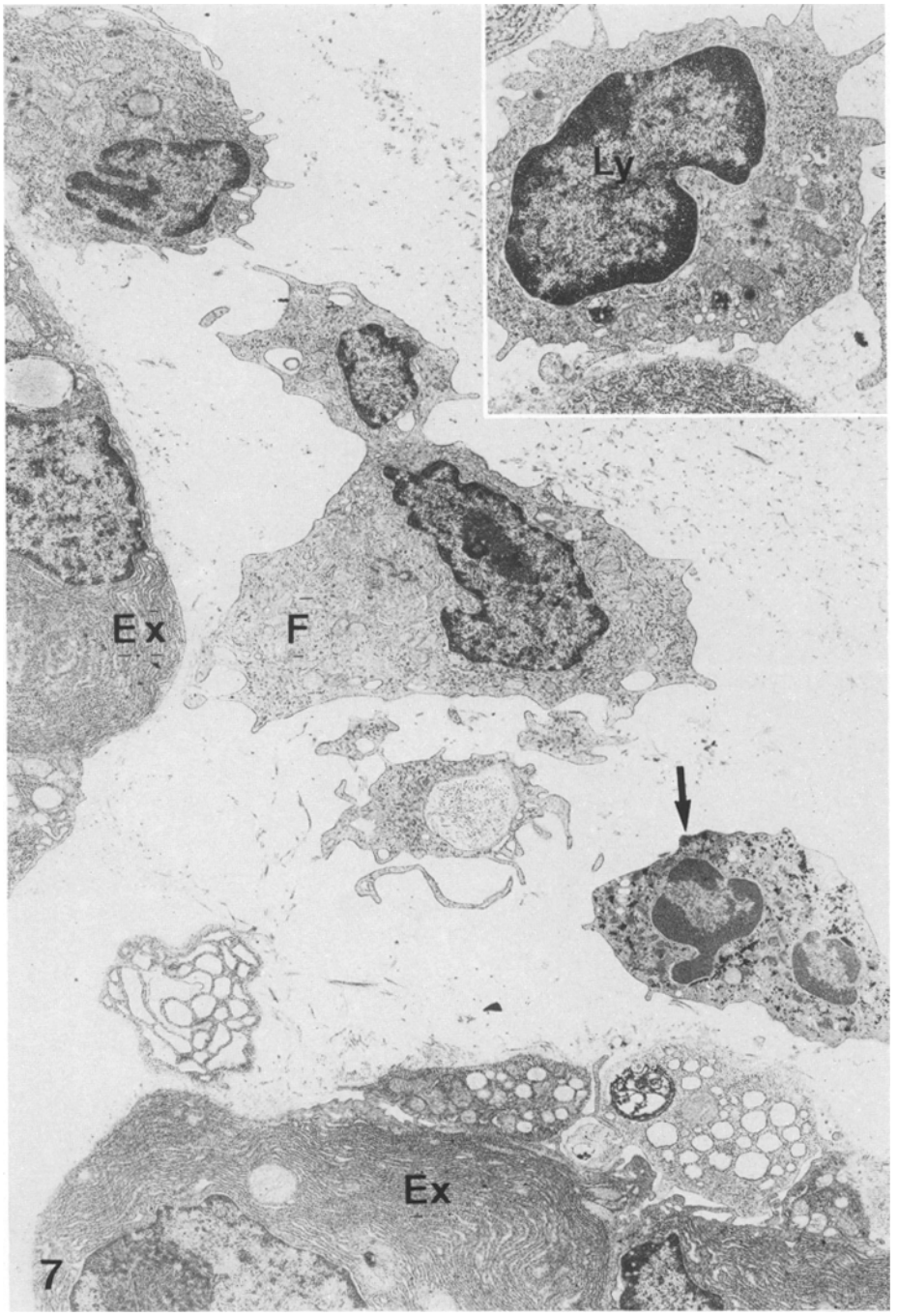

Fig. 7. Free cells in the interstitial oedema of the pancreas after 5 h caerulein infusion. The inset shows an activated lymphocyte with slender processes and some granules in the cytoplasm. Magnification ×20,000. Fine fibrillar material is observed in the vicinity of fibroblasts (*F*), the arrow points to a polymorphonuclear neutrophil cell containing a few granules and glycogen. *Ex* exocrine pancreas. Magnification ×6500

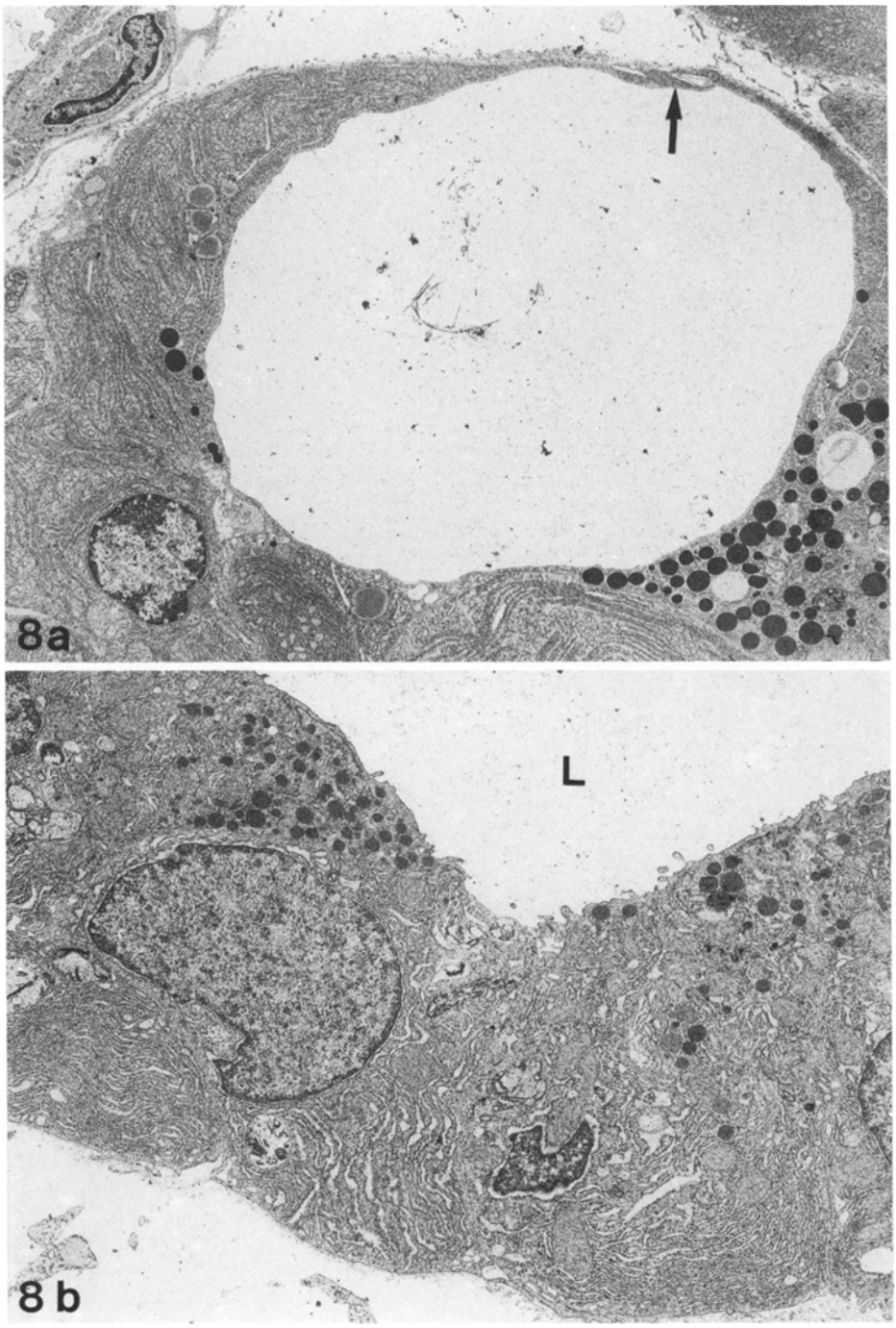

Fig. 8. a After 12 h caerulein infusion most parts of the exocrine cells are transformed into large vacuoles, which sometimes are lined by a small rim of cytoplasm (arrow). Magnification ×4500. **b** After 24 h caerulein infusion the large vacuoles have disappeared from the cytoplasm. Instead the acinar lumen (*L*) is widened considerably and lined by flat exocrine cells Magnification ×6500

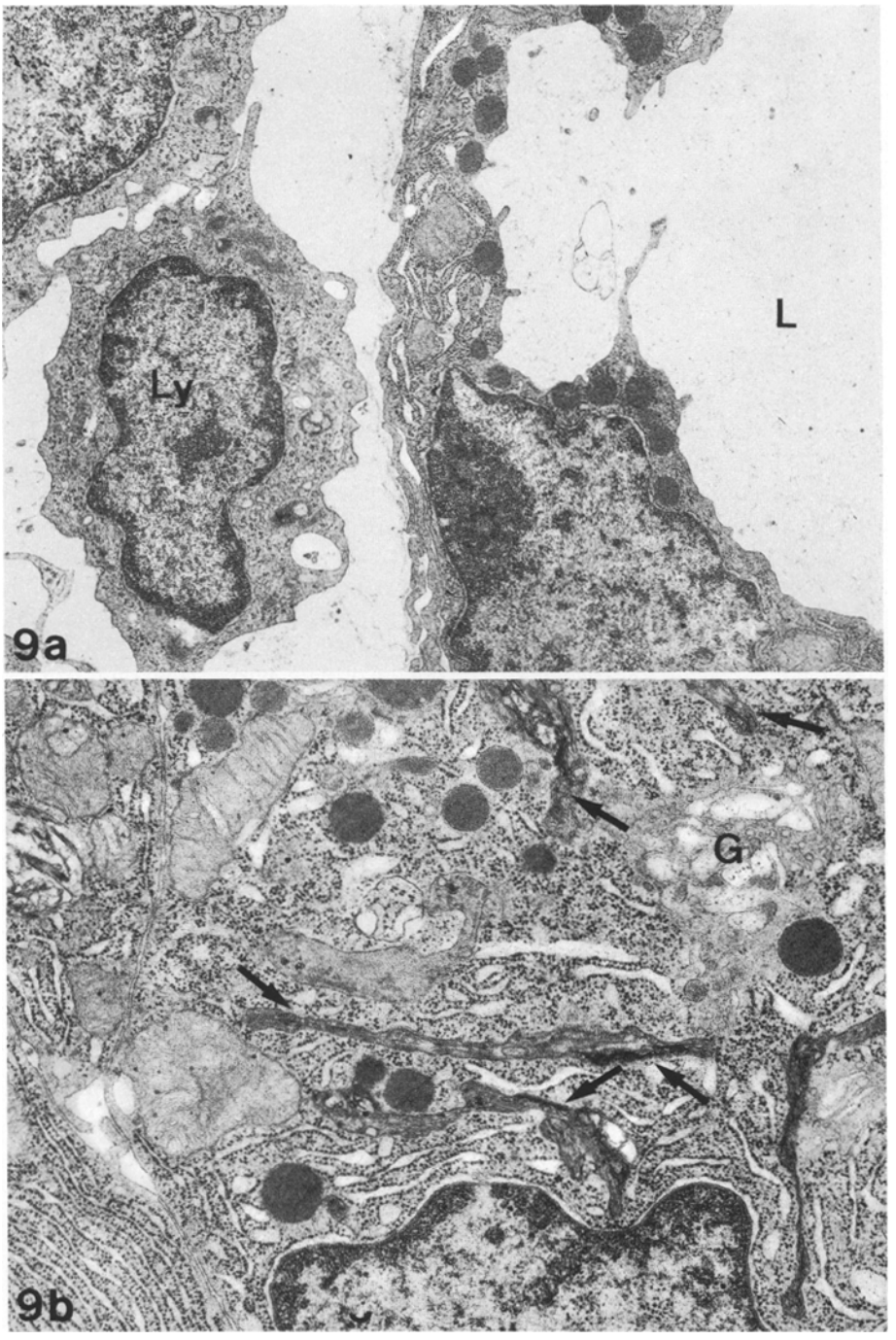

Fig. 9a and b. Details of cellular alterations after 24 h caerulein infusion. **a** In some cases the acinar lumen reaches deep into the juxtanuclear region of the cells and is lined by zymogen granules; *Ly* Lymphocyte in the interstitium. Magnification ×11,300. **b** The cytoplasm of the exocrine cells contains numerous irregular shaped electron dense bodies composed of fibrillar material and myelin figures (arrows). *G* Golgi complex. Magnification ×20,000

Table 1. Amylase content in total homogenate from pancreas after in vivo infusion of 5×10^{-6} g/kg/h caerulein. Values are expressed as IU/μg DNA

Conditions	Time of infusion	Amylase content JU/μg DNA
Control	–	5.1 ± 1.2
5×10^{-6} g/kg/h caerulein	0.5	4.7 ± 0.9
	1	4.6 ± 1.1
	3	5.1 ± 0.8
	6	4.0 ± 0.8
	12	3.3 ± 0.9
	24	2.2 ± 1.1

enzymes were only lost into the interstitium and were therefore preserved in the total homogenate of the gland. From 6 h a decrease in amylase content was observed probably due to diffusion into the blood and excretion by the urine. In Table 2 the rate of incorporation of tritiated leucine into TCA-precipitable proteins, as a measure of the biosynthetic rate and the formation of $^{14}CO_2$ from ^{14}C-palmitate as a parameter of cellular respiration, are compared. Infusion of 5×10^{-6} g/kg/h caerulein depressed both parameters after only 30 min, the effect on cellular respiration was more pronounced up to six hours and then showed a tendency to improvement. The rate of protein synthesis was uniformley depressed by about 30% during the first 6 h of infusion, decreased by 50% at 12 h and by 70% at 24 h of treatment.

Pronounced changes were observed in the discharge of amylase and labeled secretory proteins. According to Jamieson and Palade (1971) release of amylase into the incubation medium can be taken as a measure of the secretory behaviour of the total pool of stored zymogen granules. Release of tritiated proteins, which were labeled during a short pulse incubation, mimics all the consecutive steps which secretory proteins have to undergo from their site of synthesis up to packaging into storage granules. Furthermore, a comparison

Table 2. L-leucine-4.5-^{3}H incorporation into TCA-precipitable proteins after 2 h in vitro incubation of lobules from control and caerulein-treated animals. Comparison to $^{14}CO_2$-production from 1-^{14}C-palmitic acid of similar lobule preparations and incubation time. Values are expressed as percent of control, absolute values are listed in cpm/μg DNA (under control)

Condition	L-leucine-4.5-^{3}H-incorporation into TCA-precipitable proteins/2 h incubation		$^{14}CO_2$-production from 1-^{14}C-palmitic acid/2 h incubation
	Time [h]	Percent of control	Percent of control
Control	–	100 = 1505 ± 364 cpm/μg DNA	100 = 26,120 ± 745 cpm/mg DNA
5×10^{-6} g/kg/h caerulein	0.5	64.9 ± 8.8	65.0 ± 14.7
	1	66.2 ± 8.5	52.3 ± 20.6
	3	69.2 ± 13.0	33.2 ± 11.2
	6	75.0 ± 17.1	34.2 ± 5.5
	12	50.4 ± 5.0	59.0 ± 17.0
	24	29.8 ± 4.0	76.7 ± 9.5

Table 3. Comparison of in vitro discharge of amylase and labeled proteins for each period of incubation. Basal means release without secretagogue, Carb. signifies in the presence of 5×10^{-6} M carbamylcholine. The values are expressed as percent discharge of the total content in medium and tissue

Conditions	Time of infusion [h]	30′		60′		90′		120′	
		basal	Carb.	basal	Carb.	basal	Carb.	basal	Carb.
in vitro discharge of amylase [percent of total content]									
Control	–	0.8±0.09	3.3±0.9	1.3±0.17	8.3±2.0	1.8±0.22	15.1±2.7	2.5± 0.24	21.5±3.8
5×10^{-6} g/kg/h	0.5	3.3±0.15	4.6±0.9	4.8±0.3	12.6±3.9	6.2±0.5	18.4±3.1	7.8± 0.9	24.7±3.6
caerulein	1	4.2±0.6	6.4±1.1	6.5±1.0	12.3±1.8	8.3±1.1	18.4±2.2	9.9± 1.5	23.8±2.8
	3	7.7±2.1	9.3±1.6	11.0±3.2	15.2±1.8	13.4±3.8	19.9±1.4	14.9± 3.8	24.0±1.9
	6	8.7±2.0	9.6±4.2	12.4±2.5	16.1±5.7	14.7±3.4	19.9±5.0	17.8± 3.4	24.4±6.1
	12	8.8±2.6	8.0±3.1	15.9±3.9	15.2±5.7	20.0±6.5	20.6±6.4	24.9± 6.8	25.7±7.5
	24	6.8±3.1	8.0±3.0	15.2±5.2	16.1±4.5	20.6±8.1	23.4±5.6	25.7±10.5	29.6±5.7
in vitro discharge of labeled proteins [percent of total content]									
Control	–	0.2±0.08	1.3±0.7	0.9±0.3	6.3±2.2	2.0±0.6	20.7±4.9	2.8±0.7	35.6± 5.3
5×10^{-6} g/kg/h	0.5	0.7±0.1	1.5±0.2	2.3±0.5	7.3±1.0	4.0±0.8	19.8±2.6	4.7±1.1	30.3± 2.0
caerulein	1	0.7±0.1	1.5±0.5	2.5±0.4	9.4±3.1	4.2±0.3	22.1±5.9	5.2±0.3	31.5± 6.0
	3	1.0±0.4	2.7±1.4	3.2±1.2	12.1±5.2	4.9±1.6	22.9±8.2	6.2±1.9	30.2±10.2
	6	1.2±0.4	3.3±1.6	4.5±1.0	14.5±5.3	14.8±1.2	7.4±6.9	9.3±1.6	25.6± 8.1
	12	2.9±0.7	4.1±1.9	8.6±1.1	13.9±4.1	12.8±1.8	22.2±5.3	16.2±2.3	25.0± 7.1
	24	1.9±0.5	2.3±0.3	4.4±0.7	5.7±1.8	7.2±1.5	9.6±4.1	9.4±2.0	13.2± 7.0

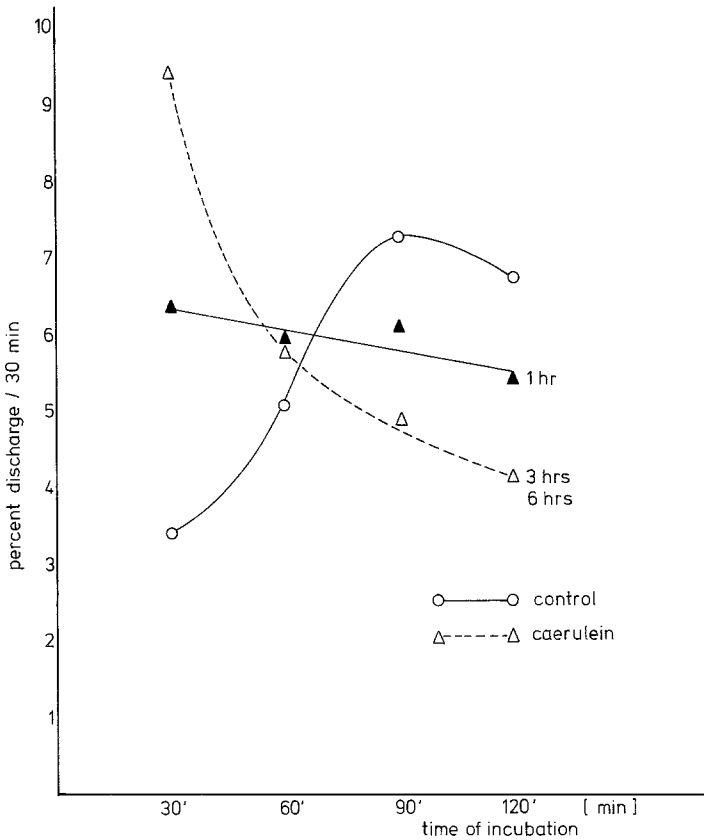

Fig. 10. Kinetics of in vitro discharge of amylase stimulated by 5×10^{-6} M carbamylcholine after in vivo infusion with 5×10^{-6} g/kg/h caerulein. The data are expressed as percent discharge of the total content for each 30 min period of incubation

between unstimulated release (so-called basal release) and discharge stimulated by 5×10^{-6} carbamylcholine in the incubation medium enables insights into the "leakiness" of the in vitro system. Table 3 lists the discharge of amylase and labeled proteins for each time point of in vitro incubation and compares basal and carbamylcholine-stimulated release. Under control conditions the basal release for both parameters over 2 h incubation amounted to about 2.5% of the total content in the tissue. If discharge is stimulated by a secretagogue a tenfold increase was observed over the same period. In vivo infusion of 5×10^{-6} g/kg/h caerulein led to an immediate and progressive increase in the rate of basal discharge of both amylase and labeled proteins (Table 3). By 24 h basal and stimulated discharge over a period of 2 h reached the same values. An interesting difference between the release of amylase compared to labeled proteins was observed if the kinetics of discharge were plotted for each 30 min-period of in vitro incubation (Fig. 10 and 11). Under control conditions there was a constant increase in the rate of discharge – in the case of amylase

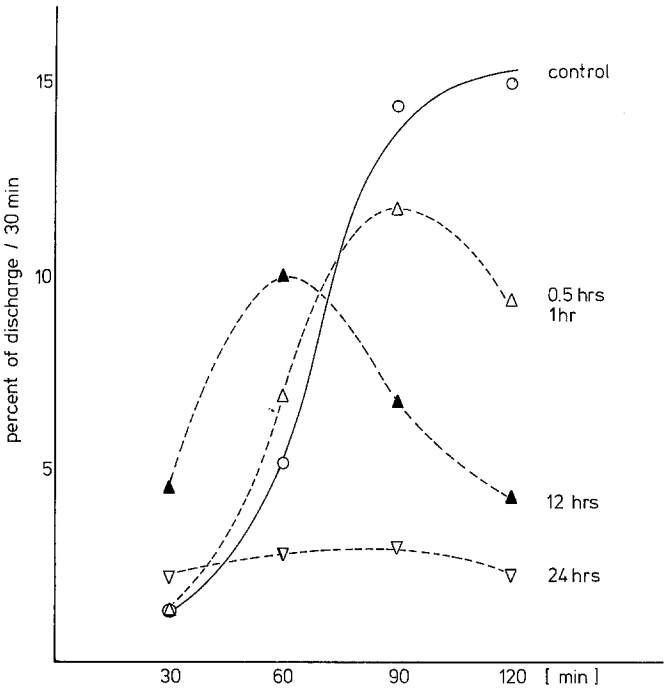

Fig. 11. Kinetics of in vitro discharge of labeled proteins stimulated by 5×10^{-6} carbamylcholine after caerulein infusion. Data are expressed as percent discharge of the total protein radioactivity released per each 30 min period of incubation

up to 90 min, with labeled proteins up to 2 h. After in vivo infusion of the high dose of caerulein a decrease of the rate of discharge of amylase was found after only 1 h, after 3 and 6 h the release follows the kinetics of wash-out (Fig. 10). The curve for 12 and 24 h had the same slope with higher percentage values (data not shown). Since discharge of labeled proteins was not obscured to the same extent as was amylase by the accumulation of the interstitial oedema, the kinetics of their release gave better insight in the relative responsiveness of the gland to in vitro stimulation. Figure 11 demonstrates that a consistent decrease in the maximal response of the pancreas to carbamylcholine in vitro was observed with increasing in vivo infusion time. After 24 h pretreatment the pancreas was practically insensitive to the in vitro action of the secretagogue.

Discussion

It has been previously demonstrated that in vivo infusion of low doses of caerulein (2.5×10^{-7} g/kg/h) leads to release of zymogen granules from the pancreas and to activation of several steps in the secretory process (Bieger et al., 1976a, b). During a total period of 72 h continuous stimulation no signs of cellular destruction or any rise in serum amylase levels have been observed.

The present study shows that excessive doses of the same synthetic secretagogue (5×10^{-6} g/kg/h) lead to severe disturbances of the secretory process and finally destroy their target organ, the exocrine pancreas. The primary event leading to this destruction is a premature fusion of condensing vacuoles and zymogen granules in the cytoplasm resulting in large vacuoles.

In general it is assumed that hormonal stimulation of the pancreas is exerted by specific binding of the secretagogue to the plasma membrane. The binding sets in motion a series of cytoplasmic events which finally lead to directional movement of granules towards the cell apex and fusion of the granule membrane to the apical plasma membrane (exocytosis). This well controlled process is obviously disturbed if excessive doses of the secretagogue are infused in vivo. Under such conditions the condensing vacuoles and the zymogen granules fuse to each other and also to the lateral plasma membrane. This leads to the formation of the large cytoplasmic vacuoles and also explains the development of interstitial oedema.

There are several reports in the literature indicating that secretory stimulation of the pancreas can induce cellular destruction in the acinar cells. Babkin et al. (1909) described the formation of large vacuoles and "inclusion bodies" in the cytoplasm after prolongued nervous stimulation of the pancreas or instillation of "soap" into the gut of anesthetized dogs. Similar results were obtained by Villaret et al. (1929) injecting acetylcholine, and these authors claimed for the first time the production of an acute pancreatitis by injection of a secretagogue (cholinergic agent). These results have been confirmed and extended in the rat by Leblond and Sergeyeva (1944). The destructive effect of repetetive injections of acetylcholine was potentiated by the injection of thyroxine, which by itself could also induce vacuolation of the exocrine pancreas, if given continuously over six days. The authors concluded that a potentiation of the normal parasympathetic stimulation of the pancreas leads to cellular destruction. Our own studies using a contineous infusion of a peptidergic secretagogue (caerulein) largely corroborate these previous findings. At low doses the pancreatic exocrine cells are stimulated to release their stored material and to activate all the consecutive steps in the secretory process (protein synthesis, intracellular transport and packaging, granule discharge). At higher doses the regulated sequence of the secretory process is quickly disturbed, the selectivity in the fusion process between the granule and the plasma membrane is lost and uncontrolled fusion occurs between individual granules and to the lateral plasma membrane. Similar changes have also been observed recently in vitro using excessive concentrations of carbamylcholine on isolated pancreatic lobules (Palade, personal communication).

Once the fusion of condensing vacuoles and secretory granules is initiated and formation of large cytoplasmic vacuoles occurs, a number of changes develop which are generally characteristic of acute interstitial pancreatitis (Doerr, 1964; Gambill, 1973). These include an immediate and progressive interstitial oedema followed by cellular infiltration, increase in serum amylase and lipase levels and cytoplasmic destruction of pancreatic cells.

It has to be concluded from these and previous studies (Bieger et al., 1976a, b) that the same synthetic secretagogue at low concentrations stimulates secretion

and at high doses can induce destruction of the target tissue. The initial event leading to this destructive effect is poorly understood. Consecutive studies (Adler et al., in preparation) were analyzing the fine structure of the internal organization of the plasma membrane by freeze-fracturing techniques. It is known that distinct differences in the distribution of membrane-associated particles exist between the lateral and apical plasma membrane, the latter showing close resemblance to the limiting membrane of zymogen granules (de Camilli et al., 1974). No obvious changes compared to control preparations were observed after various periods of caerulein infusion (5×10^{-6} g/kg/h). However, parallel studies analyzing the transport of amino acids across the plasma membrane, indicated that the cell has become "leaky" already after 30 min caerulein infusion (Adler et al., in preparation). It could be hypothesized that the premature fusion of granules inside the cell is initiated by uncontrolled movement of ions and/or other factors involved in stimulus-secretion-coupling (Case, 1974; Robberecht et al., 1974). These alterations of transport functions in the plasma membrane are not observed after infusion of low doses of caerulein (2.5×10^{-7} g/kg/h). It was recently demonstrated that the number and distribution of receptor proteins in the plasma membrane are regulated by the extracellular concentration of the hormone itself and that each binding event leads to the removal of the hormone-receptor-complex from the membrane (Gavin et al., 1974; Soll et al., 1975). Sustained circulation of an excessive concentration of a hormone could therefore induce drastic alterations in the molecular structure of the plasma membrane of the target tissue followed by severe disturbances of the secretory process.

These and our previous results (Bieger et al., 1976a, b) bear some relevance to earlier studies by Tardini et al. (1971) in which 1.2×10^{-6} g/kg/h caerulein were infused in anesthetized dogs for periods up to 12 h. This lead to release of zymogen granules from the exocrine pancreatic cells and to formation of large vacuoles in the cytoplasm. At later infusion periods autophagic vacuoles appeared, followed by fingerprint-like whorls (so-called "nebenkern"-formation) of the endoplasmic reticulum. The authors concluded that prolongued enzyme secretion leads to destruction of most of the machinery involved in the secretory process, followed by regeneration.

Our own studies in the rat (Bieger et al., 1976a, b) have shown that both the structural and biochemical reaction of the pancreas to caerulein infusion follow a close dose-response relationship. Chronic experiments injecting 10^{-6}, 5×10^{-6} and 2.5×10^{-5} g/kg/day caerulein in rats for up to 6 months gave similar results of pancreatic destruction and parenchymal atrophy as observed in this study after 24 h contineous infusion (Solcia et al., 1972). No pathological alterations were observed in the gastrointestinal tract. It was suggested by these authors that excessive release of pancreatic secretagogues might play a role in the onset of acute or chronic pancreatitis in man, which possibility can be studied by specific measurements of circulating levels of the different hormones which stimulate pancreatic secretion in man.

The infusion of excessive doses of caerulein in the rat or in other laboratory animals can be used as an experimental model for secretagogue-induced acute interstitial pancreatitis, in the analysis of its regression and in its prevention.

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