Virchows Arch. A Path. Anat. and Histol. 382, 31–47 (1979)

Virchows Archiv A

Course and Spontaneous Regression of Acute Pancreatitis in the Rat

Guido Adler, Thomas Hupp, and Horst F. Kern*

Department of Cell Biology and Cell Pathology, Philipps University, Marburg, Federal Republic of Germany

Summary. Rat exocrine pancreatic function was studied structurally and biochemically after the in vivo production of acute interstitial pancreatitis by supramaximal stimulation with caerulein. Two major phases in the reaction of the gland were observed: During the first two days after cessation of the supramaximal stimulation a progressive infiltration of the interstitium and the pancreatic tissue with polymorphonuclear leucocytes, lymphocytes and macrophages occurred which led to further destruction of the gland and to decreased functional response. From two days after the cessation of the treatment, hypertrophy of centro-acinar cells and an increased rate of mitotic activity indicated regeneration of the pancreas. This was combined with an accelerated in vitro discharge of newly synthesized proteins over a period of four days. Between days three and six after the initial treatment mitotic activity was also observed in fully differentiated exocrine cells. Total structural and functional recovery of the pancreas was achieved nine to twelve days after the cessation of the supramaximal stimulation.

Key words: Pancreatitis – Regeneration – Secretory process – Mitotic activity.

Introduction

It is generally assumed that activation of hydrolytic enzymes in the pancreas leading to autodigestion is the common pathogenetic principal of acute pancreatitis in man and in experimental animals (for review see Gambill, 1973; Dürr, 1978). Under physiological conditions autodigestion is prevented by distinct

Send offprint requests to: Prof. Dr. H.F. Kern, Department of Cell Biology and Cell Pathology, Philipps University, Robert-Koch-Str. 6, D-3550 Marburg/Lahn, Federal Republic of Germany

* Supported by a grant from Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg (SFB 122, project C 5). The expert technical assistance of Mrs. Traudl Seitz and Mrs. Hedwig Düking and the editorial help of Mrs. Gisela Lesch are gratefully acknowledged

0340-1227/79/0382/0031/$03.40

structural and biochemical protective mechanisms within the exocrine pancreatic cell:

1. From the beginning of their biosynthesis digestive enzymes are segregated from the cytosol by membranes of the various cellular compartments. Compartmental transport, packaging and storage systems, exclude free mixing with other cellular constituents (Jamieson and Palade, 1977).
2. Some of the most dangerous enzymes (mainly proteases) are synthesized as larger molecules which are biologically inactive (zymogens) and are activated only in the gut, by limited proteolysis. The concomitant synthesis, transport and packaging of specific protease-inhibitors within the pancreatic cell acts as a further protection against intracellular activation (Fritz et al., 1974).
3. The release of digestive enzymes from the pancreas by exocytosis is only possible by means of highly specific interactions between the membrane of the zymogen granule and the apical plasma membrane, thus ensuring discharge via the duct system only and not along the lateral plasma membrane into the interstitium (Meldolesi et al., 1978).

In the rat these protective mechanisms are well maintained even after pronounced secretory stimulation for several days. No destruction of exocrine pancreatic cells or symptoms of pancreatitis have been observed (Bieger et al., 1976a, b). However, supramaximal stimulation with excessive doses of a synthetic pancreatic secretagogue (caerulein) led to a quick degeneration of the regular secretory pathway, by uncontroled fusion among zymogen granules in the cytoplasm and with the lateral plasma membrane (Lampel and Kern, 1977). This resulted in a marked interstitial oedema and progressive destruction of the exocrine pancreas. Using freeze fracturing techniques it was demonstrated that only short term supramaximal stimulation induced marked alteration in the distribution of membrane associated particles and a disintegration of tight junctional networks along the acinar lumen (Adler et al., 1978). These structural changes were combined with profound disturbances in the specific transport of amino acids by the plasma membrane. The purpose of the present study was to carry out a time sequence of the course of the pancreatitis and the mechanisms of its regeneration.

Material and Methods

Acute interstitial pancreatitis was induced in male Wistar rats weighing 200 to 220 g by infusion of 2×10^{-6} M synthetic caerulein (batch TF 19750, Deutsche Farmitalia, Freiburg, Germany) via a jugular vein catheter for a standard period of 12 h. At the end of this period the rats were disconnected from the infusion system and the degree of pancreatic oedema was determined by macroscopical inspection through a small abdominal incision, under light ether anesthesia. The structural and functional state of the exocrine pancreas was assessed at the end of the 12 h caerulein infusion and after 1, 2, 3, 6, 9 and 12 days following the induction of pancreatitis and was compared to saline infused and similarly disconnected controls. The animals were killed by cardiac section under light ether anesthesia, the pancreas was removed and placed in ice cold Krebs-Ringer solution containing amino acids as specified by Eagle (1959), 14 mM glucose and 15 mM Hepes to assure constancy of pH. For biochemical studies pancreatic lobules were prepared according to the procedure of Scheele and Palade (1975). Ice cold incubation medium was injected into the interstitium with a 20 ml syringe and Nr. 20 needles, resulting in visible lobules, which were excised in a size of 1 to 2 mm^2 using watch makers forceps and fine scissors. A set of 5 lobules was 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. For analysis of in vitro discharge of enzymes and newly synthesized proteins from pancreatic lobules after in vivo pretreatment the system of Jamieson and Palade (1971) was used. They 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 10^{-10} M caerulein or 5×10^{-6} M carbamylcholine. 1-ml-samples from these incubations were taken after 10, 20, 30, 40, 50 and 60 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. The rate of protein synthesis in pancreatic lobules from the same animals was determined by the incorporation of L-leucine-4,5-^{3}H into TCA-precipitable proteins after 30, 60 and 90 min incubation. Specific proteinbound radioactivity was expressed per µg DNA.

To assess a possible relationship between the extent of pancreatic destruction and the time needed for regeneration, in a seperate set of experiments less severe changes were produced by a 3 h infusion of supramaximal doses of caerulein. The structural and biochemical aspects of the secretory process were investigated at the end of this treatment and after 3, 6, 12 and 24 h following disconnection.

Assay Procedures

Samples from incubation media and tissue homogenates were precipitated on filter discs at 4° C with 10% trichloroacetic acid (TCA) and washed twice with 5% TCA. After carefully drying the filter papers were counted in 5 ml Insta-Fluor liquid scintillation fluid in a Packard Tri-Carb counter, model 3380. (both from Packard Instrument Company, Frankfurt, Germany)

Proteins were determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin as standard. DNA was measured according to Richards (1974), amylase according to Bernfeld (1955) and lipase according to Zinterhofer et al. (1973). Chymotrypsinogen was activated with trypsin (50 µg/mg protein) and measured 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.

Morphology

The biochemical findings were correlated to the fine structure of pancreatic lobules at each experimental period. The lobules were fixed in a mixture of 2,5% formaldehyde and 2,5% glutaraldehyde containing 0.05% picric acid (Ito and Karnovsky, 1968). After standard procedures of tissue processing and cutting sections stained with uranylacetate and lead citrate were examined in a Zeiss EM 9 S electron microscope.

To analyze the process of tissue regeneration in a seperate set of experimental animals the rate of mitotic activity after induction of pancreatitis and after 1, 2, 3, 9 and 12 days was studied using two intravenous injections of 500 µg/kg vinblastine (Velbe, E. Lilly, Bad Homburg, Germany) 1 and 2 h prior to killing of the animals. The pancreas was fixed in total in Bouins fixative, and after embedding in paraffin, 5 µ thick sections were stained with hematoxylin and eosin for light microscopic counting of mitotic figures.

Results

Table 1 summarizes the tissue content of three digestive enzymes after induction of the acute pancreatitis and after various periods following discontinuation. It became evident that the content of amylase and chymotrypsin in the total homogenate of oedematous glands did not change significantly during the 12 h caerulein infusion when compared with controls, indicating that no substantial

Table 1. Enzyme content of the total homogenate of control rat pancreas, after caerulein infusion for 12 h and after various periods following the infusion. The values are expressed in International Units (IU) per DNA content in the same homogenate. They represent the means from six identical experiments in each experimental group

	Amylase content IU/μg DNA	Chymotrypsin content ImU/μg DNA	Lipase content ImU/μg DNA
Control	7.19 ± 1.14	105.3 ± 41.39	1,633 ± 409.5
12 h Caer.	6.57 ± 1.39	110.3 ± 7.02	561.6 ± 95.8
24 h post inf.	1.00 ± 0.54	56.4 ± 33.04	288.6 ± 170.2
48 h post inf.	0.35 ± 0.30	24.9 ± 14.52	294.5 ± 150.5
72 h post inf.	1.42 ± 0.95	72.2 ± 28.04	478.6 ± 205.4
6 d post inf.	4.14 ± 2.69	121.0 ± 35.5	1,292 ± 38.37
9 d post inf.	6.15 ± 0.91	138 ± 38.18	1,504 ± 177.01
12 d post inf.	6.90 ± 1.12	130.7 ± 24.1	1,600 ± 230.0

Table 2. Incorporation of L-leucine-4,5-^{3}H into TCA-precipitable proteins after 90 min in vitro incubation of pancreatic lobules from control and caerulein treated animals. Values are expressed in percent of controls, $n = 6$ in each experimental group

Control	100% = 6,280 ± 1,948 cpm/μg DNA
12 h Caer.	45.95 ± 9.5
24 h post inf.	32.39 ± 3.2
48 h post inf.	38.12 ± 8.68
72 h post inf.	50.69 ± 16.07
6 d post inf.	78.53 ± 8.71
9 d post inf.	105.24 ± 6.30
12 d post inf.	97.55 ± 10.5

release via the duct system has taken place. Only the content of lipase was found to be reduced, by about 60%. However, a marked reduction in enzyme content was observed during the first three days following discontinuation of the caerulein treatment and a slow recovery occurred after 6 and 12 days. Similar results were obtained when the rate of protein synthesis was measured by the incorporation of tritiated leucine into TCA-precipitable proteins (Table 2). As has been demonstrated previously (Lampel and Kern, 1977), supramaximal stimulation by caerulein reduced protein synthesis by about 50%, and this effect became more pronounced following discontinuation for one and two days. A total recovery of the rate of protein synthesis was evident only after nine and twelve days. The fact that functional alterations of the secretory process of the pancreas became aggrevated after discontinuation of the caerulein treatment was also evident when the release of newly synthesized proteins was compared with the discharge of amylase in vitro (Figs. 1 and 2). In the radioassay system according to Jamieson and Palade (1971) a small pool of newly synthesized and radioactively labeled proteins is compared with a large pool of stored enzymes (measured as e.g. amylase). In the absence of secretagogues in vitro about one percent of the newly synthesized proteins and two percent of amylase were released into the medium during a 30 min incubation period

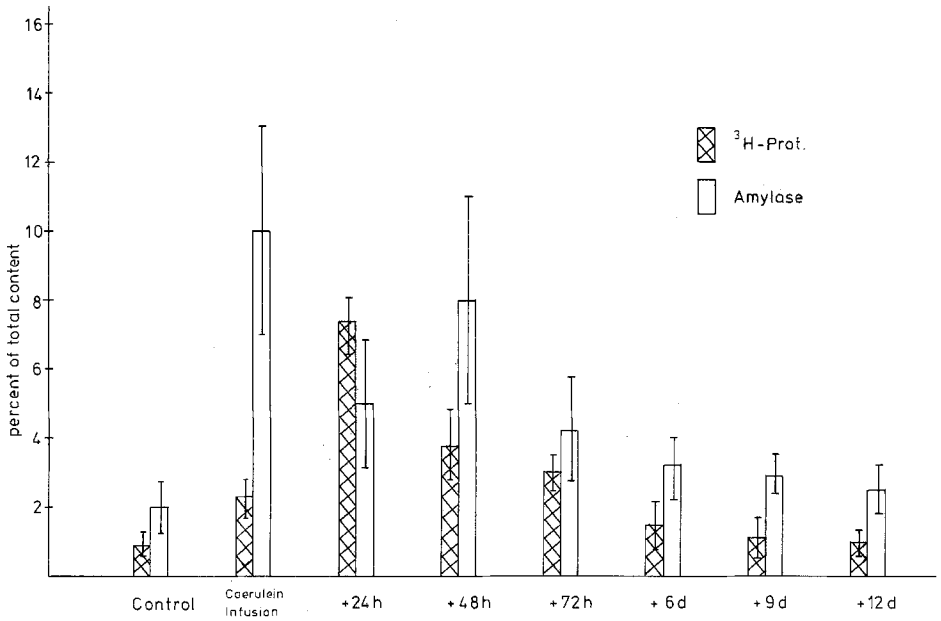

Fig. 1. Unstimulated discharge of pulse-labeled proteins (hatched column) and of amylase from pancreatic lobules of controls, after supramaximal stimulation with caerulein for 12 h, and after various periods following the treatment. Values of 30 min incubation are expressed as percent of total content in tissue and medium. The values represent means of 6 identical experiments ± standard error

(Fig. 1). After supramaximal stimulation with caerulein for 12 h this unstimulated release of amylase was increased by a factor of five, and this has been shown previously to be due mainly to wash-out of interstitial oedema (Lampel and Kern, 1977). Discharge of newly synthesized proteins at the end of the supramaximal stimulation was only slightly enhanced but became markedly increased after discontinuation for one and two days. It returned to control levels after six to twelve days following discontinuation. While these data on unstimulated discharge are generally taken as a measure for tissue integrity or uncontroled leakage, stimulated discharge serves a measure for the secretory responsiveness of the organ to specific secretagogues. Figure 2 summarizes the kinetics of in vitro discharge of pulse labeled proteins using 10^{-10} M caerulein as secretagogue. Under control conditions an increased rate of discharge occured from 30 min on with a steady increase up to 60 min. After 12 h supramaximal stimulation this secretory response was greatly diminished while one day after discontinuation the release pattern followed simple wash-out kinetics (Fig. 2, upper panel).

From two days after discontinuation up to six days the pancreas showed an increased secretory activity with a shift of the peak rate toward earlier time points. Under control conditions the highest rate of release was reached by 60 min incubation with little discharge during the initial 20 min. After 3 and 6 days following the induction of the pancreatitis a significant release of

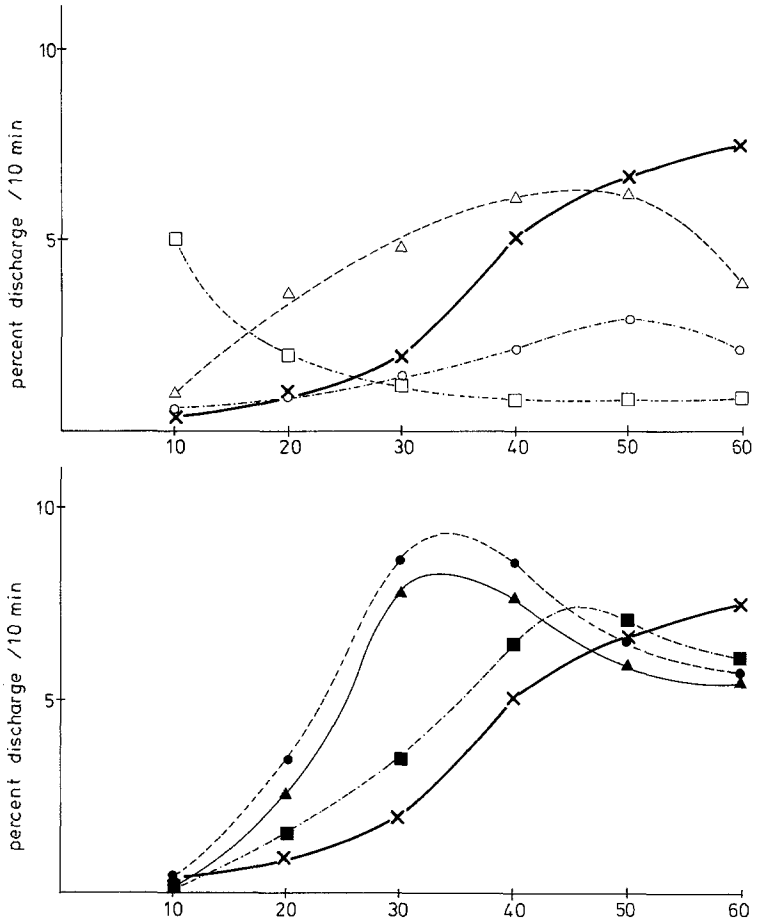

Fig. 2. Kinetics of in vitro discharge of pulse labeled proteins from controls (×——×), after induction of pancreatitis (○——○) and after various periods following the treatment. □——□ 1d, △——△ 2d, ●——● 3d, ▲——▲ 6d, ■——■ 12d. Values are expressed as percent of total content released during each 10 min incubation period and are means of 6 identical experiments

newly synthesized proteins was found after only 20 min incubation and the peak rate hat developed by 30 min and decreased thereafter (Fig. 2, lower panel).

Table 3 summarizes results from a seperate set of experiments in which animals were infused for 3 h with a supramaximal dose of caerulein and the course of the pancreatitis was studied as previously. It became evident that even this short excessive stimulation reduced enzyme content in the pancreas and the rate of protein synthesis significantly. Similarly to the results reported above unstimulated discharge of both amylase and newly synthesized proteins was increased. As in the previous experiments with 12 h supramaximal stimulation cessation of the treatment was followed by an aggrevation of the changes in most steps of the secretory process at 3 and 6 h post infusion. However, recovery was obtained much faster, namely 24 h after discontinuation of the

Table 3. Major variables in the secretory process of rat exocrine pancreas after 3 h supramaximal stimulation compared to various periods after cessation of the treatment. Values are expressed as percent of controls, discharge of amylase and labeled proteins are expressed as percent of total content released during 30 min incubation; $n=6$ in each experimental group

	Control	Caerulein infusion (3 h)	3 h post inf.	6 h post inf.	12 h post inf.	24 h post inf.
Protein synthesis	100% = 5,324 ± 990 cpm/μgDNA	74 ±14	53.9 ±4.3	24.3 ±2.0	51.1 ±7.7	70.7 ±3.2
Amylase content	100% = 8.98 ± 1.63 IU/μgDNA	61.35 ± 21.1	48.7 ±14.4	87.4 ±15.5	126.2 ±20.6	04.4 ±21.1
Discharge of amylase stimulated	16.7 ±3.7	17.4 ±2.5	12.0 ±0.6	12.0 ±2.7	17.3 ±2.0	16.5 ±1.8
(% of total content) basal	1.98 ± 0.7	11.14 ± 1.29	7.13 ± 0.7	3.9 ±0.9	1.73 ± 0.4	1.7 ±0.4
Discharge of ^{3}H-proteins stimulated	4.15 ± 1.13	7.8 ±1.2	1.87 ± 0.3	3.4 ±0.4	4.02 ± 1.5	3.55 ± 0.82
(% of total content) basal	0.99 ± 0.3	2.28 ± 1.4	1.7 ±0.4	2.06 ± 0.4	1.1 ±0.4	1.2 ±0.3

treatment the content in enzymes and their discharge in the presence or absence of a secretagogue had returned to normal conditions. Only the rate of protein synthesis was still reduced by about 30%.

These biochemical data were correlated to the structure of the pancreatic lobules during the course of the pancreatitis. Light microscopic investigation revealed distinct stages: at the end of the 12 h supramaximal stimulation most pancreatic cells contained large vacuoles and individual acini were seperated by a marked interstitial oedema (Fig. 3a). One day following discontinuation of the treatment the cytoplasmic vacuoles had disappeared, the size of the acini had significantly decreased and the interacinar space revealed numerous free cells and long tubular elements of the duct system (Fig. 3b). After three days most of the exocrine cells again showed storage of zymogen granules but cellular infiltration of the interstitium was still evident (Fig. 3c). After six days the regular structure of exocrine pancreatic acini had been restored (Fig. 3d). At the electron microscopic level initial vacuolization of most exocrine cells at the end of the 12 h supramaximal stimulation and disintegration of the regular architecture of pancreatic acini 24 h later, were obvious (Fig. 4a, b). At this stage the exocrine cells showed a variable degree of secretory granulation and severe changes in cytoplasmic fine structure, including destruction of rough endoplasmic reticulum (RER), mitochondrial damage and different stages of autophagia (Fig. 4b). There was an increasing tendency for cellular infiltration in the interstitial space, starting during supramaximal stimulation

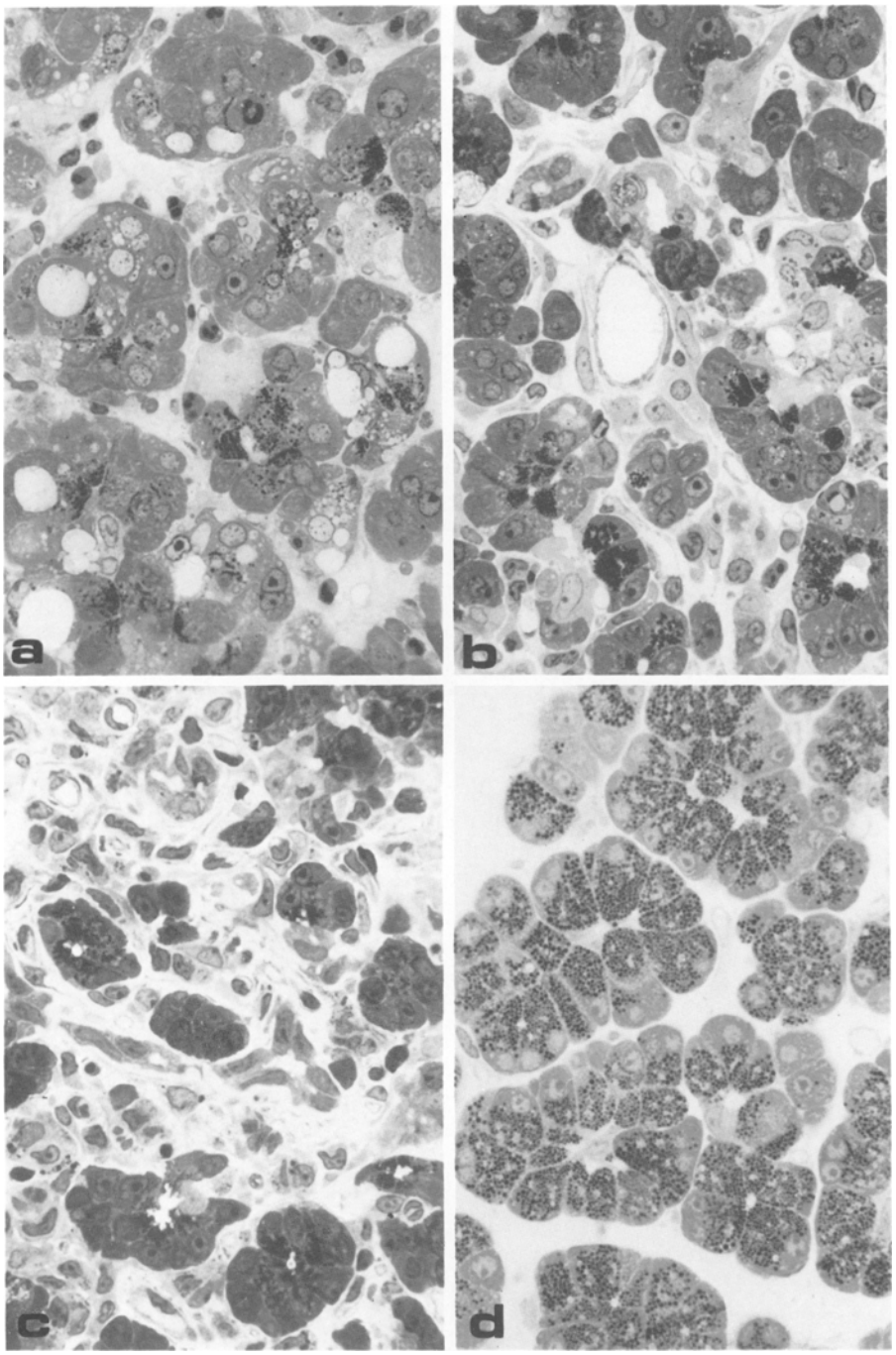

Fig. 3a–d. Light microscopic appearance of pancreatic lobules after 12 h infusion of caerulein (**a**) and after 1 (**b**), 2 (**c**) and 12 (**d**) days following the treatment. Magnification × 528

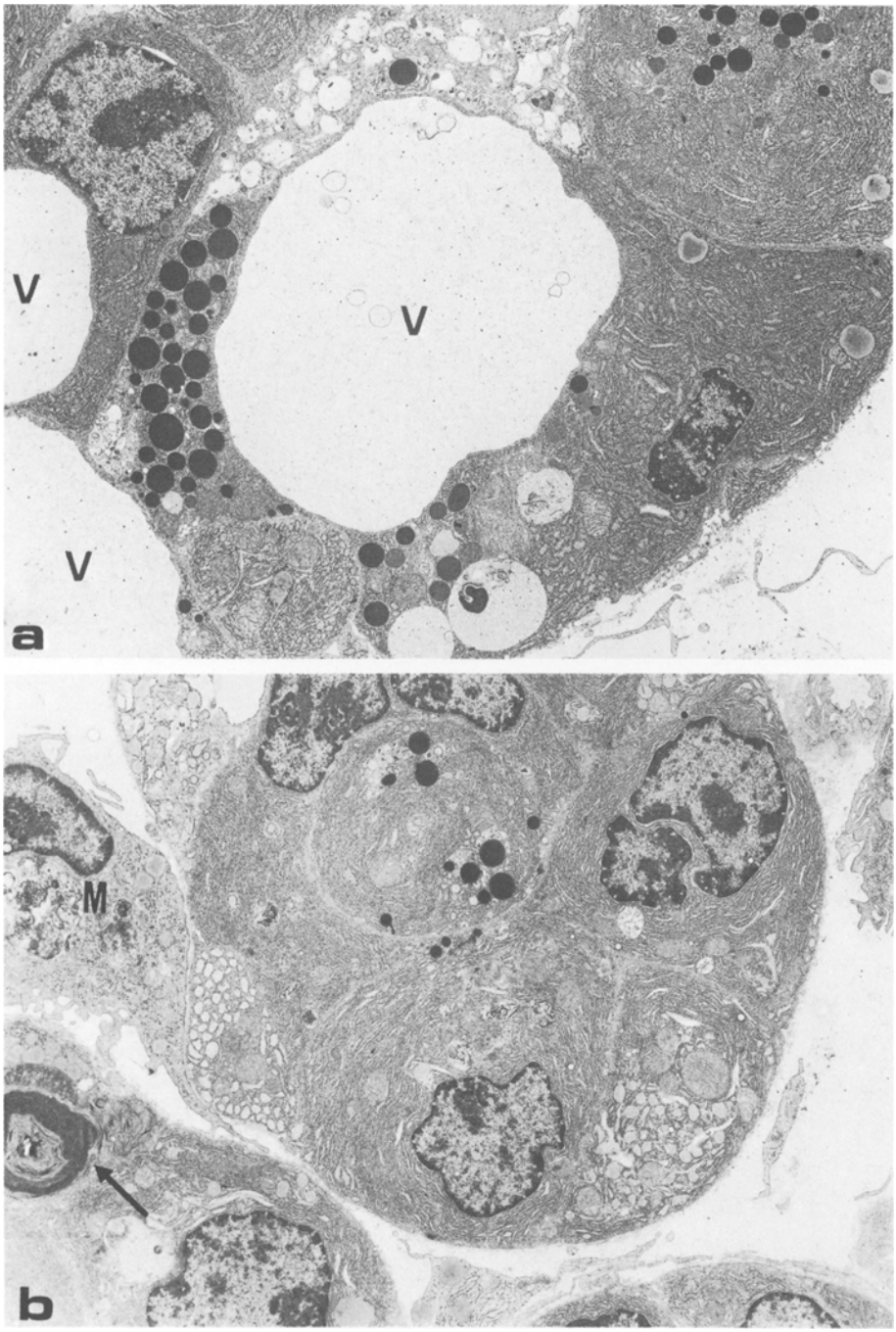

Fig. 4 a and b. Extensive vacuolization (*V*) 12 h after supramaximal stimulation (**a**) and disappearance of vacuoles combined with destruction of exocrine cells 24 h after cessation of the treatment (**b**). *M* macrophage, the arrow indicates residual body in exocrine cell. Magnification × 3,800

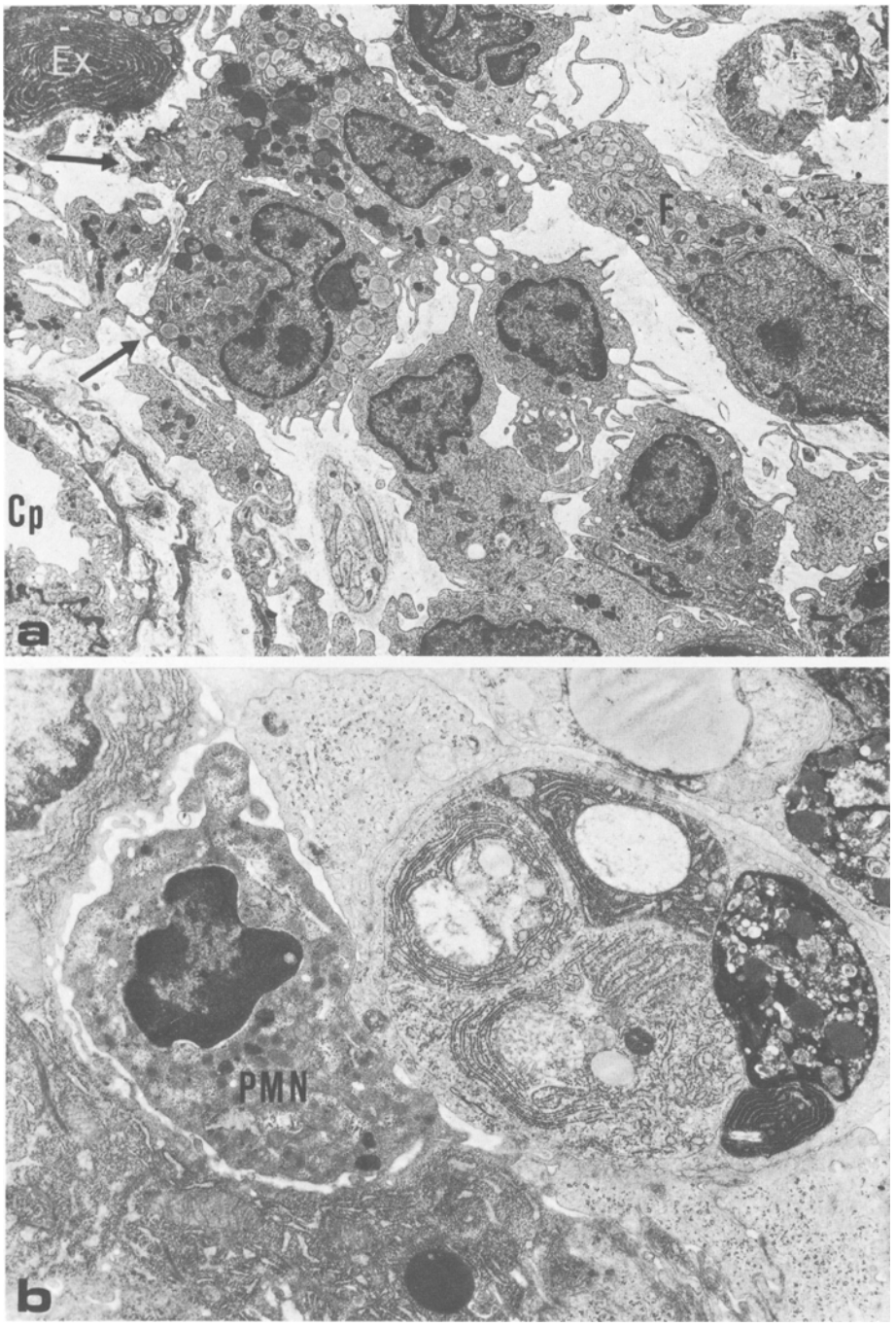

Fig. 5. a Cellular infiltration, predominantly by macrophages, 24 h after cessation of supramaximal stimulation. The arrows indicate two macrophages with numerous heterophagic bodies. *F* fibroblast; *Cp* capillary; *Ex* exocrine cell. Magnification × 3,800. **b** Polymorphonuclear leucocyte (PMN) invading exocrine tissue and macrophage phagocytosing sequestered exocrine pancreatic material, from the same animal as a. Magnification × 9,400

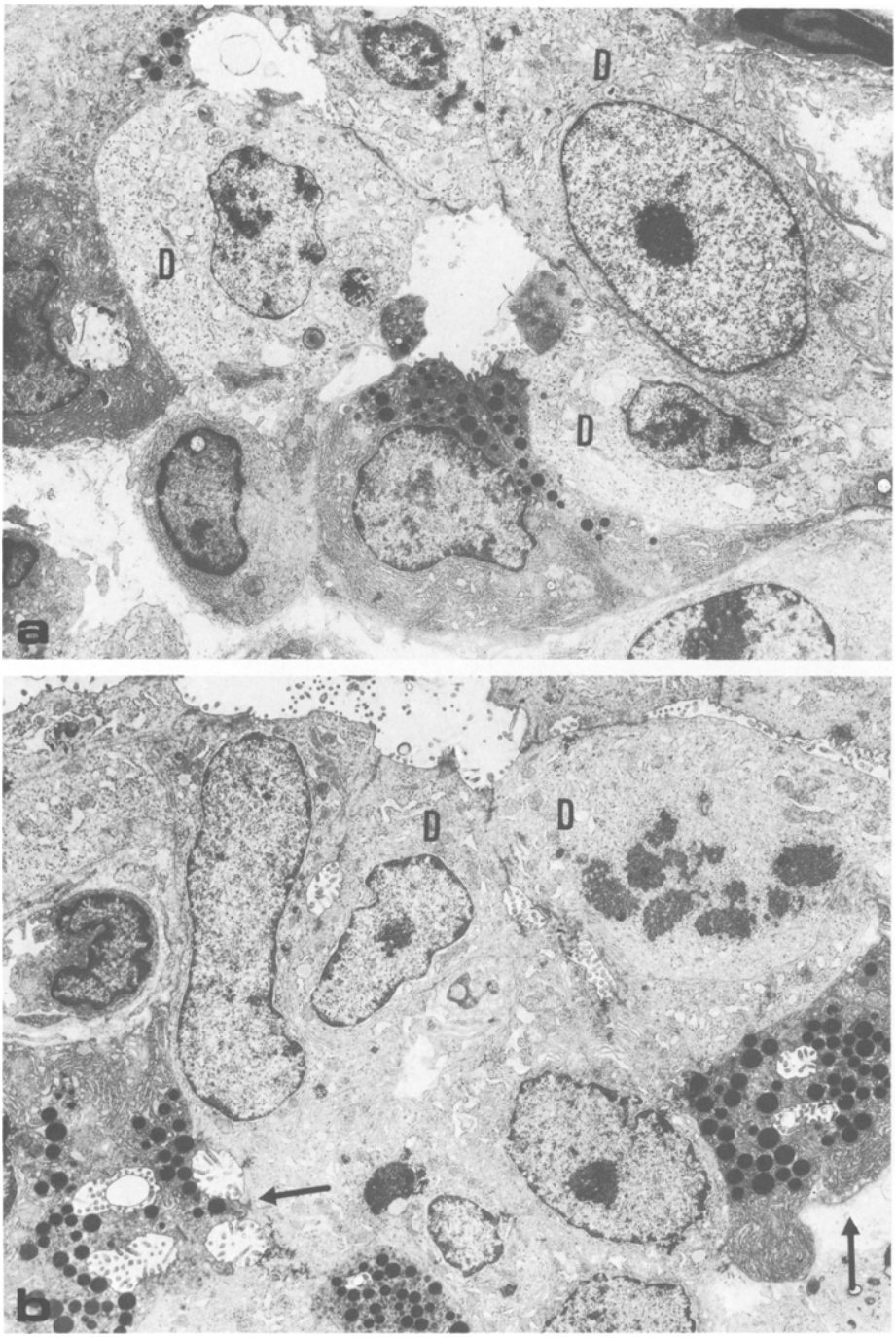

Fig. 6a and b. Hypertrophy (**a**) and mitotic activity (**b**) of centroacinar cells (*D*) 24 and 48 h after induction of acute pancreatitis. Note small irregular remnants of exocrine pancreatic cells (*arrows*). Magnification × 3,800

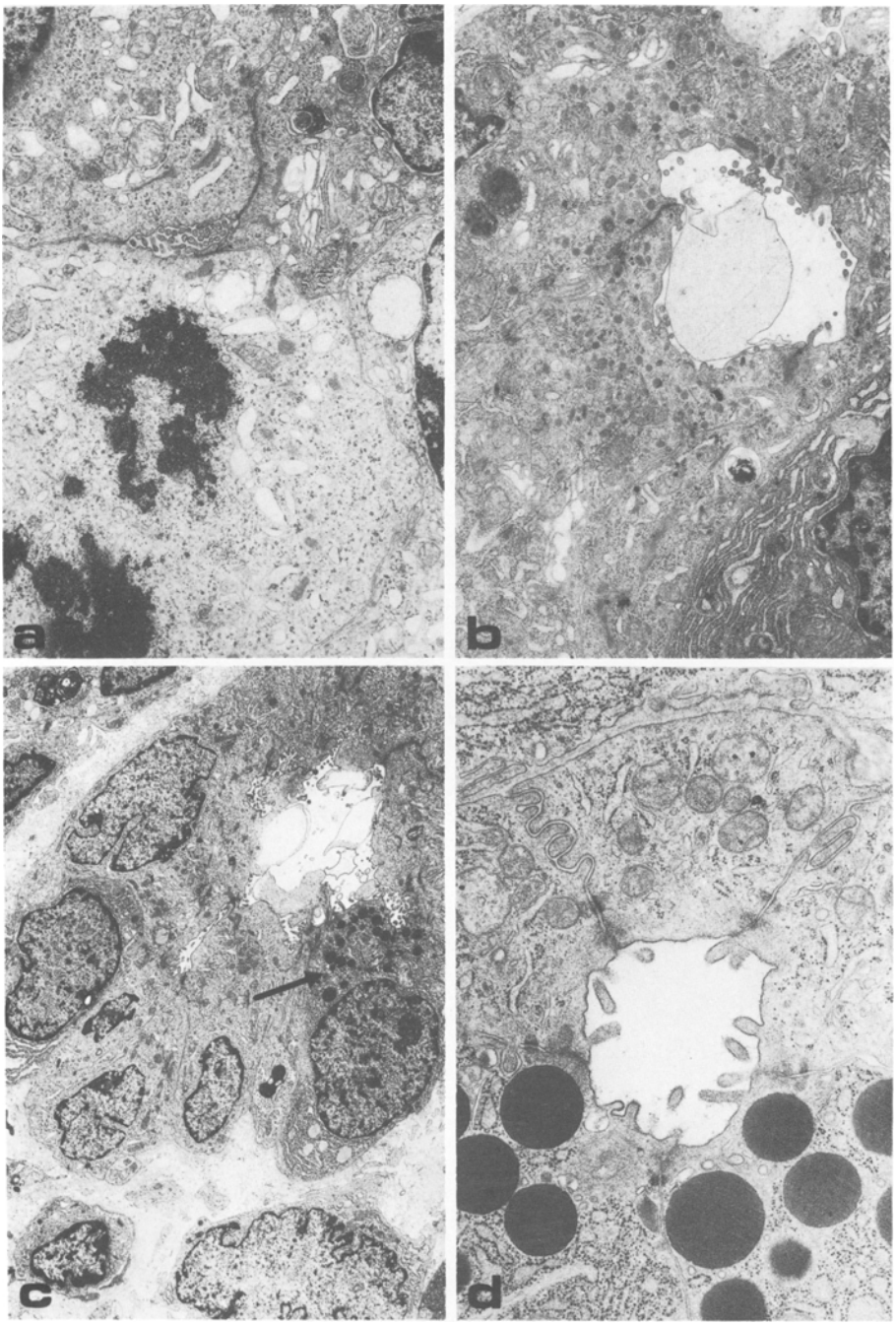

Fig. 7 a–d. Tentative stages in the differentiation of centroacinar to exocrine pancreatic cells. **a** Centro-acinar cell in mitosis at 2 days post treatment. **b** Accumulation of small secretory granules after 3 days. **c** Storage of zymogen granules in centro-acinar cell (*arrow*) at the same day. **d** Regular fine structure of centro-acinar cells 9 days after pancreatitis. Magnification: a, b, d × 12,000; c × 2,600

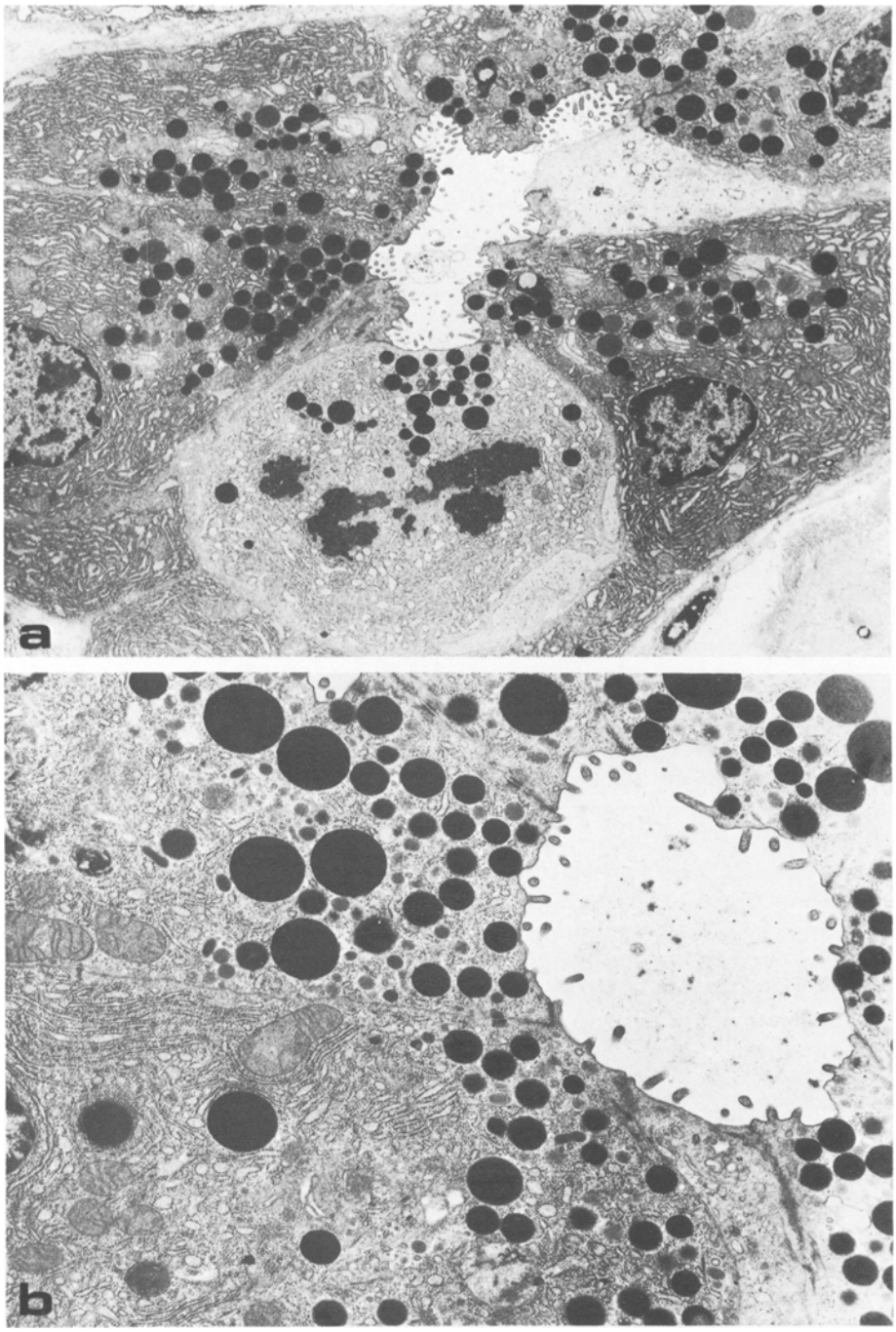

Fig. 8. **a** Mitosis of exocrine pancreatic cell 3 days after induction of pancreatitis. Magnification × 3,800. **b** storage of two populations of secretory granules at the same time. Magnification × 16,000

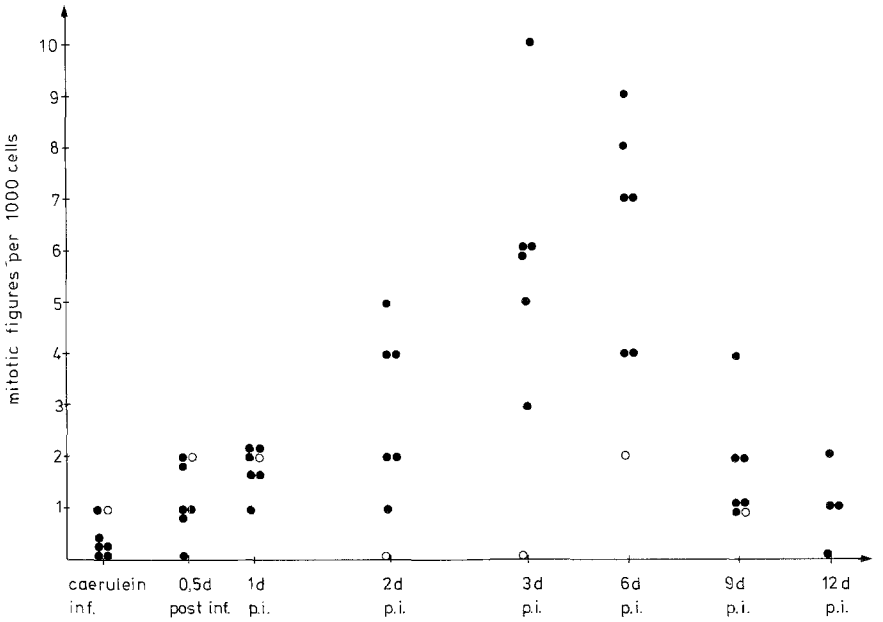

Fig. 9. Mitotic activity of exocrine pancreatic cells and duct cells of control animals (*open circles*), after induction of pancreatitis by 12 h caerulein infusion and after various periods following the treatment. Values are expressed as mitotic figures per 1000 pancreatic cells

but progressing significantly after discontinuation of the treatment. Infiltrating cells included polymorphonuclear leucocytes, lymphocytes and macrophages, which were found in large numbers in the interstitium (Fig. 5a) but also invading between exocrine cells of an acinus (Fig. 5b). Pronounced phagocytotic activity of macrophages, especially during the first two days after discontinuation of supramaximal stimulation, became evident from the numerous stages of endocytosed organelles and sequestrations from exocrine pancreatic cells (Fig. 5b). Concomitant with cellular infiltration, deposition of collagen fibers occured in the vicinity of long, slender fibroblasts.

Parallel to these destructive changes in exocrine cells which progressed during the first two days after stimulation, regenerative changes in the duct system were observed especially in centro-acinar cells. One day after the cessation of the treatment pancreatic acini often contained isolated exocrine cells which had decreased in size. However, centro-acinar cells had greatly enlarged their cytoplasm and nuclei and contained prominent nucleoli (Fig. 6a). An increased number of mitotic figures was observed starting from two to 6 days after induction of pancreatitis (Fig. 6b). From variations in the fine structure of different centro-acinar cells a tentative sequence of their transformation into secretory exocrine cells could be deduced (Fig. 7a–d). During the initial hypertrophy of centroacinar cells and during mitosis their cytoplasm mainly contained free ribosomes and only individual elements of RER (Fig. 7a). At a later stage centro-acinar cells had accumulated profiles of RER and elaborate Golgi complexes in the vicinity of which small secretory granules of moderate electron

density were observed (Fig. 7b). At a later time point such cells contained typical zymogen granules (Fig. 7c), while by six to twelve days after the supramaximal stimulations the regular fine structure of small centro-acinar cells was again observed (Fig. 7d). Around the same period the original architecture of exocrine acini had been restored, but mitotic figures in fully differentiated exocrine pancreatic cells were still encountered (Fig. 8a). It is interesting to note that from three to six days after cessation of hormone infusion the exocrine pancreatic cells contained a mostly mixed population of secretory granules: small electron translucent granules measuring 150 to 200 nm and large electron dense zymogen granules measuring between 500 to 800 nm (Fig. 8b). The process of tissue regeneration by mitotic division of centro-acinar and acinar cells is summarized in Fig. 9. There was no change in mitotic activity when compared with controls, during the first 24 h after cessation of caerulein infusion. From two days to six days the mitotic index increased significantly, to reach control levels again by nine and twelve days.

Discussion

The present study confirms and extends previous communications from our laboratory which indicated that supramaximal hormonal stimulation of the rat exocrine pancreas leads to self-destruction of the gland (Lampel and Kern, 1977). The primary event and original pathogenetic principal is a random and uncontrolled fusion among zymogen granules within the cytoplasm and with the lateral instead of the apical plasma membrane (Adler et al., 1978). This is combined with a destruction of the network of tight junctions sealing off the acinar lumen from the interstitial space. Both mechanisms contribute to the development of a pronounced interstitial oedema which is mainly composed of digestive enzymes. Furthermore the formation of large cytoplasmic vacuoles, described in an earlier communication (Lampel and Kern, 1977), could induce activation of digestive enzymes inside the pancreatic cells and result in their destruction. As a secondary event the oedematous pancreas is infiltrated by polymorphonuclear leucocytes, lymphocytes and macrophages and this inflammatory reaction leads to a further aggrevation of the syndrome and finally to pancreatitis. It might be assumed, that the progressive infiltration of the pancreas by inflammatory cells following the formation of interstitial oedema is mediated by the chemotactic influence of denatured proteins from destroyed pancreatic cells and by components of the complement system (for review see Zigmond, 1978), but this hypothesis remains to be tested. The complement system has recently been implicated as a pathogenetic factor in the formation of acute pancreatitis in laboratory animals (for review see Seelig and Seelig, 1975a, b). Proteolytic enzymes from the interstitial oedema may activate components of the complement system which then damage cell membranes, in particular the plasma membrane of the exocrine cells, and thus potentiating the original hormonal effect.

Both the biochemical and fine structural results of this study indicate that the acute interstitial pancreatitis induced is aggrevated after discontinuation

of the supramaximal stimulation. This is evident from the more pronounced inhibition of the rate of protein synthesis one and two days after supramaximal stimulation (Table 2) and from the significantly enhanced rate of unstimulated release of pulse labeled proteins during the same time period when compared with the end of the caerulein infusion (Fig. 1). There was a close correlation between the degree of interstitial oedema produced by various periods of caerulein infusion and the time needed for a full structural and biochemical recovery of the pancreas. Table 3 summarizes results from a separate set of experiments in which the course of the pancreatitis after 3 h caerulein infusion was followed. After the 12 h supramaximal stimulation full functional restitution took 9 and 12 days. However a shorter period (3 h) induced intracellular vacuolization and moderate interstitial oedema, with only slight infiltration by inflammatory cells. Most inidators of the secretory process had returned to control levels 24 h after such a treatment.

It is therefore assumed that the extent of the secondary inflammatory reaction largely determines the course and time sequence of post-stimulatory pancreatitis and its regeneration. Since most exocrine cells had been damaged by this secondary reaction, regeneration started predominantly from elements of the duct system, mainly from centro-acinar cells. These appeared to be hypertrophied two days after induction of oedema and a high rate of mitotic activity was found at six to nine days. Formation of elements of the RER and elaborate Golgi complexes in the vicinity of which numerous small granules were observed suggested the transformation of centro-acinar cells into exocrine pancreatic cells. In this respect our findings are in agreement with the extensive experimental studies by Fitzgerald and his group on pancreatic regeneration (Fitzgerald, 1960; Fitzgerald et al., 1968a, b; Marsh et al, 1968; Lehv and Fitzgerald, 1968). It is interesting to note that during this period of tissue regeneration the pattern of in vitro discharge of newly synthesized proteins suggests an increased rate of release and also accelerated transport of these proteins through the pancreatic cells (Fig. 2). Similar results had been obtained using maximal secretory stimulation with caerulein without cellular damage (Bieger et al., 1976a, b). It remains to be seen whether during recovery from acute interstitial pancreatitis the exocrine pancreas proceeds through different phases of secretory responsiveness to hormonal stimuli. This might indicate increased sensitivity to physiological stimulation.

The original cause for the induction of acute pancreatitis in the rat after supramaximal hormonal stimulation remains unknown. In vitro studies analyzing amino acid transport accross the plasma membrane indicate severe changes in its structural and functional integrity (Adler et al., 1978) which could lead to uncontroled ion fluxes into the cytoplasm. This in term would initiate random fusion of zymogen granules and would interfer with the controled sequence of the regular steps in the secretory process.

References

Adler, G., Bieger, W., Kern, H.F.: Amino acid transport in the rat exocrine pancreas. III. Effect of maximal and supramaximal hormonal stimulation in vivo. Cell Tiss. Res. **194**, 447–462 (1978)

Bernfeld, P.: Amylases α and β. In: Methods in enzymology, Vol. 1, pp. 149–158. New York: Academic Press 1955

Bieger, W., Martin-Achard, A., Bassler, M., Kern, H.F.: Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. IV. Stimulation by in vivo infusion of caerulein. Cell Tiss. Res. **165**, 435–453 (1976a)

Bieger, W., Seybold, J., Kern, H.F.: Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. V. Kinetic studies on accelerated transport following caerulein infusion in vivo. Cell Tiss. Res. **170**, 203–220 (1976b)

Dürr, H.K.: Acute pancreatitis, including relapsing acute pancreatitis. In: The exocrine pancreas, H. Sarles and H.T. Howar (eds.). London: Saunders (in press)

Fitzgerald, P.J.: The problem of the precursor cell of regenerating pancreatic acinar epithelium. Lab. Invest. **9**, 67–85 (1960)

Fitzgerald, P.J., Herman, L., Carol, B., Roque, A., Marsh, W.H., Rosenstock, L., Richards, C., Perl, D.: Pancreatic acinar cell regeneration. I. Cytologic, cytochemical, and pancreatic weight changes. Am. J. Pathol. **52**, 983–1011 (1968a)

Fitzgerald, P.J., Vinijchaikul, K., Carol, B., Rosenstock, L.: Pancreatic acinar cell regeneration. III. DNA synthesis of pancreas nuclei as indicated by thymidine-^{3}H autoradiography. Am. J. Pathol. **52**, 1039–1065 (1968b)

Fritz, H., Tschesche, H., Greene, L.J., Truscheit, E.: Proteinase inhibitors. Berlin, Heidelberg, New York: Springer 1974

Gambill, E.E.: Pancreatitis, Saint Louis: C.V. Mosby Comp. 1973

Hummel, B.C., A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. Canad. J. Biochem. **37**, 1393–1399 (1959)

Ito, S., Karnovsky, M.J.: Formaldehyde-glutaraldehyde fixatives containing trinitro compounds. J. Cell Biol., **39**, 168a–169a (1968)

Jamieson, J.D., Palade, G.E.: Synthesis, intracellular transport and discharge of secretory proteins in unstimulated pancreatic exocrine cells. J. Cell Biol. **50**, 135–158 (1971)

Jamieson, J.D., Palade, G.E.: Production of secretory proteins in animal cells. In: International cell biology 1976–1977, B.R. Brinkley and K.R. Porter (eds.) pp. 308–317. New York: The Rockefeller University Press 1977

Lampel, M., Kern, H.F.: Acute interstitial pancreatitis in the rat induced by excessive doses of a pancreatic secretagogue. Virchows Arch. A Path. Anat. and Histol. **373**, 97–117 (1977).

Lehv, M., Fitzgerald, P.J.: Pancreatic acinar cell regeneration. IV. Regeneration after surgical resection. Am. J. Pathol. **53**, 513–535 (1968)

Marsh, W.H., Goldsmith, S., Crocco, J., Fitzgerald, P.J.: Pancreatic acinar cell regeneration. II. Enzymatic, nucleic acid, and protein changes. Am. J. Pathol. **52**, 1013–1037 (1968)

Meldolesi, J., Borgese, N., DeCamilli, P., Ceccarelli, B.: Cytoplasmic membranes and the secretory process. In: Cell surface reviews, G. Poste and G.L. Nicolson, (eds.) Vol. 5, pp. 510–598. Amsterdam, New York, Oxford: North-Holland Publishing Comp. 1978

Reggio, H.A., Palade, G.E.: Sulfated compounds in the zymogen granules of the guinea pig pancreas. J. Cell Biol. **77**, 288–314 (1978)

Richards, G.M.: Modifications of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. Analyt. Biochem. **57**, 369–376 (1974)

Scheele, G.A., Palade, G.E.: Studies on the guinea pig pancreas. Parallel discharge of exocrine enzyme activities. J. Biol. Chem. **250**, 2660–2670 (1975)

Seelig, R., Seelig, H.P.: The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. I. Immunopathogenetic pancreatitis, local Schwartzman – Sanarelli phenomenon, endotoxin induces pancreatitis. Acta Hepato-Gastroenterol. **22**, 263–268 (1975a)

Seelig, R., Seelig, H.P.: The possible role of serum complement system in the formal pathogenesis of acute pancreatitis. II. Cobra venom factor pancreatitis, sodium taurocholate and deoxycholate pancreatitis. Acta Hepato-Gastroenterol. **22**, 335–346 (1975b)

Zigmond, S.H.: Chemotaxis by polymorphonuclear leukocytes. J. Cell Biol. **77**, 269–287 (1978)

Zinterhofer, L., Wardlaw, S., Jatlow, P., Seligson, D.: Nephelometric determination of pancreatic enzymes. II. Lipase. Clin. Chim. Acta **44**, 173–178 (1973)

Received October 16, 1978