

G. JURKOWSKA, G. RYDZEWSKA, A. ANDRZEJEWSKA*.

THE INFLUENCE OF POLYAMINES SYNTHESIS INHIBITION ON PANCREAS REGENERATION AND PHOSPHOLIPASE D ACTIVITY AFTER ACUTE CAERULEIN INDUCED PANCREATITIS IN RATS. BIOCHEMICAL AND ULTRASTRUCTURAL STUDY

Gastroenterology Department, *Pathomorphology Department, Medical School, Bialystok, Poland.

The importance of polyamines in tissue growth and regeneration was shown. The involvement of phospholipase D (PLD) activity in pancreas recovery after acute pancreatitis (AP) was postulated. Thus, the aim of present study was to evaluate: the effect of polyamines synthesis inhibition on pancreas regeneration after AP and possible relationship between polyamines metabolism and PLD activity during recovery after AP.

AP was induced by s.c. injection of caerulein (Cae.) in gelatin (12 µg/kg; t. i. d.) during 2 days. After AP induction rats were treated with the irreversible inhibitor of polyamines synthesis α -difluoromethylornithine (DFMO) and/or putrescine or saline for 2, 7 and 14 days. Pancreatic weight, total protein, enzymes, nucleic acids contents were evaluated and ultrastructural examination was performed. Also pancreatic acini were prepared and loaded with [3 H] myristic acid to measure 3 H phosphatidic acid (PA), a marker of PLD activity. For *in vitro* study the pancreatic acini from healthy rats were preincubated with 1 mM DFMO and stimulated with Cae.

AP results in pancreas destruction, followed by spontaneous recovery within 14 days. We found that treatment with DFMO during AP induction did not produce more severe tissue damage. However, when this treatment was prolonged (up to 14 days) during the recovery period after pancreatitis injury reduced the spontaneous regeneration. Microscopic examination showed also the more prominent signs of acinar cell injury in AP-DFMO treated rats vs. AP animals especially after 7 and 14 days of treatment. The signs of microscopic injury, lower pancreatic weight and RNA content in acute pancreatitis rats treated with DFMO during 14 days vs. control group correspond with the increased PLD activity observed after 7 and 14 days of treatment. PLD activity increased significantly also in healthy rats treated with DFMO already after 2 days and remained at significantly elevated level after 7 and 14 days of treatment vs. control. The obtained results indicate the involvement of polyamines in pancreas recovery after acute pancreatitis and in unspecified pancreas injury with concomitant increase of PLD activity. However the modulation/elevation of enzyme activity does not seem to be directly related to polyamines metabolism in the pancreas as the lack of DFMO effect on PLD activity *in vitro* study was found. The results suggest rather indirect modulatory influence of polyamines on intracellular signaling pathway.

Key words: acute pancreatitis, regeneration, polyamines, phospholipase D.

INTRODUCTION

The injury to the pancreatic tissue during acute pancreatitis (AP) is followed by spontaneous reparative/regenerative process, which is completed after different time periods depending on the type of animals or model of AP used in the study (1, 2). The mechanisms and factors responsible for the recovery after AP are still not fully elucidated. It was shown that caerulein (CCK analogue) or endogenous CCK are able to accelerate pancreas regeneration after AP (2). The finding that specific and potent CCK receptor antagonist L364,718 significantly delayed the pancreas recovery after AP (3) support the role of endogenous CCK in this process.

During the last decade the existence of a new cellular signaling pathway involving phospholipase D (PLD) activation was described in many tissues including exocrine pancreas (4—6). This pathway can be selectively activated by the most potent trophic factor for the pancreas — CCK (4, 7) and as well as by different growth factors (6). The significant activation of PLD accompanied with growth associated process, stimulated by pancreatic juice diversion or pancreatectomy was demonstrated and the involvement of PLD in pancreatic growth and regeneration was postulated (7). During acute pancreatitis induction the activity of PLD increases significantly already 6 h after first caerulein injection and remain elevated even 3 days later (when the pancreas destruction is maximal). The treatment with caerulein at a trophic dose, known to accelerate the regeneration process (which is completed after 5 days of caerulein treatment) was connected with the increased PLD activity observed during 4 days of treatment. These results strongly suggest the involvement of PLD in the recovery process after AP (5).

Regulation of the regenerative growth is, in general, poorly understood. However, it is known that polyamines synthesis is required for the regeneration in a number of tissues (8—12). Polyamines (putrescine, spermidine, spermine) are the series of naturally occurring (in all eucariotic cells) aliphatic amines and play an essential role in cell proliferation and differentiation. It was clearly shown that polyamines are regulators of both DNA and protein synthesis (10, 13, 14) and may also affect the expression of genes, which regulate cell division (15). Biosynthesis of polyamines is controlled by the activity of ornithine decarboxylase (ODC), the first and rate limiting enzyme of this pathway (10). While this enzyme is present in rather small amounts in quiescent cells, its activity could be increased several fold within few hours after exposition to injury or trophic stimuli (8—12, 16, 17).

Tabor and Tabor (10) proposed that the inhibition of ODC, the rate-limiting enzyme for polyamine *de novo* synthesis, may be essential during growth stimulation. It was shown that pancreas growth stimulating effect of caerulein (at a trophic dose) was inhibited by ODC inhibitor

α -difluoromethylornithine (DFMO) and this inhibition was reversed by simultaneous putrescine treatment (18, 19).

Dubé *et al.* (16) observed the increases of ODC activity and ODC gene expression early in the course of acute pancreatitis followed by the elevation in DNA synthesis.

Indeed both elevations of ODC and PLD activities preceded the augmentation in DNA synthesis during the recovery after AP injury, and both enzymes are assumed to be involved in the regeneration process.

Thus, the present study was designed to investigate whether the potent ODC inhibitor α -difluoromethylornithine (DFMO) is able 1) to affect the pancreas regeneration after acute pancreatitis and 2) to modulate phospholipase D activity during the pancreas recovery after acute inflammation injury.

MATERIAL AND METHODS

Animals

Male Wistar rats ($n = 102$) weighing 200–220 g were used in the study. The animals were given a standard chow diet. Care was provided in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals (NIH publication # 85–23, 1985).

Materials

Purified collagenase (1424 units/mg) was from Worthington Biochemicals. Silica gel TLC plates (LK6D) were obtained from Whatman. [^3H]myristic acid (56 Ci/mmol) was from Du Pont. Phosphatidylethanol was from Avanti Polar Lipids. α -difluoromethylornithine was kindly supported from Marion Merrell Dow Research Institute (Strasbourg-Cedex, France). Caerulein was from Farmitalia Carlo Erba and soluble starch from Merck Darmstadt (Germany). All other reagents including standards and solvents for thin layer chromatography (TLC) were purchased from Sigma Chemical Co.

Experimental design

Acute pancreatitis (AP) was induced by sc. injections of caerulein $12 \mu\text{g kg body weight}^{-1}$ every 8 h for 2 days. This analogue of cholecystokinin (CCK) was dissolved in gelatin (16% w/v) to prolong its absorption (2). The control animals were injected with saline in gelatin sc.

Rats were divided into six treatment groups: 1) control + saline (C), 2) Control + ornithine decarboxylase inhibitor α -difluoromethylornithine (DFMO) (C-DFMO), 3) AP + saline (AP), 4) AP + DFMO (AP-DFMO), 5) AP + putrescine (AP-P), 6) AP + DFMO + putrescine (AP-DFMO-P). Rats were treated for 2, 7 or 14 days. DFMO was given i.p. every 8 h at a dose 300 mg/kg during 2, 7 or 14 days of treatment in C-DFMO, AP-DFMO, AP-DFMO-P groups and additionally for the first 3 days: 1 day before and during 2 days of caerulein injections, as 2% solution in drinking water plus $3 \times 300 \text{ mg/kg b.w. i.p.}$ Putrescine was given at a dose 4 mg/kg b.w. intraperitoneally (i.p.) three times pr day (t.i.d).

The animals were fed *ad libitum* with a standard diet during whole experiment with a 12 hour light-dark cycle. After an overnight fast with free access to water, rats were decapitated. The pancreas was dissected as quickly as possible, free of connective tissue, fat, lymph nodes and weighed.

The representative specimens of pancreatic tissue from 5 rat of each group were fixed in 3,6% glutaraldehyde at pH = 7,4 and postfixed in Milloning's phosphate buffered 1% osmium tetroxide. After dehydration they were embedded in Epon 812. Ultrathin sections were contrasted with lead citrate and uranyl acetate and examine in Opton 900 PC transmission electron microscope.

Biochemical assays

The pieces of pancreas were homogenized using motor-driven ground-glass homogenizer either in 0.6 mol/L perchloric acid for nucleic acids determinations or in the ice-cold 0.1 mol/L Tris-HCl buffer (pH = 8.0) for protein, α -amylase and chymotrypsin assays. One piece of pancreas (at least 300 mg) was taken to prepare freshly dispersed acini for PLD activity determination.

DNA and RNA were extracted according to Mainz *et al.* (20). DNA was assayed according to Volkin and Cohn (21) using calf thymus DNA as a standard. RNA was measured after overnight hydrolysis in 0.3 mol/L KOH according to Munro and Fleck (22). Tissue α -amylase and chymotrypsin activities were determined according to the Bernfeld (23) and Hummel (24), respectively. Protein content was measured according to Lowry *et al.* (25).

Preparation of pancreatic acini

Acini were prepared as reported by Peikin *et al.* (26) from 300 mg piece of tissue. They were pooled and resuspended in 32 ml of an enriched HEPES buffered solution [in mM: 24.1 HEPES, 98 NaCl, 6 KCl, 2.1 KH_2PO_4 , 0.5 CaCl_2 , 1.2 MgCl_2 , 5 sodium pyruvate, 5 sodium fumarate, 5 sodium glutamate, 11.4 glucose and 0.01% (wt/vol.) SBTI, 0.5% (wt/vol.) fatty acid-free bovine serum albumin (BSA), adjusted to pH 7.4].

Phospholipase D activity

PLD activity was measured by the determination of the phosphatidic acid (PA) formation from labeled phosphatidylcholine (PC) (as described) (4). Briefly, after an hour of incubation with 5 μCi of [^3H] myristic acid, 5 mL of acini suspension in each flask was washed twice in freshly oxygenated incubation medium and was incubated separately for an additional 20 min in the presence of 200 μM propranolol to favor PA accumulation (27). At the end of the incubation period, 1 mL of acini was removed and quickly centrifuged at 100,000 g in a microcentrifuge for 15 s. The supernatant was removed and the pellet was washed once with the incubation medium and then detached mechanically with spatula. To this methanol- glycine mixture, 1 ml of chloroform was added and mixed; 1 ml of chloroform and 1 mL of water were then added to this mixture and the phases were separated by a 5 min. centrifugation at 1,000 \times g (28). The radioactivity present in the chloroform phase was measured. The samples of the chloroform phase with standards added were dried under a stream of nitrogen and redissolved in 50 μL of chloroform to be applied on TCL plates. PA was separated in a solvent system containing chloroform/acetone/methanol/acetic acid/water (50:20:15:10:5, vol./vol.) (4). After separation and exposure to iodine vapor, the area containing PA was scraped and radioactivity was counted. Radioactivity in PA is expressed as a percentage of the total radioactivity in the chloroform phase.

Statistical analysis

All values were expressed as means \pm SE. Statistical significance of the difference between the groups was assessed using t test for unpaired observations and Fischer test. $P < 0.05$ was considered statistically significant. For PLD activity results were analyzed by one-way analysis of variance followed by Fischer test. Results were considered significantly different at $p < 0.05$.

RESULTS

Animals body weight in corresponding groups were not significantly different during the whole experiment.

DFMO treatment of control animals during 2 and 14 days after AP induction resulted in no significant changes of any, but α -amylase trophic parameters measured in comparison to C (Figs 1—3). α -Amylase content after 2 days of treatment was significantly increased (by 78.4%) in C-DFMO vs. C rats (Fig. 2).

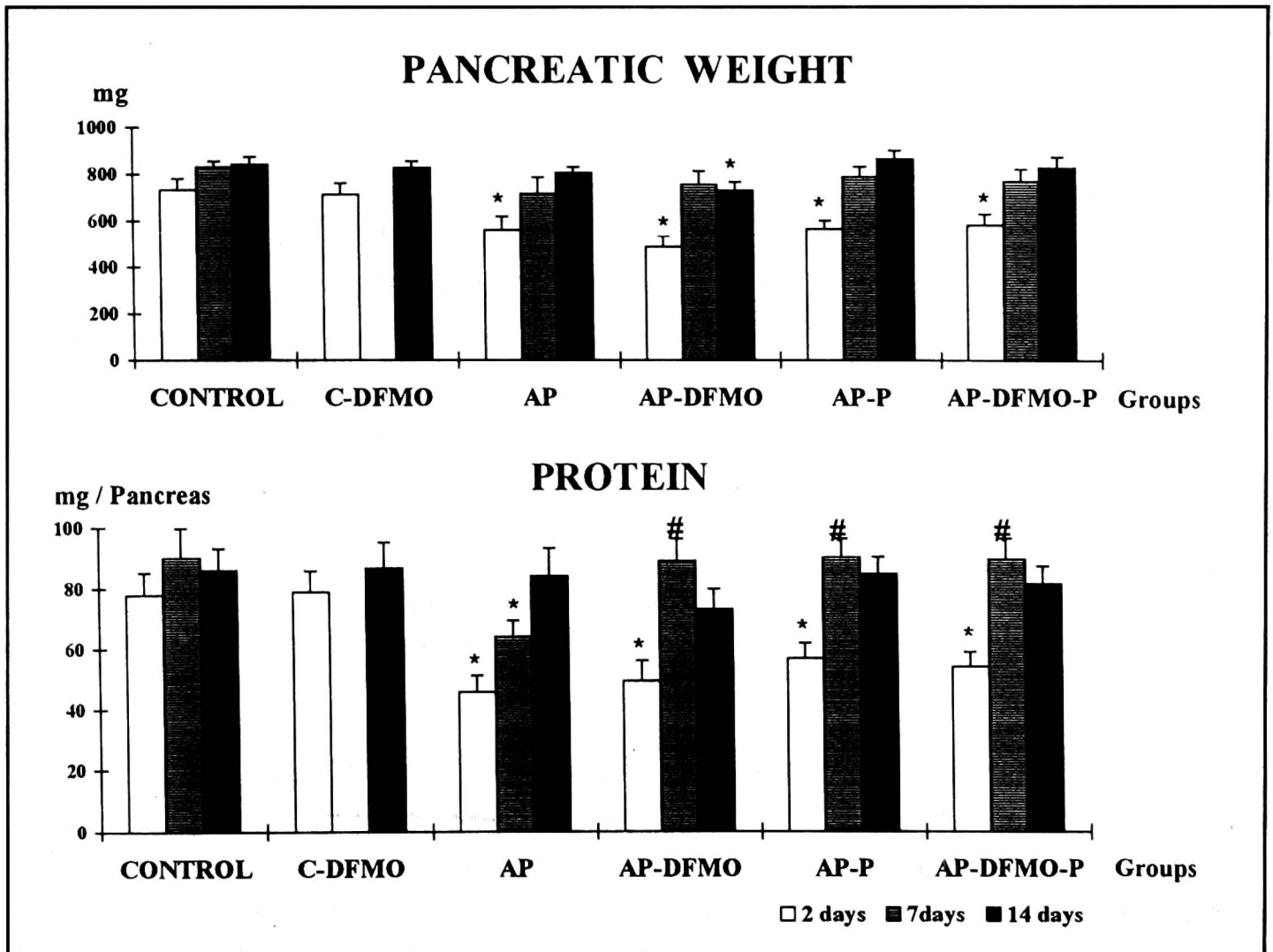


Fig. 1. Pancreatic weight and total content of protein in pancreata of healthy (C) and caerulein-induced acute pancreatitis (AP) rats untreated or treated with α -difluoromethylornithine (DFMO) (C-DFMO; AP-DFMO) or/and putrescine (AP-P; AP-DFMO-P) during 2, 7 or 14 days after AP induction. DFMO was given 3×300 mg/kg b.w. i.p. during first 3 days (1 day before and during 2 days of caerulein injections) and during 2, 7 and 14 days of treatment and additionally as 2% solution in drinking water during the first 3 days. Putrescine was given at a dose 4 mg/kg t.i.d. i.p. Values are means \pm SE of six rats per group. *Significantly different from control; # Significantly different from AP group; o Significantly different from C-DFMO group.

The pancreas destruction two days after pancreatitis induction was evident in all acute pancreatitis groups, as different degree decreases of all trophic parameters measured were observed when compared to C. Pancreatic

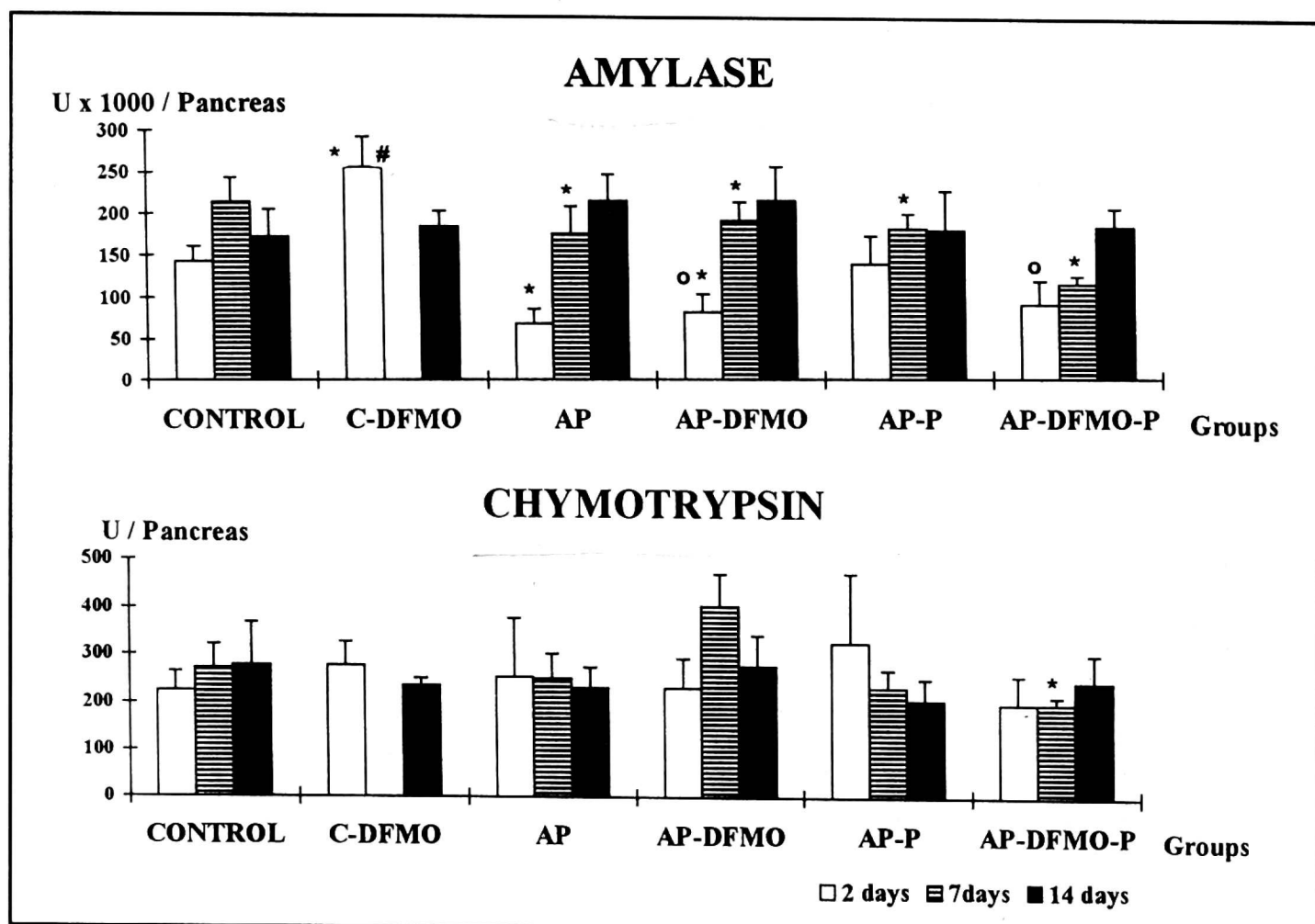


Fig. 2. Total contents of α -amylase and chymotrypsin in the pancreata of healthy (C) and caerulein-induced acute pancreatitis (AP) rats untreated or treated with DFMO (C-DFMO; AP-DFMO) or/and putrescine (AP-P; AP-DFMO-P) during 2, 7 or 14 days after AP induction. Treatment protocol is described in Fig. 1. note. Value are means \pm SE of six rats per group. *Significantly different from control; #Significantly different from AP group, o Significantly different from C-DFMO group.

weight decreased significantly in AP, AP-DFMO, AP-P and AP-DFMO-P groups by 23%, 33.4%, 23% and 21%, respectively in comparison to C group (Fig. 1). Pancreatic protein contents (at this time point of experiment) were also significantly lowered (by 27%—41%) in all acute pancreatitis rats when compared to C (Fig. 1). Pancreatic α -amylase content in AP and AP-DFMO rats decreased by 52.6% and 43.9%, respectively, in comparison to control ($p < 0.05$) (Fig. 2). Total chymotrypsin content was not significantly different in all experimental groups when compared to C (Fig. 2). Acute pancreatitis rats injected with putrescine during 2 days after pancreatitis induction showed elevated chymotrypsin content by 45.5% in comparison to healthy animals (n.s.). Total content of RNA decreased by 33.8% in AP group and by more than 50% in AP animals treated with DFMO, DFMO and P or P alone in comparison to healthy rats (Fig. 3). Total DNA content was significantly lowered in AP, AP-DFMO, AP-P and AP-DFMO-P groups vs. C (Fig. 3). There were no significant changes of parameters measured between AP and AP-DFMO rats two days after acute pancreatitis induction.

Seven days of treatment resulted in the partial improvement of trophic parameters measured, and 14 days after pancreatitis induction nearly complete recovery of the pancreatic tissue was observed.

Pancreatic weight in all acute pancreatitis groups (AP, AP-DFMO, AP-P, AP-DFMO-P) were not significantly different from values observed in the control rats after 7 days of treatment (*Fig. 1*). After 14 days of treatment only pancreatic weight of AP rats treated with DFMO was significantly lower than C.

Total pancreatic protein content still remained under control values after 7 days of treatment in AP rats ($p < 0.005$) (*Fig. 1*). No evident changes of this parameter were found in pancreata of all AP groups 14 days after pancreatitis induction. However, the lowest values were observed in AP-DFMO rats when compared with C, this difference was not statistically significant.

Total pancreatic α -amylase contents in AP, AP-DFMO, AP-P and AP-DFMO-P groups decreased significantly in comparison to C rats 7 days after pancreatitis induction, but after 14 days of treatment no evident differences between groups were found (*Fig. 2*).

Pancreatic chymotrypsin content in AP-DFMO-P group was significantly lowered when compared to C after 7 days of treatment, however any differences were found for chymotrypsin content between all groups after 14 days of treatment (*Fig. 2*). Total pancreas RNA content, which decreased 2 days after pancreatitis induction in all pancreatitis rats remained significantly lower than in C also after 7 days of treatment ($p < 0.05$) (*Fig. 3*). Fourteen days of treatment resulted in the increase of this parameter toward control values in all but acute pancreatitis rats treated with DFMO (AP-DFMO). The significant decrease of pancreatic RNA content when compared with healthy animals (C) was observed only in AP-DFMO group.

No significant differences of pancreatic DNA content between all groups were found after 7 and 14 days of treatment (*Fig. 3*).

Phospholipase D activity

In vivo study

DFMO treatment of control animals (C-DFMO) during 2 and 14 days resulted in increased PLD activity vs. healthy untreated rats (C) (*Fig. 4*).

The increased PLD activity was observed 2 days after pancreatitis induction in AP, AP-P and AP-DFMO-P rats (by 58.7%; 50.9% and 51.5%, respectively) vs. Control. PLD activity of acute pancreatitis rats treated with DFMO (AP-DFMO) showed not significant elevation (by 27.5%) when compared with healthy animals 2 days after pancreatitis induction (*Fig. 4*).

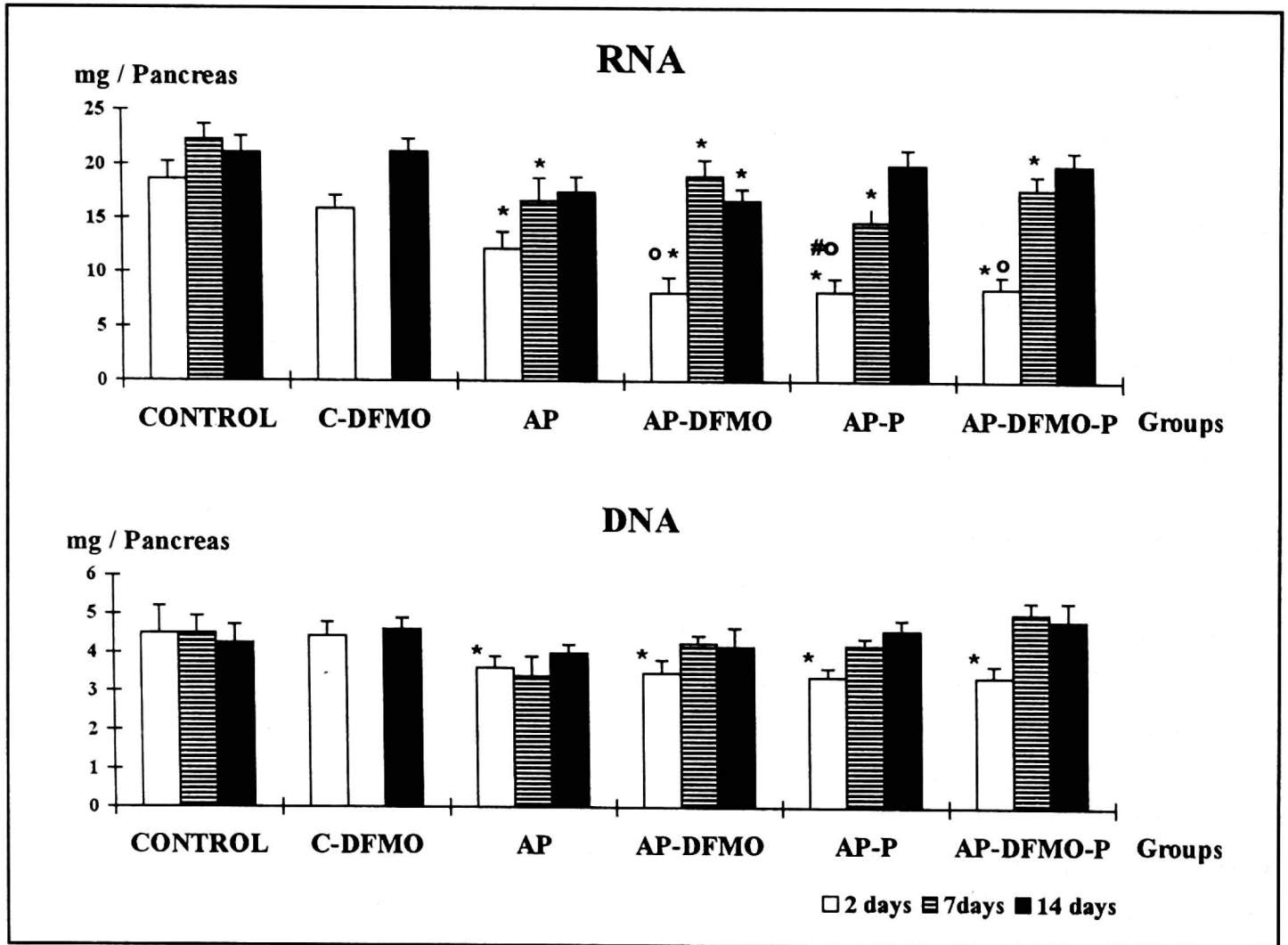


Fig. 3. Total contents of DNA and RNA in pancreata of healthy (C) and caerulein-induced acute pancreatitis (AP) rats untreated or treated with DFMO (C-DFMO; AP-DFMO) or/and putrescine (AP-P; AP-DFMO-P) during 2, 7 or 14 days after AP induction. Treatment protocol is described in Fig. 1. note. Values are means \pm SE of six rats per group. *Significantly different from control; #Significantly different from AP group, o Significantly different from C-DFMO group.

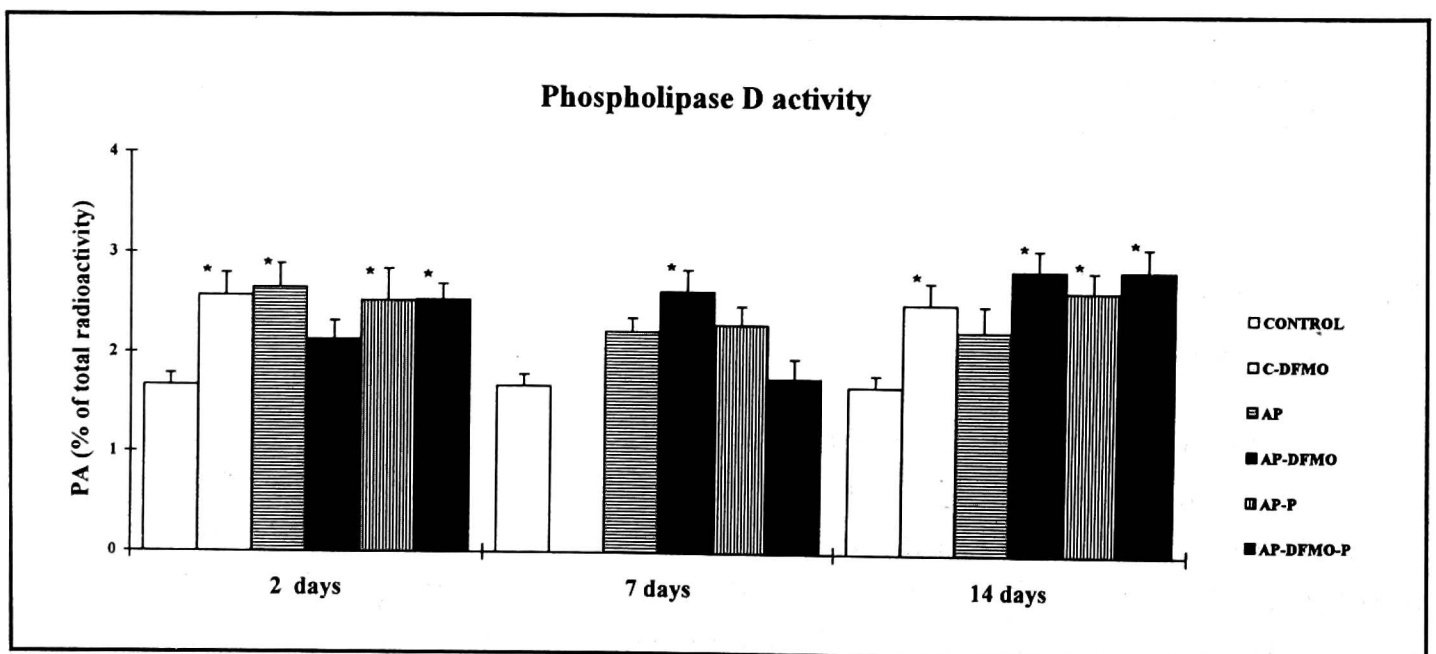


Fig. 4. The influence of DFMO and putrescine treatment on Phospholipase D activity in the course of caerulein-induced acute pancreatitis in rats. Treatment protocol is described in Fig. 1 note. Values are means \pm SE of six rats per group. *Significantly different from control.

After seven days of treatment the PLD activity returned to Control value in AP, AP-P and AP-DFMO-P groups (*Fig. 4*). At this time point of experiment the elevated enzyme activity was observed in AP-DFMO group *vs.* C (by 56.9%) and this increase sustained 14 days after pancreatitis induction. Also PLD activity of AP-DFMO-P and AP-P rats increased significantly after 14 days of treatment in comparison to C. The enzyme activity in AP rats after 14 days remained at control level as was observed 7 days after AP-induction.

In vitro study

Pancreatic acini from healthy rats were preincubated with 1 mM DFMO for 30 min., and stimulated with 500 pM of caerulein. Caerulein stimulated PLD activity over control (basal) value by 135%. DFMO did not influence the basal and caerulein-stimulated PLD activity (*Fig. 5*).

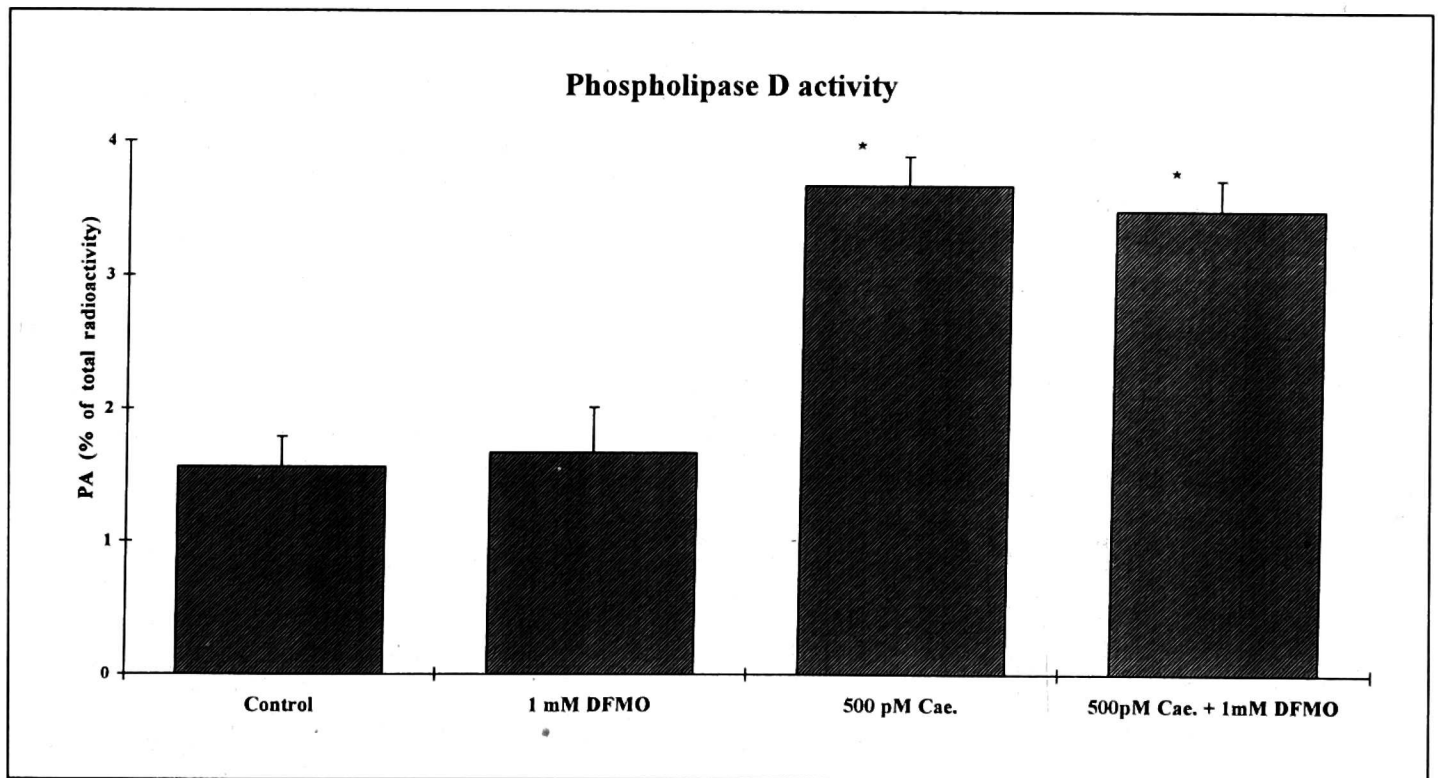


Fig. 5. The influence of DFMO pretreatment on basal (Control) and caerulein-stimulated Phospholipase D activity in isolated rat pancreatic acini. Values are means \pm SE of six rats per group. *Significantly different from control.

ELECTRON MICROSCOPE EXAMINATION

Two days after pancreatitis induction interstitial edema, inflammatory cell infiltration (mainly with granulocytes and macrophages) and signs of acinar cell injury were observed in all acute pancreatitis rats (*Fig. 6*) when compared to healthy animals (*Fig. 7*). Also numerous mitoses were seen in all acute pancreatitis rats in comparison to C and C-DFMO rats. Mitoses were not

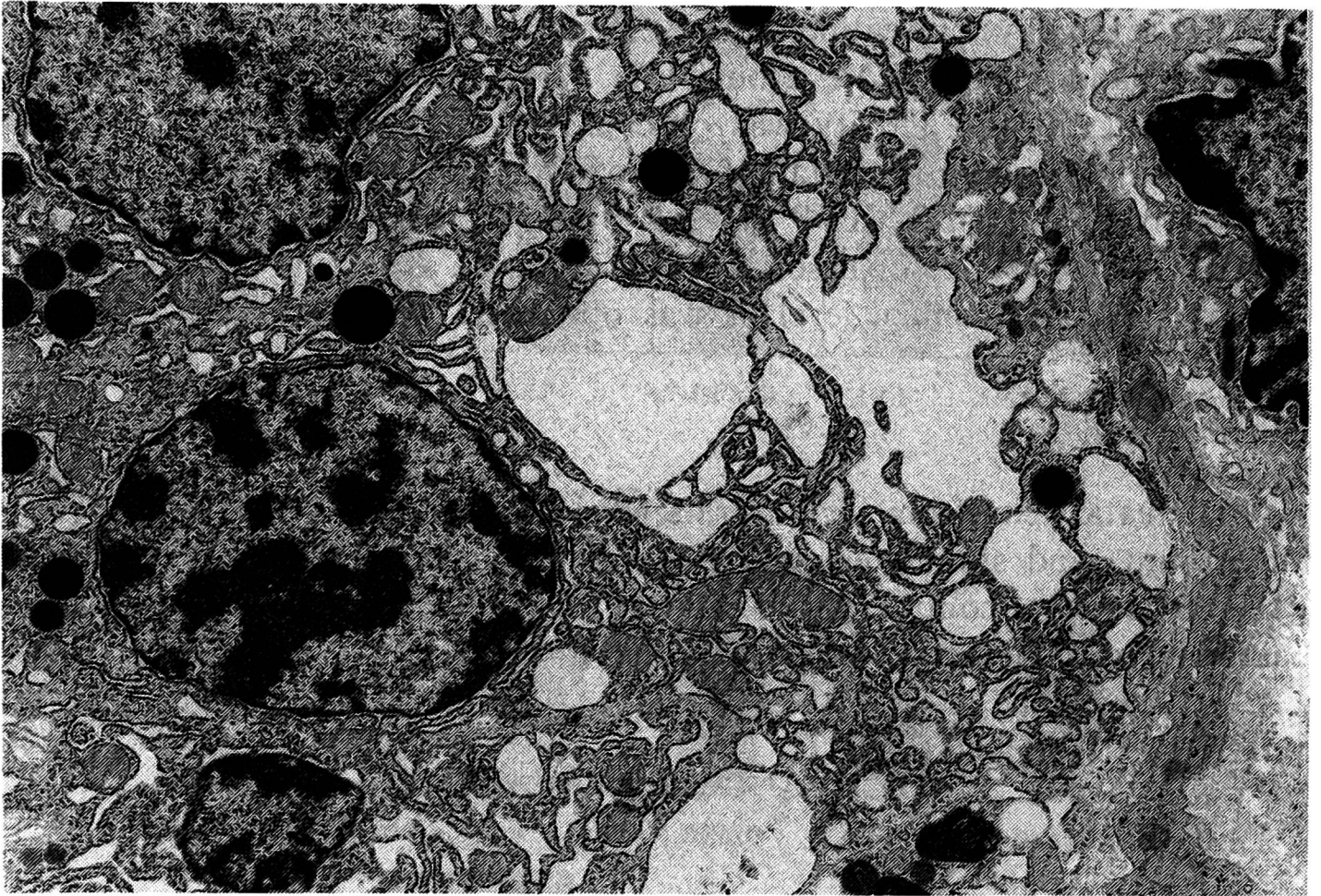


Fig. 6. The fragment of acinar cell; dilated canals of RER, irregularly dispersed zymogen granules are seen. AP group, 2 days after acute pancreatitis induction (Mag. 3000 \times).

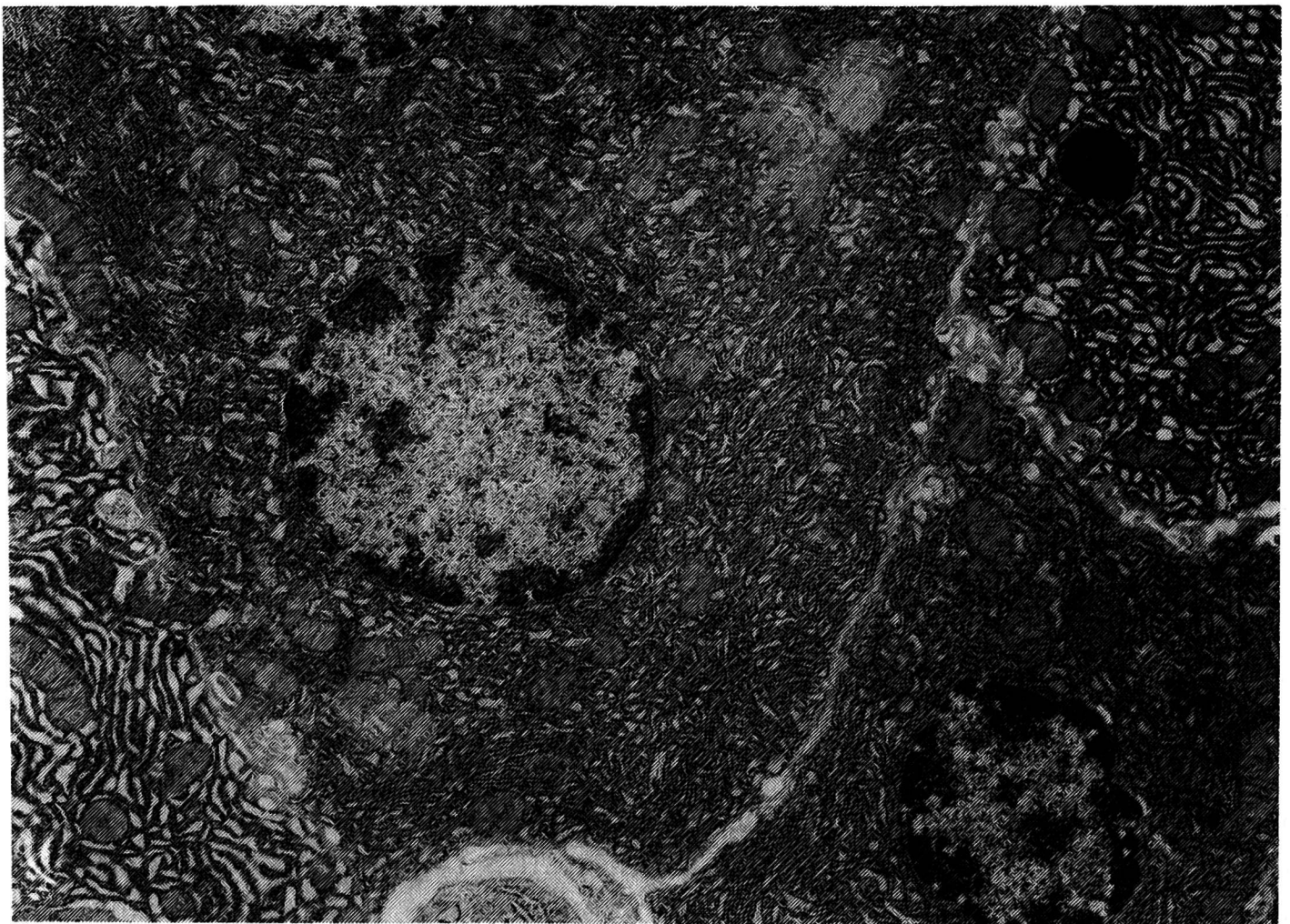


Fig. 7. Normal ultrastructure of acinar cell. Control group (Mag. 3000 \times).

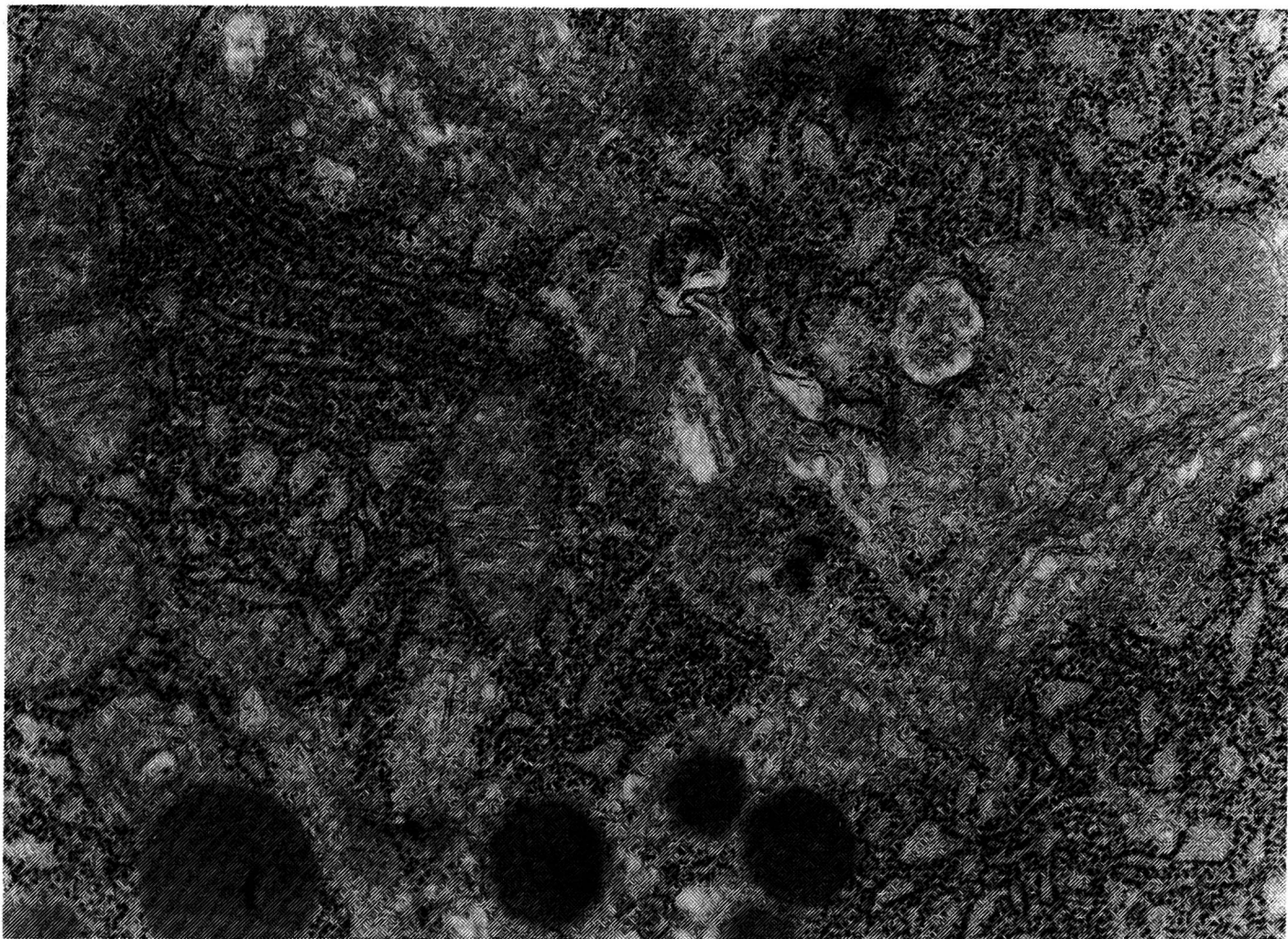


Fig. 8. Large, dilated cisterns of Golgi apparatus filled with amorphous material in acinar cell of acute pancreatitis rats treated with DFMO (AP-DFMO), 14 days after pancreatitis induction (Mag. 12 000 ×)

observed in the later time points of experiment. After 7 days and especially after 14 days of treatment the interstitial oedema and inflammatory cell infiltration were not found, rather quite large amount of collagen fibers in the interstitial spaces was seen in pancreatitis *vs.* control animals. After 14 days nearly complete recovery of pancreas ultrastructure in AP rats was observed. DFMO treatment of acute pancreatitis rats (after 7 and 14 days) was connected with more prominent acinar cell injury. The acinar cells of all treated groups had prominent nucleoli, and nucleus shape showed some irregularity only in DFMO treated animals in three time periods (i.e. 2, 7 and 14 days after pancreatitis induction). Mitochondria of all pancreatitis rats were generally normal, however the evident polymorfism of these organelles was seen in AP-DFMO and AP-DFMO-P (especially after 7 days of treatment). The cisterns of rough endoplasmatic reticulum (RER) were densely arranged in acinar cells of all pancreatitis rats at three time periods (i.e. 2, 7 and 14 days after pancreatitis induction). When the treatment with DFMO was applied (mainly in AP-DFMO group) the disorganization and degranulation areas of RER were seen. Large, dilated cisterns of Golgi apparatus filled with amorphous material were observed in DFMO treated animals (in AP-DFMO after 14 days, in AP-DFMO-P after 2 and 7 days) (*Fig. 8*). Also acinar cells of healthy

animals treated with DFMO during 14 days showed enormously dilated cisterns of Golgi apparatus with membrane shadows of condensing vacuoles and enzymatic destruction of surrounding cytoplasm. No significant changes of RER or Golgi apparatus in acinar cells of AP rats were found. The number of zymogen granules (ZG) in acinar cells were different during the study. The largest amount of ZG was found 2 days after pancreatitis induction (in all pancreatitis rats), but decreased after 7 and 14 days, especially in AP-DFMO group when compared to AP and C animals (sometimes zymogen granules in acinar cells of AP-DFMO rats were absent). Fagosomes were found rather in small amount and no significant differences of its number, shape and content between groups were observed.

DISCUSSION

The supramaximal dose of caerulein given three times a day for 2 days resulted in acute edematous pancreatitis. During this destructive period pancreatic tissue is already undergoing tissue repair as the cellular events normally observed during tissue growth (elevation of ODC activity and DNA synthesis) occur during AP induction (16). The increase of ODC activity and ODC mRNA expression early in the course of acute pancreatitis (already 2 h after first caerulein injection) followed by increased titrated thymidine incorporation into DNA at 48 h suggest, that polyamines metabolism play a role in pancreas regeneration after acute pancreatitis (16).

Although ODC activity and polyamines contents were not determined in our study, the amount of DFMO given during AP induction was shown to reduce significantly the increased ODC activity and pancreatic putrescine content in response to camostate-induced pancreatic growth (mediated *via* endogenous CCK release) (17) and reduced the polyamines level after hormonal stimulation of pancreatic growth (18). Even lower dose of this compound injected during the regeneration period was similar to dose of DFMO used in CCK-stimulated growth of pancreas study, and was shown to reduce significantly ODC activity and putrescine concentration (29).

We found that treatment with DFMO during AP induction do not produce more severe tissue damage as trophic parameters and electron microscope evaluation of acute pancreatitis rats untreated or treated with DFMO were not significantly different 2 days after AP induction. When this treatment was prolonged during the recovery period, it slightly reduced the spontaneous regeneration process. Indeed, when spontaneous recovery was already completed after 14 days in AP untreated rats, the DFMO treatment resulted in lower PW and RNA content in acute pancreatitis rats in comparison to C. Also acinar cell ultrastructure of AP rats injected with DFMO showed signs of destruction when compared to AP and especially to control rats.

The injuring influence of DFMO on pancreas regenerating after AP was also observed in rats treated with putrescine, however trophic parameters changes were less evident (and not significant) in comparison with Control.

DFMO treatment of healthy rats during 2 days resulted in the significant increase of amylase content in comparison to C. Because RNA, DNA, protein and chymotrypsin contents were not affected by DFMO treatment, it could be assumed that inhibition of polyamine synthesis affects rather secretory processes than synthesis of this enzyme, followed by the transient accumulation of amylase in the pancreatic tissue. When healthy rats were treated with DFMO during 14 days, small degree disorganization of acinar cell ultrastructure without concomitant differences of trophic parameters were found in comparison to C. Other authors did not find the significant changes of trophic parameters of healthy rats treated with DFMO (17, 18, 30), however electron microscope examinations were not performed in their studies.

These results suggest the involvement of polyamines metabolism in tissue recovery after pancreatitis injury, but DFMO at given dose and route of administration did not inhibit the spontaneous regeneration — rather reduced this process. Similarly DFMO treatment did not completely inhibit the pancreatic growth of healthy rats stimulated with CCK or with camostate feeding (18, 30). It is interesting to note that the elevated ODC activity during pancreatic growth stimulation with camostate was significantly inhibited already after 6 hours of DFMO treatment. However, the significant decreases of trophic parameters as: pancreatic weight and protein content in camostate + DFMO versus camostate fed rats were found at day 1 and latter after 30 and 20 days of treatment, respectively for each parameter (17). Total DNA content was significantly lower after 30 days of DFMO treatment vs. Camostate fed animals. Thus the pancreatic growth stimulated by endogenous CCK was not completely inhibited by DFMO, only delayed, as was in the case of our study when DFMO treatment of acute pancreatitis rats reduced the pancreas regeneration, but not completely inhibits tissue recovery after pancreatitis injury.

The evident pancreas destruction found 2 days after AP induction resulted in elevated PLD activity in all acute pancreatitis rats. Because the increase of enzyme activity (vs. Control) was not significant only in DFMO treated animals, thus some weak inhibitory effect of DFMO treatment on PLD activity during early phase of regeneration could be suspected. When treatment with DFMO was prolonged up to 7 days and especially up to 14 days, we found significantly elevated PLD activity in pancreas of AP-DFMO rats vs. Control. At these time points, PLD activity of acute pancreatitis untreated rats decreased and remained at control level. It could be assumed, that elevated PLD activity 7 and 14 days after pancreatitis induction occurs as delayed response to AP damage or possibly as a consequence of injuring impact

(evaluated on the basis of morphological and biochemical criteria) of prolonged DFMO treatment of acute pancreatitis rats. The increased PLD activity was also observed in the pancreata of healthy rats receiving DFMO, where only small degree of acinar cells ultrastructure changes were found. It may supports some detrimental effect of DFMO treatment on the pancreas, even in healthy rats. As there is not known other action of DFMO than inhibition of ODC activity, it could be speculated that increased pancreatic PLD activity in DFMO treated rats results from the inhibition of ODC.

The obtained results suggest that DFMO treatment results in the disturbances of pancreas reparative process after acute pancreatitis or even results in low degree of pancreatic injury when the treatment is prolonged with concomitant increase of PLD activity. However, the lack of DFMO-induced basal and caerulein-stimulated PLD activity changes in *in vitro* study suggests that the modulation of enzyme activity is not directly related to polyamines metabolism in the pancreas. Rather unspecific tissue injury evoked by DFMO treatment, similarly to pancreatectomy (7) or AP destruction (5) serves as a stimulus for pancreas reparative process, which involves also increased PLD activation.

The lack of complete inhibitory effect of DFMO on pancreas recovery process after AP observed in our study can result from the fact that animals were fed a standard diet containing putrescine and its availability and uptake from systemic circulation minimize the effect of DFMO (17, 31, 32). It is also possible, that in recovery process after AP the increased uptake of polyamines from systemic circulation may take place. Elevated pancreatic polyamines concentration and pancreatic growth was observed in rats fed lectins with minimal influence on ODC activity (33).

Also insufficient uptake of DFMO into the cells *in vivo* or rapid excretion of DFMO, as it was shown that DFMO is more active in blocking the ODC activity *in vitro* than *in vivo* (34), could be responsible for lack of complete inhibitory effect of DFMO on pancreas recovery after AP induction. It is also possible that even small amount of ODC, formed despite of DFMO treatment, allow the occurrence of recovery process, however, in reduced fashion. There exist also another possibilities, that during the regeneration process in pancreas the polyamine metabolism is regulated mainly via interconversion pathway activity and/or putrescine *de novo* synthesis is activated in much lesser extent or even may not be significantly involved as it was shown in azaserine-induced increase of putrescine content (35). These possibilities remain to be investigated. Ultimately, other than polyamines metabolism mechanisms are involved in tissue regeneration after injury, thus they might underline this phenomenon.

Our results suggest the involvement of polyamines in pancreatic tissue recovery process after pancreatitis damage. DFMO treatment resulted in the reduction of pancreas regeneration after acute pancreatitis and in unspecific pancreas injury with concomitant increase of PLD activity. However the

modulation/elevation of enzyme activity does not seem to be directly related to polyamines metabolism in the pancreas. The obtained results suggest rather indirect modulatory influence of polyamines metabolism on intracellular signaling pathway.

Acknowledgments: This study was supported by the grant KBN 4 S402 0306. The results of the present paper were partially presented during 5-th United European Gastroenterology Week, Paris, 1996.

REFERENCES

1. Elsasser H-P, Lutcke H, Kern HF. Acinar and duct cell replication and regeneration. In *The exocrine pancreas biology, pathobiology and diseases*. VLW Go, JD Gardner, FP Brooks, E, Lebenthal ED Di Magno, GA, Scheele (eds.) New York: Raven, 1986: 45—53.
2. Jurkowska G, Grondin G, Masse S, Morisset J. Soybean trypsin inhibitor and cerulein accelerate recovery of cerulein-induced pancreatitis in rats. *Gastroenterology* 1992; 102: 550—562.
3. Jurkowska G, Grondin G, Morisset J. Involvement of endogenous cholecystokinin in pancreatic regeneration after cerulein-induced acute pancreatitis; *Pancreas* 1992; 7: 295—304.
4. Rydzewska G, Rossignol B, Morisset J. Involvement of phospholipase D in caerulein-induced phosphatidylcholine hydrolysis in isolated rat pancreatic acini. *Am J Physiol* 1993; 265: G725—G734.
5. Rydzewska G, Rivard N, Morisset J. Dynamics of pancreatic tyrosine kinase and phospholipase D activities in the course of cerulein-induced acute pancreatitis and during regeneration. *Pancreas* 1995; 10: 382—388.
6. Rydzewska G, Morisset J. Activation of pancreatic acinar cell phospholipase D by epidermal, Insulin-like and basic Fibroblast Growth Factors involves Tyrosine Kinase. *Pancreas* 1995; 10: 59—65.
7. Rivard N, Rydzewska G, Boucher C, Lods J—S, Calvo E, Morisset J. Cholecystokinin activation of tyrosine kinase, Ptd Inositol 3-kinase and phospholipase D: a role in pancreas growth induction? *Endocrine J* 1994; 2: 393—401.
8. Diehl AM, Wells M, Brown ND, Thorgeirsson SS, Ster CJ. Effect of ethanol on polyamine synthesis during liver regeneration in rats. *J Clin Invest* 1990; 85: 385—390.
9. Poso H, Pegg AE. Effect of α -difluoromethylornithine on polyamine and DNA synthesis in regenerating rat liver. Reversal of inhibition of DNA synthesis by putrescine, *Biochim Biophys Acta* 1982; 696: 179—186.
10. Tabor CW, Tabor H. Polyamines, *Ann Rev Biochem* 1984; 53: 749—790.
11. Luk G. D. Polyamines in normal and adaptive gastrointestinal growth. In *Polyamines in gastrointestinal tract*. R H Dowling, UR, Folsh C. Loser (eds.) Lancaster: Kluwer Academic, 1992: 205—216.
12. Wang J—Y, Johnson LR. Role of ornithine decarboxylase in repair of gastric mucosal stress ulcers. *Am J Physiol* 1990; 258 (21): G78—G85.
13. Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. *Biochim Biophys Acta* 1978; 473: 241—293.
14. Jacob ST, Duceman BW, Rose KM. Spermine mediated phosphorylation of RNA polymerase I and its effect on transcription. *Med Biol* 1981; 59: 381—388.
15. Luscher B, Eisenman RN. c-myc and c-myb protein degradation: effect of metabolic inhibitors and heat shock. *Mol Cell Biol* 1988; 8: 2504—2512.

16. Dube M, Sarfati P, Pradyrol L, Vaysse N, Morisset J. Dynamics of ODC activity, ODC mRNA expression and DNA synthesis during caerulein-induced pancreatitis in rats. In: *Polyamines in gastrointestinal tract*. RH Dowling, UR, Folsh C. Loser (eds.) Lancaster: Kluwer Academic, 1992: 229—237.
17. Loser Ch, Folsh UR, Cleffman U, Nustede R, Creutzfeldt W. Role of ornithine decarboxylase and polyamines in camostate (Foy-305)-induced pancreatic growth in rats. *Digestion* 1989; 43: 98—112.
18. Benrezak O, Morisset J. Effects of α -difluoromethylornithine on pancreatic growth induced by caerulein. *Reg Pept* 1984; 9: 143—153.
19. Morisset J, Benrezak O. Reversal of α -difluoromethylornithine inhibition of caerulein-induced growth by putrescine. *Reg Pept* 1985; 11: 201—208.
20. Mainz DL, Black O, Webster PD. Hormonal control of pancreatic growth. *J Clin Invest* 1973; 52: 2300—23004.
21. Volkin E, Cohn WE. Estimation of nucleic acids. *Biochem Anal* 1954; 91: 287—303.
22. Munro HF, Fleck A. Recent developments in the measurement of nucleic acids in biological materials. *Analyst* 1966; 91: 78—88.
23. Bernfeld P. Amylase α and β . *Methods Enzymol* 1955; 1: 149—158.
24. Hummel BC. A modified spectrophotometric determination of chymotrypsin, trypsin and thrombin. *Can J Biochem* 1959; 37: 1393—1399.
25. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 59—70.
26. Peiken SR, Rothman AJ, Batzri S, Gardner JD. Kinetics of amylase release by dispersed acini from guinea pig pancreas. *Am J Physiol* 1978; 23: E743—749.
27. Witter B, Kaufer JN. Hydrolysis of endogenous phospholipids by rat brain microsomes. *J Neurochem* 1985; 44: 155—162.
28. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959; 37: 911—918.
29. Haarstadt H, Petersen H. Polyamines in initiation of pancreatic growth. *Digestion* 1990; 46 (suppl. 2): 458.
30. Loser Ch, Folsh UR, Sahelijo-Krohn P, Creutzfeldt W. Ornithine decarboxylase and polyamines in cholecystokinin-induced pancreatic growth in rats: effects of α -difluoromethylornithine and the CCK receptor antagonist L-365—718. *Eur J Clin Invest* 1989; 19: 448—458.
31. Loser Ch, Folsh UR. Importance of various intracellular regulatory mechanisms of polyamine metabolism in camostate-induced pancreatic growth in rats. *Digestion* 1993; 54: 213—223.
32. Hinuma K, Maghsoudloo M, Murphy GM, Dowling RH. Dietary and intestinal polyamines in the rat: in vitro transport studies. In *Polyamines in gastrointestinal tract* RH Dowling, UR, Folsh closer (eds) Lancaster: Kluwer Academic, 1992: 463—472.
33. Pusztai A, Ewen SWB, Grant G *et al.* Stimulation of growth and polyamine accretion in the small intestine and pancreas by lectins and trypsin inhibitors. In *Polyamines in gastrointestinal tract* RH Dowling, UR, Folsh C. Loser (eds.) Lancaster: Kluwer Academic, 1992: 473—483.
34. Pegg AE. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res* 1988; 53: 749—790.
35. Loser Ch, Stuber E, Folsh UR. Dissimilar effect of the carcinogenic agent azaserine on pancreatic and hepatic polyamine metabolism in rats. *Pancreas* 1995; 10: 44—52.

Received: March 24, 1997

Accepted: September 9, 1997

Author's address: G. Jurkowska, Gastroenterology Department, Medical School, M. C. Skłodowskiej 24a Str., 15-276 Białystok, Poland.