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Exocrine Pancreas in Parathyroprivic Hypocalcemia: Morphofunctional Characteristics

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Hypocalcemia caused by removal of the parathyroid glands results in abnormal secretory activity of pancreatic acinar cells, with secretion accumulating in these cells and interstitium. In addition, focal necrosis develops in the pancreas by day 30 after parathyroidectomy. These abnormalities are accompanied by a decrease in blood levels of pancreatic enzymes. Possible mechanisms of these changes are discussed.

Key Words: *parathyroid glands; hypocalcemia; pancreas; pancreatic enzymes*

There is evidence that calcium ions play an important role in mediating the exocrine pancreatic function. Calcium is involved in pancreatic secretion at the level of exocrine pancreatic cells (EPC), both directly and indirectly, via various stimulatory substances. It has been demonstrated that cytoplasmic calcium, which is the major cation of EPC, is implicated in the formation of zymogen granules excreted in the pancreatic juice, where Ca^{2+} levels are paralleled by those of enzymes [10,11]. Pancreatic secretory activity is determined by Ca^{2+}, as evidenced by a markedly reduced enzyme release from isolated pancreas or its fragments in a calcium-free medium [7]. An indirect influence of Ca^{2+} on pancreatic secretion is confirmed by increased $^{45}Ca^{2+}$ influx into cells of intact pancreas under the action of some stimulator of secretion [8]. It has been shown that pancreatic secretion is equally activated by cholecystokinin-pancreozymin and by an ionophore facilitating Ca^{2+} transport through the plasma membrane of acinar cells [9].

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Bearing in mind the role of parathyroid glands in the regulation of Ca^{2+} levels in the body, we evaluated the morphofunctional state of the pancreas under conditions of hypocalcemia caused by removal of the parathyroids.

MATERIALS AND METHODS

Albino rats (n=110) weighing 200-250 g were used. Specific hypocalcemia was produced by removal of the parathyroid glands. On days 5, 15, 30, and 60 after parathyroidectomy, the rats were decapitated. Before sacrifice, they were deprived of food for 18-24 h fast. Intact rats served as controls. The pancreas was fixed in 10% Lillie's formalin and embedded in paraffin. Paraffin sections (4 μ) were stained with hematoxylin and eosin or by the method of Jenner—Giemsa. Zymogen granules and fibrin were visualized with Gram—Weigert stain. Amylolytic activity of the serum was measured as described [6]. Trypsin-like activity and the level of trypsin inhibitor were measured by the method [5]. Serum concentrations of total and ionized calcium were determined, respectively, with spectrophotometry and ion-selective analysis. The Ca^{2+}-absorbing capacity of pancreatic tissue was assessed as described previously [3]. The results were analyzed using Student's t test.

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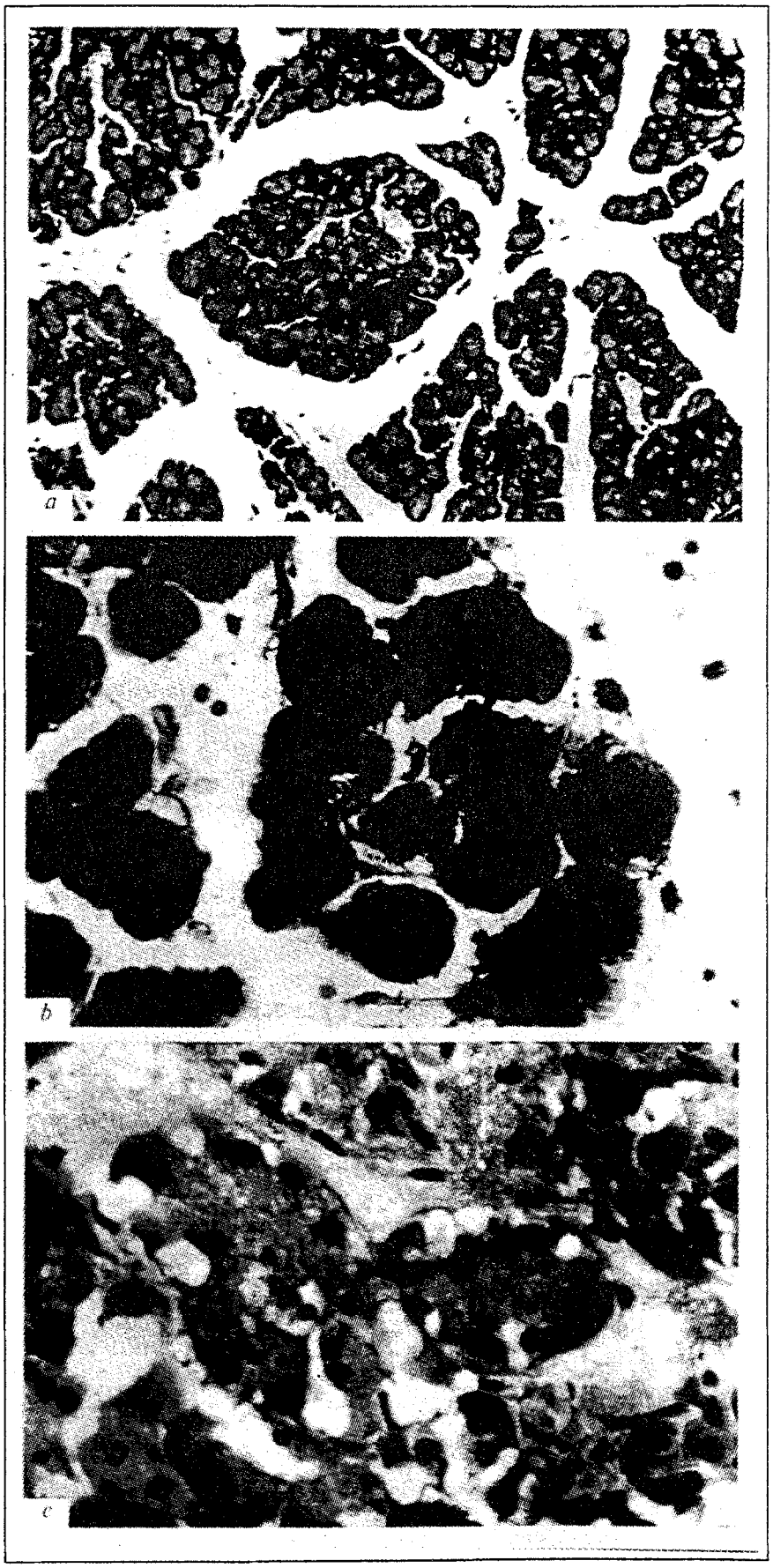

Fig. 1. Pancreas on day 5 after parathyroidectomy. *a*) narrowed basal and dilated apical areas of the cytoplasm of an exocrine pancreatic cell (EPC) (hematoxylin and eosin, ×60); *b*) zymogen granules in the EPC cytoplasm and in the pancreatic interstitium (Gram—Weigert stain, ×200); *c*) vacuolization and focal lysis in the basal portion of the EPC cytoplasm (Jenner—Giemsa stain, ×400).

TABLE 1. Levels of Amylase, Trypsin, Trypsin Inhibitor, Total Calcium, Ionized Calcium, and Phosphorus in Control and Parathyroprivic Rats

Compound	Control group	Days after parathyroidectomy			
		5th	15th	30th	60th
Amylase, U	2335.0±110.3 (n=32)	1563.2±79.85 (n=8)	1428.8±79.49 (n=8)	2018.97±70.24 (n=6)	1303.67±59.27 (n=3)
Trypsin, mU	6.4±0.2 (n=45)	4.167±0.351 (n=6)	4.3±0.35 (n=6)	3.4±0.926 (n=7)	4.4±0.32 (n=3)
Trypsin inhibitor, mU	503.6±12.5 (n=45)	432.1±12.6 (n=7)	461.16±10.38 (n=8)	413.53±13.68 (n=9)	422.03±20.93 (n=3)
Total calcium, mM	2.288±0.031 (n=24)	1.655±0.081 (n=20)	1.98±0.077 (n=20)	2.153±0.019 (n=25)	2.099±0.051 (n=13)
Ionized calcium, mM	1.133±0.015 (n=21)	0.545±0.037 (n=17)	0.77±0.018 (n=18)	0.59±0.029 (n=25)	0.758±0.021 (n=13)
Phosphorus, mM	2.13±0.03 (n=20)	3.578±0.152 (n=15)	2.838±0.157 (n=5)	3.104±0.066 (n=9)	2.797±0.123 (n=11)

Note. All values are significant (p<0.001) in comparison with the control group.

RESULTS

Five days after parathyroidectomy, blood levels of both total and ionized calcium were significantly reduced (Table 1). Focal breakdown of the acini and isolated round or oval EPC devoid of zymogen was observed. In preserved acini, EPC were dilated in the apical pole and narrowed in the basal pole (Fig. 1, *a*); considerable accumulations of zymogen granules were seen at the apical pole. Disoriented zymogen granules were present in some EPC, including their basal cytoplasmic areas, and zymogen granules were found in the interstitium (Fig. 1, *b*). Large vacuoles looking like large or small drops as well as focal necroses were seen in the basal parts of some EPC (Fig. 1, *c*). The lumens of excretory ducts were filled with homogeneous protein masses throughout the entire experimental period (60 days). In some pancreatic regions, interacinar vessels were congested, while capillaries showed signs of erythrocyte stasis and contained hyaline thrombi. Formed elements of the blood were separated in interlobular vessels. Sinusoidal capillaries of pancreatic islets were also markedly congested. Similar changes were observed later; on day 30 and particularly on day 60 they were more pronounced and differed qualitatively from those seen earlier. Thus, the acini containing EPC with "amputated" apical cytoplasmic portions and showing the signs of oxyphilic or basophilic degeneration as well as EPC with small areas of coagulation necrosis and/or lysis were seen (Fig. 2). On days 30 and 60, tissue basophils were markedly degranulated; the walls of small arteries were impregnated with plasma proteins and contained fibrin deposits. Presumably, the entire gland was congested. Stromal edema had developed, and small focal diapedetic hemorrhages were seen. Small accumulations of polymorphonuclear leukocytes were scattered over the stroma; on day 60, infiltrates composed of mononuclear cells producing cytotoxic and cytolytic effects of EPC were detected.

Blood levels of pancreatic enzymes were lowered throughout the experimental period (Table 1). After a 15-sec incubation of tissue specimens in the presence of $^{45}Ca^{2+}$, calcium uptake by the tissues from parathyroidectomized rats decreased: 36.4±1.76 *vs.* 89.0±8.3 pg/mg in the control group; p<0.001).

Hypocalcemia not only impaired the release of pancreatic enzymes, but also led to their deficiency or inadequate synthesis. This is indirectly confirmed by lowered serum levels of pancreatic amylase despite the release of zymogen granules into pancreatic interstitium.

Calcium ions from either intracellular or extracellular sources have been ascribed a key role in the mechanism responsible for acinar cell secretion [1,2]. In any case, alterations of calcium homeostasis occurring in parathyroprivic hypocalcemia are probably implicated in the mechanism by which the secretion from acinar cells is impaired. This hypothesis is supported by decreased ability of "parathyroprivic" pancreatic cells to uptake Ca^{2+} as well as by homeostatic Ca-dependent mechanisms for activating chemoexcitable sodium channels in the pancreatic cell membrane, which maintains pancreatic secretion at an optimal level [4].

Inhibition of EPC secretion in hypocalcemia may also result from reduced sensitivity of EPC

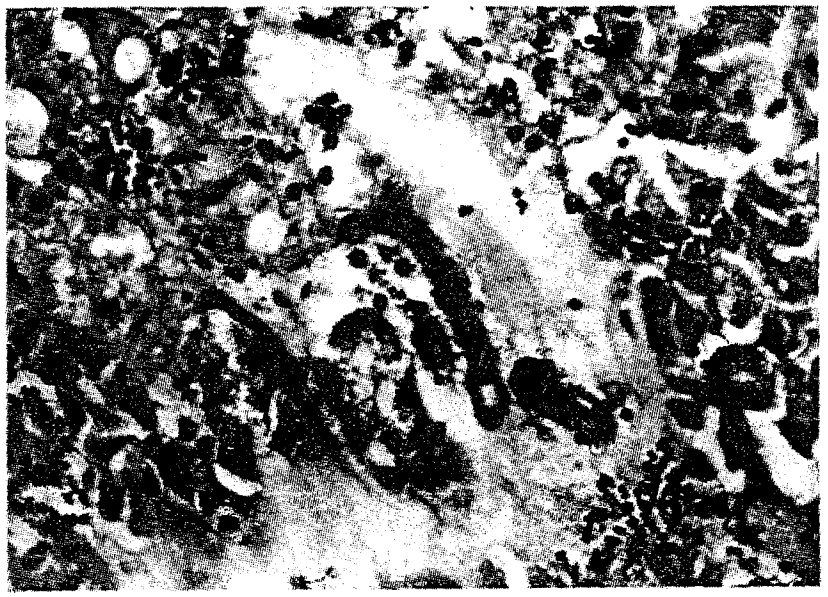

Fig. 2. Small necrotic areas and lysis of exocrine pancreatic cells. Hematoxylin and eosin, ×200.

receptors to naturally occurring stimulators of secretion, since this process is mediated by Ca^{2+}-dependent cyclic nucleotides [1,2].

Overload of EPC with secretory products in the presence of pronounced microcirculatory disorders leads to profound degenerative changes in the EPC and eventually to necrobiosis and necrosis of pancreatic parenchyma. Until recently, hypercalcemia has been considered to be one of the factors in the etiology of pancreatic necrosis; however, our results suggest that focal necrosis of the pancreas can be also caused by hypocalcemia.

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