EFFECT OF SODIUM THIOSULFATE ON THE PANCREAS IN EXPERIMENTAL PANCREATITIS

A. S. Kanayan and P. S. Simavoryan

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Histological and histochemical investigations of the pancreas on albino rats with experimental pancreatitis showed that sodium thiosulfate largely inhibits the development of necrotic changes and circulatory disturbances. The compound prevents a second attack of pancreatitis and sclerosis of the gland. As a result of administration of sodium thiosulfate regeneration is stimulated and is accompanied by the development of regeneration hypertrophy and marked epimorphosis.

KEY WORDS: *experimental pancreatitis; pancreas; sodium thiosulfate.*

Substances with antienzyme action are widely used nowadays among measures for the treatment of pancreatitis. However, experience of their use has shown that although these substances promote quiescence of the acute manifestations and shorten the period of hospital treatment of patients with edematous pancreatitis, they have no appreciable effect on the course and outcome of destructive types of pancreatitis [3, 6, 13].

The object of the present investigation was to study the effect of sodium thiosulfate on the pancreas in acute experimental pancreatitis. This substance was chosen because of its antitoxic, anti-inflammatory, and desensitizing action [11] and its marked antiproteinase activity [15].

EXPERIMENTAL METHOD

Acute pancreatitis was induced in rats by cooling the splenic part of the pancreas with ethyl chloride [10]. Experiments were carried out on 90 noninbred albino rats weighing 130-150 g. Thirty of these animals received a 30% aqueous solution of sodium thiosulfate by intraperitoneal injection in a dose of 50 mg/100 g body weight in the course of 30 days. The remaining rats with pancreatitis, not treated with thiosulfate, served as the control. The experimental and control animals were killed on the 1st, 3rd, 7th, 14th, 30th, and 60th days of the experiment after preliminary starvation for 18 h. Pieces of pancreas were fixed in 10% neutral formalin by Lillie's method and embedded in paraffin wax. Sections were stained with hematoxylin-eosin, picrofuchsin by Van Gieson's method, and fuchselin by Weigert's method and impregnated with silver by the method of Gordon and Sweet. Glycosaminoglycans were detected by toluidine blue at pH 3.6 and pH 4.6, by alcian blue and by dialyzed iron by Hale's method. Glycogen was determined by Lillie's method, RNA by Brachet's method, protein sulfhydryl groups by the method of Barnett and Seligman, protein amino groups by the method of Yasuma and Ichikawa, total protein by Bonhag's method, and fibrin by the Gram–Weigert method. Zymogen granules were stained with azure-II-eosin by Lillie's method and were also clearly detected on staining by the Gram–Weigert method. Gomori's test with aldehyde-fuchsin was carried out to reveal β cells of the islets of Langerhans.

EXPERIMENTAL RESULTS

Because of cooling of the pancreas all the animals developed hemorrhagic pancreonecrosis, affecting the entire splenic part of the gland. Later in the course of the pathological process, however, substantial differences were observed between the treated and control animals. For instance, after 24 h the necrobiotic changes in the experimental rats were less widespread than in the control animals. Necrotic areas directly bounded healthy areas, well-

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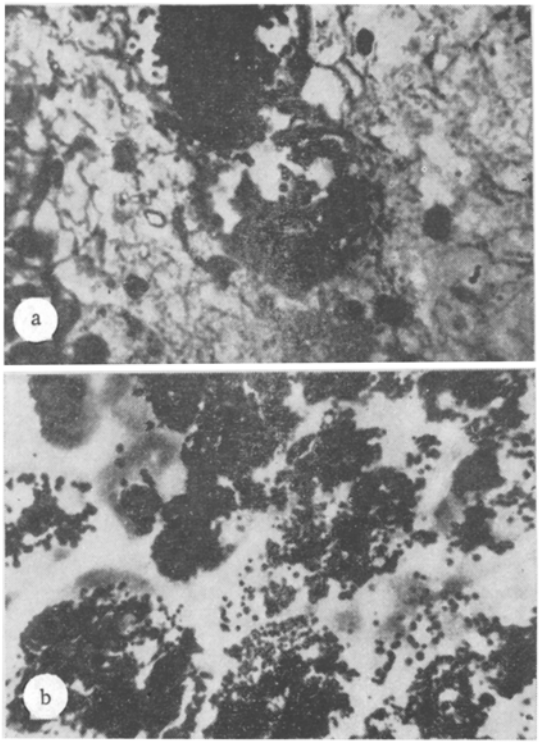

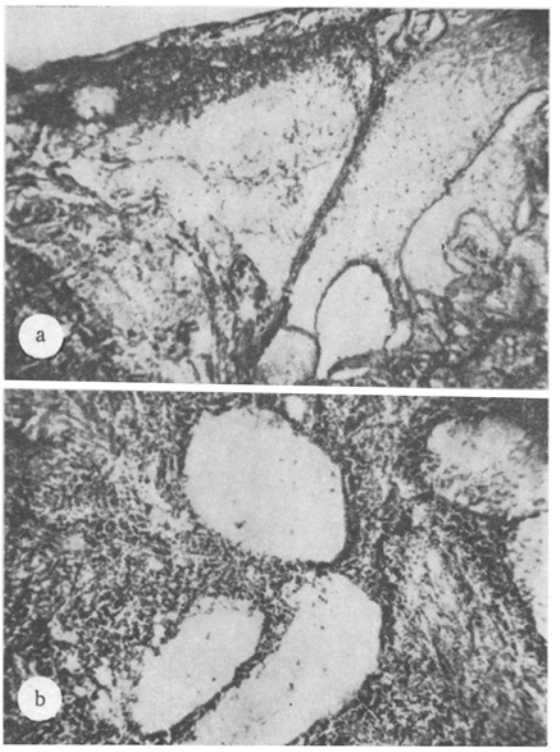

Fig. 1

Fig. 2

Fig. 1. Pancreas of control rat 24 h after production of pancreatitis. a) Zymogen granules completely fill cytoplasm of acinar cells; b) spread of zymogen granules outside region of acini. Gram—Weigert method; 400×.

Fig. 2. Pancreas of control and experimental rats on 14th day of experiment. a) Control rat: leukocytic infiltration poorly defined, pseudocyst formed; b) experimental rat: leukocytic infiltration well marked, necrotic masses undergoing resorption. Hematoxylin-eosin, magnification: a) 30, b) 20.

preserved lobules surrounded by a zone of necrosis were frequently discovered, and there was no leukocyte demarcation barrier. In the uninjured duodenal part of the pancreas in the experimental animals the edema, degenerative changes in the connective-tissue structures and walls of the blood vessels, and the circulatory disturbances were all much less marked. The argyrophilic framework was well preserved. Focal lysis of argyrophilic fibers was observed only in the zone of necrosis. Under the influence of thiosulfate, the content of RNA and of protein and its functional amino and sulfhydryl groups in the cytoplasm of the acinar epithelium and cells of the islets of Langerhans was higher than in the control. The cytoplasm of the β cells was mainly saturated with specific granules. In the control animals during the same period of the experiment different stages of necrobiosis were observed. In the zone of necrobiosis the acinar cells were rinsed in zymogen granules, which packed the basal part of the cells (Fig. 1a) and spread outside the region of the acini into surrounding tissues (Fig. 1b). Intensive edema, fibrinoid changes in the stroma, and seepage of plasma into the walls of the blood vessel developed in the duodenal segment. The cytoplasm of the β cells of the islets contained few specific granules.

On the **third to** seventh days of the experiment leukocytic infiltration aimed at resorption of the necrotic masses was sharply intensified in the experimental rats. Active growth of granulation tissue rich in vascular buds was observed. Its layers contained large numbers of undifferentiated epithelial tubes and they invaded the zone of necrosis along the course of the infiltrating leukocytes. The epithelial tube was cubical and its cytoplasm gave the diffuse but intense reaction for RNA and for amino and, in particular, sulfhydryl groups of proteins. A similar trend of reparative changes was observed in the pancreas of the control rats, but they were much weaker and were accompanied by involvement of new lobules and groups of acini in the pathological process.

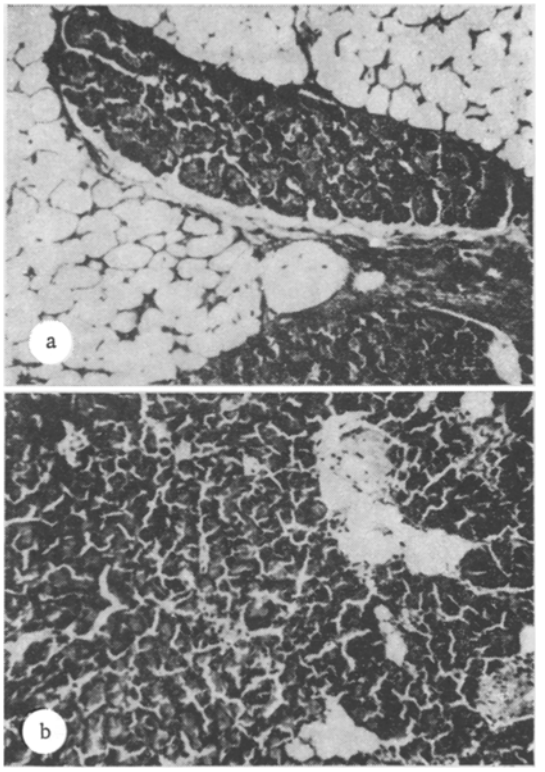

Fig. 3. Splenic portion of pancreas of control and experimental rats on 60th day of experiment. a) Control rat: sclerosis and lipomatosis; b) experimental rat: segment consists of regenerated gland tissue with small foci of lipomatosis. Hematoxylin-eosin, 100×.

On the 14th day of the experiment leukocytic infiltration in the pancreas of the experimental animals affected a large part of the necrotic foci (Fig. 2b), whereas in the control rats this reaction was inhibited and pseudocysts were formed in them (Fig. 2a), traces of which could still be seen even on the 60th day of the experiment.

On the 30th-60th days complete resorption of the necrotic masses, inhibition of development of granulation tissue, and marked degeneration of the acinar and islet tissues were observed in the damaged segment of the gland in the experimental rats, and only very small foci of lipomatosis could be detected (Fig. 3b). In the control rats, however, marked sclerosis and lipomatosis of the splenic part of the pancreas developed (Fig. 3a).

Foci of undifferentiated epithelium persisted for a long time in the pancreas of the experimental animals. Among the adipose tissue surrounding the gland individual epithelial tubules, groups of formed acini, and islets of Langerhans could be observed. Meanwhile layers of undifferentiated epithelial cells resembling centroacinar cells were observed to be invading the adipose tissue.

In both experimental and control animals repair processes in the gland developed from the earliest stages of the experiment and could be traced until its end. However, the regenerative powers of the pancreas were manifested particularly clearly in rats receiving thiosulfate. Intensification of dedifferentiation of the acinar cells and proliferation of the epithelium of the small efferent ducts and epithelial tubules could be regarded as the expression of this fact. Islets of Langerhans were formed from the epithelium of the tubules and efferent ducts and by acinar-islet transformation. Consequently, all functional units of the pancreatic parenchyma, whether located close to the focus of injury or remote from it, took part in the repair process. A similar reaction of the gland has also been described after its resection [2, 14]. In experimental rats regeneration took place chiefly through hyperplasia of the cells. According to Sarkisov [9], this hyperplasia in turn facilitates resorption of the excess connective tissue and prevents the development of sclerotic changes in the organ. Sodium thiosulfate, which helps to preserve the activity of thiol enzymes [4],

maintained the viability of the acinar cells throughout the experiment. Possibly for this reason the relations between stroma and parenchyma remained normal, and because of the high desmolytic activity of the parenchyma no scar was formed and the granulation tissue underwent involution [12]. Thiosulfate itself evidently also delayed maturation, collagenization, and coarsening of the connective tissue, for its use in the treatment of severe suppurative wounds [5] promotes their early cleansing and healing, with the formation of a delicate, elastic scar. In the stage of secondary differentiation of the epithelium of the regenerating pancreas sulfur is utilized actively [7, 8] and the use of preparations containing sulfur has a very effective influence on regeneration of the resected gland [1]. Sodium thiosulfate, a supplier of sulfur, evidently had a stimulating effect on repair processes taking place in the injured pancreas.

Because of the desensitizing properties of thiosulfate, it was able to prevent a second attack of the disease, such as was observed in the control animals on the 30th-60th days of the experiment. This fact was confirmed by the discovery of focal infiltration by lymphocytes and histiocytes, with injury to the acini, the developemnt of edema, seepage of plasma into the blood vessel walls and their fibrinoid necrosis, and by necrosis of individual lobules of the gland.

The use of sodium thiosulfate for the treatment of experimental pancreatitis in rats thus showed that the compound inhibits progression of the pathological process in the pancreas, increases the regenerative potential, and facilitates restoration of the organ by a combination of regenerative hypertrophy and marked epimorphosis. The use of sodium thiosulfate also led to the prevention of second attacks of pancreatitis, and at the end of the process prevented sclerosis of the organ.

LITERATURE CITED

1. V. V. Anisimova-Aleksandrova and V. M. Starshinova, in: Regulatory Mechanisms of Regeneration, (edited by A. N. Studitskii and L. D. Liozner) [in Russian], Moscow (1973), pp. 184-195.
2. E. Sh. Gerlovin and V. F. Ivanova, in: Development, Regeneration, and Transplantation of the Digestive Glands, (edited by E. Sh. Gerlovin) [in Russian], Leningrad (1972). pp. 56-60.
3. V. M. Mavrodi, "The diagnosis and treatment of acute pancreatitis," Author's Abstract of Candidate's Dissertation, Krasnodar (1970).
4. G. V. Matinyan, "Changes in enzyme systems of protein metabolism and in the functions of certain organs in chloroprene poisoning and the antichloroprene action of thiosulfate," Author's Abstract of Doctoral Dissertation, Erevan (1970).
5. U. M. Mukhanov, Zdravookhr. Kaz., No. 6, 70 (1974).
6. V. A. Nikolaev, "The combined treatment of various forms of acute pancreatitis," Author's Abstract of Candidate's Dissertation, Moscow (1972).
7. T. I. Polyakova, in: Development, Regeneration, and Transplantation of the Digestive Glands, (edited by E. Sh. Gerlovin) [in Russian], Leningrad (1972), pp. 65-70.
8. A. A. Puzyrev, in: Development, Regeneration, and Transplantation of the Digestive Glands, (edited by E. Sh. Gerlovin) [in Russian], Leningrad (1972), pp. 60-65.
9. D. S. Sarkisov, Regeneration and its Clinical Importance [in Russian], Moscow (1970).
10. P. S. Simavoryan, "Some aspects of the pathophysiology of pancreatitis," Doctoral Dissertation, Erevan (1973).
11. F. P. Trinus, Pharmacotherapeutic Handbook [in Russian], Kiev (1976).
12. Yu. G. Tsellarius, in: The Morphology of Adaptive Processes of Cells and Tissues [in Russian], Moscow (1971), pp. 17-21.
13. V. V. Chaplinskii and A. I. Gnatyshak, Acute Pancreatitis [in Russian], Moscow (1972).
14. A. V. Shapkina, in: Development, Regeneration and Transplantation of the Digestive Glands, (edited by E. Sh. Gerlovin) [in Russian], Leningrad (1972), pp. 51-55.
15. B. Rasche and W. Ulmer, Z. Ges. Inn. Med., 158, 229 (1972).