MORPHOLOGICAL REACTION OF VARIOUS TISSUES AND ORGANS TO THE OCCLUDING MATERIAL "RABROM"

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The performance of more extensive operations on the pancreas has led to the development of materials occluding the duct system in order to block exocrine secretion and to preserve the internal secretory function. The demands to be met by occluding materials and evaluation of materials of both synthetic and biological origin are reflected in [1, 2]. At the present-time intensive studies of occluding materials based on proteins of plant and animal origin are in progress [3, 4, 5, 6]. The ability of these materials to undergo biodegradation means that they can be used for temporary occlusion of the pancreatic ducts, thus preventing the development of postoperative complications. Similar kinds of occluding materials have not so far been available in the USSR.

The aim of this investigation was to study the morphological reaction of various tissues and organs to injection of an antibacterial x-ray contrast material of protein origin ("Rabrom"), developed at the A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR (for which an author's certificate No. 4610894/30-14 (155783) was granted on February 26, 1990).

EXPERIMENTAL METHOD

Experiments were carried out on 100 albino rats weighing 150-170 g and 24 dogs weighing from 24 to 37 kg. The experiments and removal of the material were carried out under endotracheal ether anesthesia. There were four series of experiments.

Series 1-3 were carried out on rats, series 4 on dogs. In series 1, 0.5 ml of "Rabrom" was injected subcutaneously on the lateral surface of the thigh. In series 2 0.3 ml of Rabrom was injected intraperitoneally on to the surface of the liver. In series 3, 0.3 ml of "Rabrom" was injected into the liver, and in series 4 the pancreatic ducts were occluded (2-3 ml). The material was polymerized for 3-8 min and its viscosity was 0.003 ± 0.001 Pa. The low viscosity ensured complete filling of the whole duct system of the pancreas, including ducts of the 2nd and 3rd orders. Parallel with the morphological investigation, blood levels of activity of the enzymes transaminase, histidase, and urokinase, reflecting the presence of destructive changes in the cells of the kidneys, pancreas, and liver were determined. Material from the rats was studied on the 3rd, 6th, 14th, and 30th days, from dogs after 12 and 24 h, 7, 14, and 21 days, and 4.5 and 5 months. The material was fixed in 12% neutral formalin solution and embedded in celloidin and paraffin wax. Sections 7 μ thick were stained with hematoxylin and eosin and by Van Gieson's method, and for fibrin by the picro-Mallory and Masson's methods. On staining with hematoxylin and eosin the material had the appearance of dense homogeneous pink masses whereas after staining for fibrin it appeared in shades of crimson.

EXPERIMENTAL RESULTS

Series 1 – Subcutaneous Injection. After 3 days the material remained quite inert beneath the skin, with no evidence of necrosis in the adjacent tissues and with no reaction from it. By the 6th day a thin connective-tissue capsule

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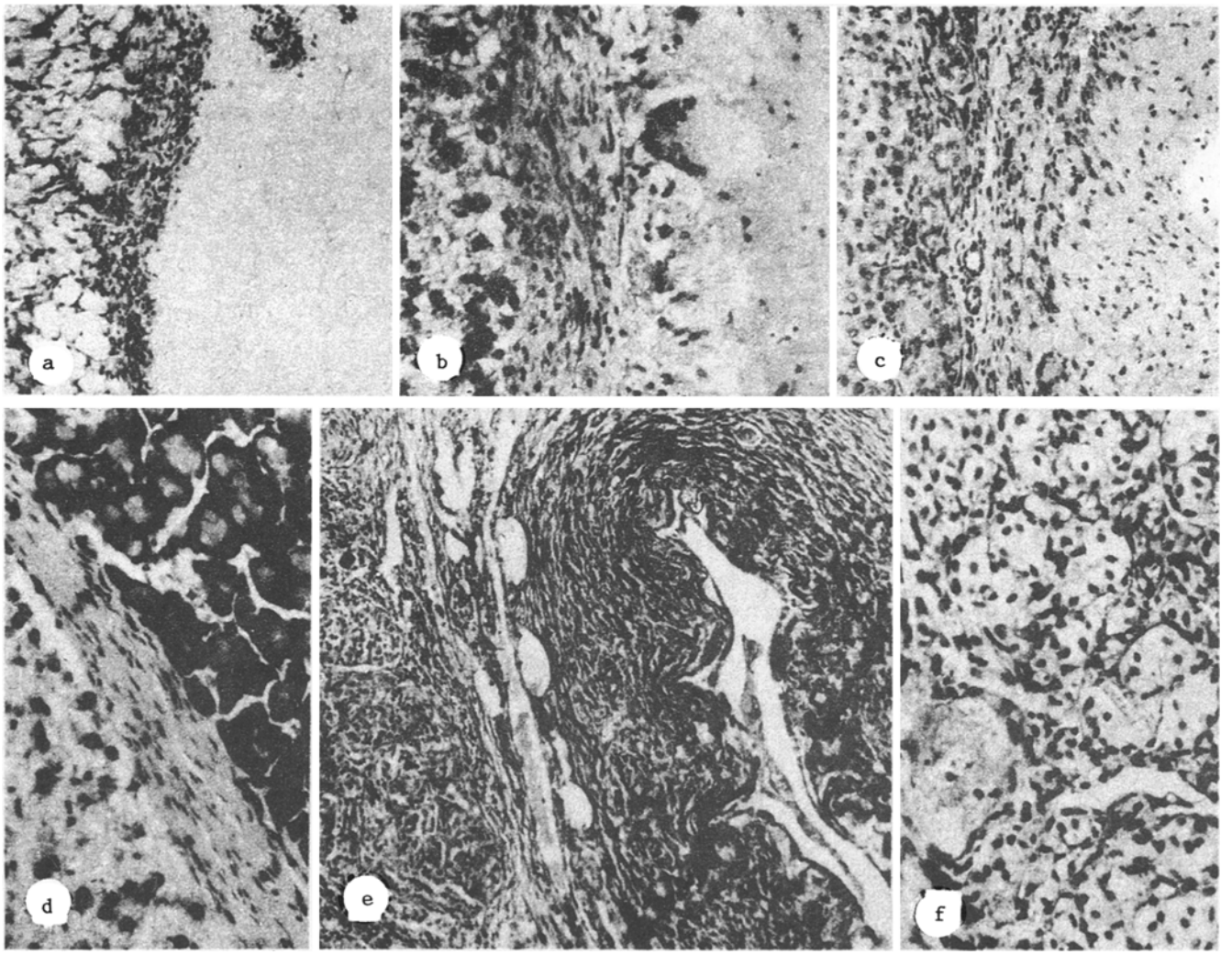

Fig. 1. a. Subcutaneous injection, 1 month, rat. Material has the appearance of dense homogeneous eosinophilic masses, surrounded by a thin connective-tissue capsule. Weak macrophagal reaction. Hematoxylin-eosin, ×90. b. Intraperitoneal injection, 6 days, rat. Marked giant-cell reaction around Rabrom material. Thin connective-tissue capsule, blood vessels and inflammatory infiltration not present in thickness of capsule. Hematoxylin-eosin, ×260. c. Injection of Rabrom inside hepatic parenchyma, 6 days, rat. Thin connective-tissue capsule formed around material. Many multinuclear giant foreign body cells present in subcapsular regions, occluding material invaded by fibroblasts. Proliferation of bile ducts of small caliber between capsule and hepatic parenchyma. Hematoxylin-eosin, ×160. d. Intraperitoneal injection, 1 month, rat. Glisson's capsule at site of application of "Rabrom" occluding material sclerosed. Pancreatic tissue adherent to liver without morphological changes. Hematoxylin-eosin, ×260. e. Occlusion of pancreatic ducts, 21 days, dog. Occluding material in lumen of Wirsung's duct. Lumen of duct slitlike, walls severely sclerosed. Exocrine parenchyma atrophied, neurovascular carcass denuded. Van Gieson, ×90. f. Occlusion of pancreatic ducts, 4.5 months, dog. Field of endocrine cells with numerous vessels of capillary type. Hematoxylin-eosin, ×260.

was formed due to proliferation of fibroblasts, and these cells penetrated to a small depth into the outer layers of the material. The macrophagal reaction on the inner surface of the capsule was well marked. There was no inflammatory infiltration of the capsule or blood vessels in it. After 1 month the quantity of material in the lumen of the capsule was somewhat reduced. Around it a thin connective-tissue capsule had formed, demonstrating its inertia (Fig. 1a). The degree of resorption of the material around its circumference also varied. In the experiments of series 2, after application of the

material in a thin layer on the surface of Glisson's capsule of the liver, by the 6th day organization was taking place due to proliferation of fibroblasts. Application of the material in a thicker layer in the form of drops led to the formation of a thin connective-tissue capsule around it by the 6th day, with invasion of fibroblasts into the outer layers of the "Rabrom." Inflammatory infiltration of the material was not present, but there was a marked giant-cell macrophagal reaction around it (Fig. 1b). After 1 month, no thickening of Glisson's capsule had occurred at the site of application of the material compared with the initial times of the investigation. The occluding material could be detected in certain fields of vision in the form of homogeneous dense eosinophilic masses. Discovery of remnants of the material was helped by the presence of giant multinuclear foreign body cells around it. In series 3, with injection of material inside the liver, a demarcation zone consisting of one or two layers of fibroblasts could be clearly seen as early as on the 3rd day. By the 6th day (Fig. 1c) a thin connective-tissue capsule, rich in fibroblasts, had formed around the occluding material, and a few thin collagen fibers also were seen. Vessels and inflammatory infiltration were not present in the thickness of the capsule. On the boundary of capsule and Parenchyma of the liver, many proliferating bile ducts of small caliber could be seen. In the subcapsular regions, in the thickness of the material, invading fibroblasts were present, together with many giant multinuclear foreign body cells. The parenchyma of the liver adjacent to the capsule, which had formed around the Rabrom material, was normal in structure. After intraperitoneal injection of the material, in some cases not only were loops of intestine glued together, but adhesion of the pancreatic gland tissue, diffusely distributed in the thickness of the mesentery, to Glisson's capsule at the site of application of the material, took place in some cases after intraperitoneal injection of the material. Between the adherent loops of intestine a very thin layer of collagenized connective tissue could be detected, and where the pancreatic tissue was adherent to the surface of the liver there was only slight thickening of Glisson's capsule (Fig. 1d), the gland tissue preserving its normal structure.

The good results obtained by subcutaneous, intraperitoneal, and intrahepatic injection of "Rabrom" material made it possible to move on to the main series of experiments, namely occlusion of the pancreatic duct system in dogs. A marked decrease in size of the pancreas was observed by the 21st day, and after 4.5-5 months elements of the gland could be found in the form of single groups of small lobules only in the region of the head of the gland, and in the thickness of the layers of peritoneum along the course of the former tail of the gland. On histological investigation of sections obtained after 21 days, marked atrophic processes were seen in the exocrine parenchyma (Fig. 2), in the form of breakdown of the structural complexes and atrophy of the acini, and an increase in the number of endocrine tissue cells between the atrophied cells of the exocrine parenchyma. Occluding material could be seen in the lumen of Wirsung's duct and the ducts of small caliber in the form of a narrow band; the lumen of the ducts was greatly narrowed and their walls sclerosed. Atrophic and sclerotic processes in the pancreas were well defined, and neurovascular bundles were arranged in the substance of the connective-tissue layers. Inflammatory infiltration was not present in the thickness of the duct walls or between the atrophied parenchyma, and there was likewise no giant-cell macrophagal reaction. After 4.5-5 months the pancreatic tissue consisted entirely of fields of endocrine cells (Fig. 1f) and neurovascular bundles arranged between them; sclerosis was well marked. No inflammatory infiltration or macrophagal reaction was present. The only remains of the duct system was Wirsung's duct with a slitlike lumen, immured in fibrous tissue. A very small amount of occluding material was present in the lumen of the duct, and staining for fibrin revealed a whole series of colors ranging from crimson to lilac and violet.

The following conclusions can be drawn from these results. "Rabrom" material is inert in the sense of not leading to the development of inflammatory changes when applied to the surface or into the thickness of organs and tissues in rats, and also into the lumen of the pancreatic duct system in dogs. Necrosis of tissues and organs in response to its injection was not found. When injected into tissues and organs a thin connective-tissue capsule formed around it, and a well-marked giant-cell macrophagal reaction developed to it, i.e., it underwent resorption. In response to injection of the material into the duct system of the dog's pancreas complete atrophy of the exocrine part of the gland and hyperplasia of its endocrine system developed.

The normal blood levels of transaminase, histidase, and urokinase confirm that Rabrom has no damaging effect on tissues and organs.

LITERATURE CITED

1. D. F. Blagovidov, T. T. Daurova, M. V. Danilov, et al., Vestn. Khir., **129**, No. 9, 44 (1982).
2. I. R. Karapetyan, A. A. Adamyan, N. I. Ostretsova, et al., Khirurgiya, No. 2, 137 (1989).
3. R. Estourgie, J. Surg. Res., **34**, No. 2, 164 (1983).

4. C. Gebhardt and M. Stolte, Gang. Arch. Chir., **346**, No. 35, 149 (1978).
5. L. F. Hollender, J. Bahnini, and J. C. Bedini, Z. Gastroenterol., **24**, No. 4, 218 (1986).
6. N. Sochendra, H. Grimm, and H. W. Schreiber, Dtsch. Med. Wschr., **111**, No. 19, 727 (1986).

MECHANISM OF FORMATION OF THE INTEGRAL HYPOTHALAMIC RESPONSE DURING PSYCHOEMOTIONAL OVERSTRAIN

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Psychoemotional overstrain is an important factor in the development of many pathological processes. Investigations have shown that the mechanism of realization of psychoemotional overstrain (PEO) is triggered in the hypothalamus by nervous impulses arriving from the cerebral cortex, reticular formation, and limbic system [1-3], which may have either an excitatory or an inhibitory action. However, there is as yet no single concept which can explain the general response of the hypothalamic region to various kinds of PEO (the stress reaction). It was accordingly decided to analyze changes in hypothalamic structure and function in rats exposed to various kinds of PEO, differing in intensity and duration.

EXPERIMENTAL METHOD

Experiments were carried out on 63 noninbred male albino rats initially weighing 180-220 g. The animals were divided into 4 groups. Group 1 (control) comprised 12 intact animals. The 18 rats of group 2 were repeatedly immobilized for 3-4 h at a time. Emotional stress was induced in the 10 rats of group 3 by subjecting them to hypokinesia by artificially restricting their movements by keeping them in especially made cages. The rats of groups 1-3 were killed under superficial ether anesthesia on the 1st, 7th, 20th, 30th, 45th, and 60th days of the experiment. The rats (15) of group 4 were intermittently starved. The animals were isolated in separate cages and given nothing but water for 5-7 days, after which they were put back on a normal diet. These animals were thus subjected to starvation 1, 2, 3, 4, and 5 times and killed after 7, 20, 30, 45, and 60 days of the experiment. The rat's brain was fixed in Bouin's fluid, and after hardening the hypothalamic region was embedded in paraffin wax. From a single block of hypothalamus 600-800 serial frontal sections were cut and stained by Missl's method. Some of the material was fixed in a 4% solution of paraform ("Fluka," Switzerland), made up in 0.1 M cacodylate buffer, followed by postfixation in buffered 1% osmium tetroxide solution and embedded in resins. Ultrathin sections were studied in EVM-100LM and PEM-100 electron microscopes. Negatives were standardized under magnification of 6000 and 15,000 times. A wide range of morphometric methods of investigation was used: the volume of the nuclei of the neurons, the internuclear distance, bulk density and specific surface area of the neurons, and their number per unit volume were determined. The degree of damage to the neurons was studied by counting the unchanged nerve cells, those with slight and severe changes, and those absent altogether. Some of the material was studied on a "Microvideomat-2" microscope ("Opton," Germany) with a microcomputer (USA), using the "Stereo-1" program. The numerical results were analyzed by computer. The hypothalamic region of the rats was investigated in accordance with the classification suggested by Szentagothai and co-workers [2]: the suprachiasmatic nucleus (SCH), supraoptic nucleus (SO), paraventricular nucleus, parvocellular part and magnocellular part (PV, not distinguished in Figs. 2 and 3), ventromedial nucleus (VM), dorsomedial

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