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EFFECT OF THYMALIN ON THYMUS MORPHOLOGY AND FUNCTION IN MICE

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In the modern view the thymus produces hormone-like factors which take part in the regulation of the immunocompetent system [5, 8, 9, 12]. Most workers state that these factors are polypeptide in nature [7, 10, 14] and they consider that, as products of the epithelial cells of the thymus, they promote differentiation of stem cells into T lymphocytes both inside and outside the thymus [6, 11, 13].

Since there is little information in the literature on the morphology of the thymus under the influence of thymus factor *in vivo* [12], it was decided to study the effect of thymus polypeptide factor (thymalin) on the morphology and function of the gland in mice.

EXPERIMENTAL METHOD

Thymalin was obtained from the calf thymus by ion-exchange chromatography [2]. It has been shown that thymalin is a substance of basic character with molecular weight of about 5000 daltons and that it consists of 38 amino acid residues [3].

The experimental study was carried out on 36 male CBA mice weighing 18-20 g, divided into two equal groups. Each group of mice was subdivided into experimental and control animals. Thymalin was injected subcutaneously into the experimental mice of group 1 in a dose of 0.05 mg/g body weight in 0.2 ml physiological saline daily for 3 days, and into the experimental mice of group 2 daily for 10 days. This dose was chosen because its administration to mice caused marked stimulation of the immune response to a thymus-dependent antigen, namely sheep's red blood cells (SRBC) [1]. Control animals of both groups received physiological saline by the same scheme. On the 4th and 11th days of the experiment the mice were decapitated. The thymus was weighed, fixed in 10% neutral formalin solution, and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, azure II-eosin, methyl green–pyronine (by Unna's method), and alcian blue, the PAS reaction was carried out, and alkaline phosphatase activity was determined (by Gomori's method) in fresh frozen sections. In the microscopic analysis of the material, areas of cross-section through nuclei of the epithelial cells of cortex and medulla were measured by a graphimetric method using the RA-7 drawing apparatus; the thickness of the cortex and medulla was measured with an ocular micrometer and the ratio between them calculated. The rate index of the thymus (weight of the gland as a proportion of body weight) was determined. The numerical data were subjected to statistical analysis by Student's criterion.

EXPERIMENTAL RESULTS

After injection of physiological saline for 3 days the weight index of the thymus was 2.72 ± 0.21. Lobes of the thymus were large, with clear division into cortex and medulla

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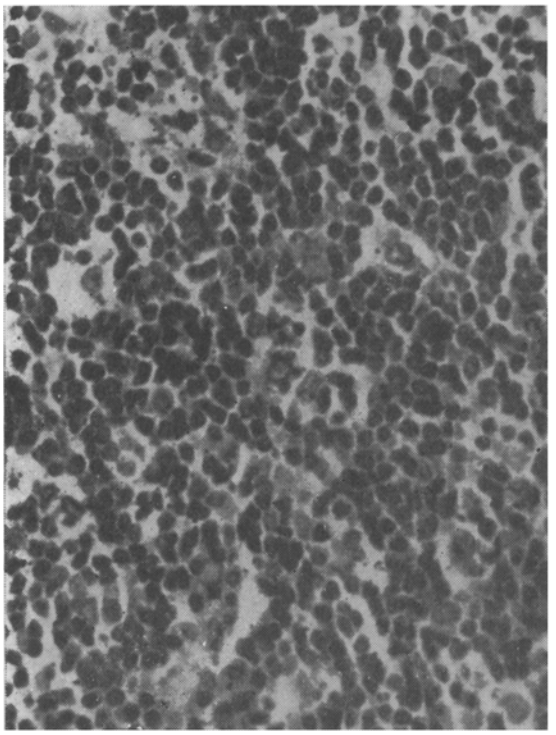

Fig. 1

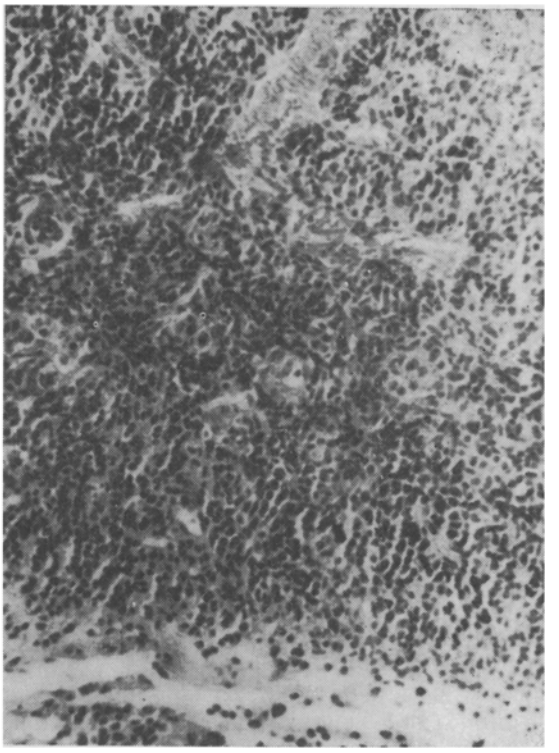

Fig. 2

Fig. 1. Intensification of macrophagal reaction in cortex of lobule of mouse thymus after injection of thymalin for 3 days. Azure II-eosin, 250 ×.

Fig. 2. Absence of division into layers and dilatation of vessels of central zones in mouse thymus lobule after administration of thymalin for 10 days. Hematoxylin–eosin, 120 ×.

(ratio 0.87 ± 0.035). The cortex presented a slightly "moth-eaten" appearance, for as a result of ligation of the thymocytes, the large reticuloepithelial cells, as a rule arranged singly, became clearly visible. Cells containing PAS-positive granules were seen in small numbers throughout the lobules of the thymus, but they differed in the size of their nuclei. Cells in the medulla had larger nuclei. The mean area of cross section of nuclei of reticuloepithelial cells was 0.0874 ± 0.0019 conventional unit (c.u.) in the cortex and 0.1317 ± 0.0037 c.u. in the medulla.

After injection of physiological saline for 10 days the weight index of the thymus fell to 1.88 ± 0.11 ($P < 0.05$). Besides large lobules, small ones also were seen. Division into layers still remained clear (ratio 0.94 ± 0.70). In the cortex foci of translucency resulting from a decrease in the number of thymocytes began to appear both immediately beneath the capsule and in deeper zones, so that reticuloepithelial cells became more clearly visible. The mean area of cross section of their nuclei was 0.0894 ± 0.0021 c.u. The cytoplasm of many cells appeared to be vacuolated and the boundaries could be traced with difficulty. Near the capsule epithelial cells with pycnotic nuclei and a small rim of cytoplasm were found more frequently. Phagocytosis was present to only a mild degree in the cortex. The mean area of cross section of nuclei of the reticuloepithelial cells of the medulla was somewhat greater than after administration of physiological saline for 3 days (0.1408 ± 0.0034 c.u., $P < 0.05$), whereas nuclei of the cortical reticuloepithelial cells remained almost the same size. Cells with PAS-positive granules were found more often; Hassall's corpuscles at different stages of maturation were found in almost the same number. The number of mast cells was appreciably increased and they were arranged mainly along the course of the vessels and in the interlobular bands of connective tissue or near the capsule. The lumen of the small vessels appeared dilated and they contained many blood cells, somewhat with focal aggregation of erythrocytes. High alkaline phosphatase activity was found in the reticuloepithelial cells of the capillaries.

Injection of thymalin for 3 days caused a marked decrease in the weight index of the

thymus compared with the control at the same time (1.08 ± 0.18, $P < 0.01$). The lobules were greatly reduced in size. The medulla was larger than the cortex (ratio 0.59 ± 0.055). There were some lobules in which division into layers was absent. Obliteration of the boundaries was explained both by migration of the thymocytes and by their mass destruction. Signs of phagocytosis were strongly evident (Fig. 1), and phagocytosis of unchanged thymocytes by macrophages was observed quite often. Many of the reticuloepithelial cells of the cortex were closely surrounded by thymocytes which, as a rule, contained pycnotic nuclei. The mean area of cross section of nuclei of the cortical reticuloepithelial cells differed only a little from that of the control (0.0927 ± 0.0057 c.u., $P > 0.05$), but nuclei of the reticuloepithelial cells of the medulla were mainly appreciably reduced in size (0.1158 ± 0.0025 c.u.) ($P < 0.01$). Among them some very large cells with pale, clearly distinguishable cytoplasm and a very large nucleus, 2 or 3 times larger than the rest of the nuclei, were constantly found among them. Vacuoles were often observed in the cytoplasm of these cells. The number of Hassall's corpuscles in different stages of maturation was increased. In size and shape they were distinctly variable. An increase in the content of PAS-positive material, often homogeneous in appearance, was observed in the central zones of the corpuscles. Besides dilated vessels in the medulla, dilatation of small vessels in the cortex was observed, especially in areas adjacent to the medulla. In the PAS reaction the basement of the capillaries stained a deep crimson color. In Gomori's test high alkaline phosphatase activity was found in the reticuloepithelium.

Injection of thymalin for 10 days caused an even greater decrease in the weight index compared with the control (0.94 ± 0.09, $P < 0.01$). Small lobules were clearly predominant in the thymus, often without division into layers (Fig. 2), and in cases when such a boundary could be identified, the ratio of cortex to medulla was 0.53 ± 0.053 ($P < 0.01$). The area of cross section of nuclei of the reticuloepithelial cells in the peripheral zones of the lobules was 0.0821 ± 0.0030 c.u. ($P < 0.01$), and in the central zones 0.0976 ± 0.0079 c.u. ($P < 0.01$). After injection of thymalin for this period an even greater decrease in size of the nuclei of most reticuloepithelial cells was observed, although as before, among them there were still some very large cells, distinguished by their large pale nucleus. Disintegration of the thymocytes and phagocytosis were less well marked than after injection of thymalin for 3 days. In lobules with no division into layers, Hassall's corpuscles were found in both central and peripheral zones, sometimes almost beneath the capsule. The numerous dilated vessels were mainly in the central zones of the lobules (Fig. 1). The walls of the vessels were rather swollen, and on staining with alcian blue they gave a delicate bluish green color, which was not observed at other times.

Injection of thymalin thus did not give rise to evident antigenic reorganization of the gland, but a considerable decrease in weight of the thymus was constantly found. Injection of thymalin, especially in the early stages, was accompanied by intensive disintegration of the thymocytes and stimulation of phagocytosis. The reticuloepithelial cells of the thymus responded variously, but most cells responded with a decrease in size of the nuclei, indirect evidence of depression of their functional activity. The impression was created that the thymocytes seemed to be released from control of the epithelial cells, so that delymphatization of the thymus took place on account of both destruction and migration. It can be postulated on the basis of these results that thymalin induces changes in the mouse thymus indicative of stimulation of thymocyte function.

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EFFECT OF TREATMENT IN A CONTROLLED GERM-FREE ENVIRONMENT ON MORPHOLOGY OF HEALING OF EXTENSIVE SUPPURATING WOUNDS

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A new open method of treatment of suppurating wounds in a controlled germ-free environment (GFE) is now being successfully developed. The method of treatment in a GFE, described by the writers previously [2], includes the following stages: 1) active surgical treatment of the purulent focus; 2) placing the affected part of the body in a plastic isolator with GFE until healing of the wound is complete; 3) intensive pre- and postoperative treatment.

Under the influence of the microclimate in the isolator the wound surface quickly becomes covered with a loose scab of dried exudate. Both the wound exudate and the surface areas of the tissues are exposed to the drying action of a current of air. During the first 24 h of treatment, the classical signs of inflammation regress, and by the end of the 2nd and beginning of the 3rd day, granulation tissue can be detected macroscopically in the wound, covering the wound defect whatever its area by the 6th-7th days. The number of microorganisms in the wound decreases from 10^8-10^9 to 10^2-10^3/g tissue, or the wound becomes sterile. In other words, the clinical course of wound healing during treatment in a GFE is speeded up. It was decided to confirm the clinical features of the course of wound healing by the results of morphological studies of healing of extensive suppurating wounds during treatment in a GFE.

EXPERIMENTAL METHOD

A morphological study was made of wound healing in a GFE by histochemical and cytological investigation of biopsy specimens from an extensive suppurating wound and of squash preparations from wounds.

The duration of treatment of 25 patients with extensive (from 1000 to 1500 cm^2 in area) suppurating wounds in an isolator with GFE was 17-26 days. Biopsy material was studied before treatment and after treatment in the GFE for 1, 3, 5-7, and 10-15 days. Material was fixed in 10% neutral formalin, absolute alcohol, and Carnoy's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and by Van Gieson's method and the following histochemical tests were carried out: for glycogen and neutral polysaccharide (PAS reaction), for glycosaminoglycans (with alcian blue and toluidine blue), for RNP (by Brachet's method), for lipoids and lipids (by Goldman's method and with Sudan III). Acid phosphatase was determined by Gomori's method. Staining for microorganisms was carried out by the Gram–Weigert method. Squash preparations from wounds were stained by the Romanovsky–Giemsa method.

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