of resorbing necrotic masses more rapidly and thus stimulating the development of the connective tissue of the liver.

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LYMPH NODE AND SPLEEN MORPHOLOGY IN GNOTOBIOTIC RATS

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The lymph nodes, spleen, and adrenal cortex of 12 gnotobiotic and 12 control Wistar rats were investigated by the ordinary histological and histochemical methods. Absence of the normal microflora was shown to inhibit the development of the zone of B lymphocytes of the lymph node and to cause thickening of the adrenal cortex and enlargement of the lipid inclusions in its cells; no effect on the structure of the lymphoid follicles of the spleen could be found. Inhibition of lymphopoiesis in the rat lymph node takes place as a result of absence of direct microbial stimulation and its mechanism involves a hormonal principle. These factors have no effect on lymphopoiesis in the spleen, which is stimulated within the organ itself.

KEY WORDS: gnotobiotic animals; lymph nodes; spleen; adrenals.

Lymphoid tissue is known to maintain the normal immunological homeostasis of the organism [1]. Ecological factors, among which an important place is occupied by microorganisms, have a great influence on the structure of the lymphoid organs. Gnotobiotic animals are the only objects by which the action of the microbial factor on the systems of the body can be standardized; the need thus has arisen for the organs of such animals to be studied with a view to determining their norms and studying the role of the microbial factor in the histophysiology of the tissue of the protective lymphoid system.

The importance of the normal microbial factor for the state of the lymphoid tissue and adrenal cortex was studied.

EXPERIMENTAL METHOD

The mesenteric lymph node, spleen, and adrenal cortex of 12 gnotobiotic Wistar rats aged 4 months were studied. Gnotobiotic animals were obtained from the Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR. Similar organs were investigated from ordinary conventional animals of the same strain. Material was fixed in Carnoy's fluid and in 10% formalin solution. Sections 5-7 μ thick were stained with hematoxylin and eosin, for RNA by Brachet's method with the appropriate control, for free iron in the spleen by Perls' method, and for lipids in the adrenal cortex by Sudan III and IV.

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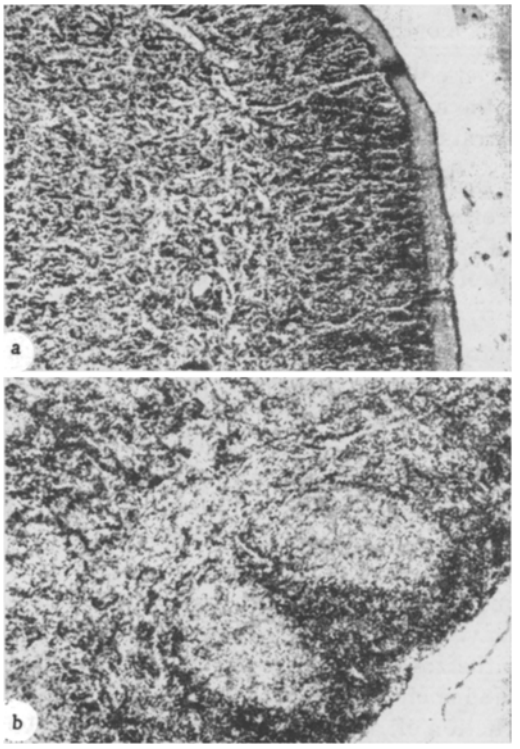

Fig. 1

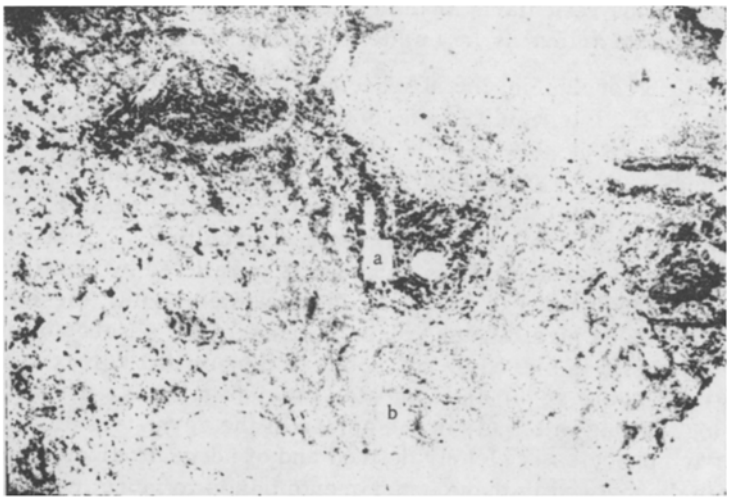

Fig. 2

Fig. 1. Mesenteric lymph node of gnotobiotic (a) and control (b) rats. Fixation in Carnoy's fluid and stained with hematoxylin–eosin, 120 ×.

Fig. 2. Spleen of gnotobiotic rat. Fixation in Carnoy's fluid and stained by Brachet's method, 120 ×: a) central artery, periarterial zone of T lymphocytes; b) zone of B lymphocytes.

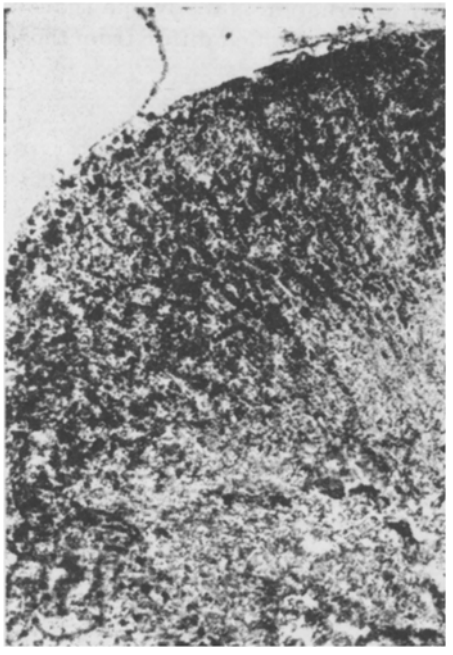

Fig. 3. Adrenal cortex of gnotobiotic rat. Fixation in 10% formalin solution and stained with Sudan III and IV, 120 ×.

EXPERIMENTAL RESULTS

The investigations showed that the mesenteric lymph node of gnotobiotic rats has no clearly identifiable cortical layer, no secondary lymphoid follicles, and no reactive centers in them (Fig. 1a). In conventional animals (Fig. 1b) the cortical layer is fairly thick and contains large secondary lymphoid follicles with reactive centers forming a wide zone of B lymphocytes. The impression was obtained that the peripheral zone of B lymphocytes and the paracortical zone of T lymphocytes in the germ-free animals were disproportionally reduced: A sharp decrease in the former was accompanied by a small decrease in the latter. Lymphocytes in the T-lymphocyte zone showed very weak pyroninophilia of their cytoplasm.

Lymphoid follicles in the spleen of the gnotobiotic rats were indistinguishable in size and structure from those in the control animals. The central artery, the periarterial zone of T lymphocytes (a), and the peripheral zone of B lymphocytes (b) could be clearly distinguished in them (Fig. 2). The latter differed from the former in their clearer nucleus and the deep pyroninophilia of their cytoplasm. Reaction centers with dividing large lymphocytes were frequently seen in the zone of B lymphocytes. Inhibition of lymphopoiesis thus was not observed in the spleen of animals in a germ-free state. In sections of the organ stained by Perls' method, very many crystals of free iron were seen in the reticular stoma of the red pulp from the gnotobiotic rats. They filled the cytoplasm of macrophages and reticulum cells and lay freely in the loops of fibers.

Iron could be seen in the spleen of conventional animals at the age of 4 months as infrequent small crystals.

One of the functions of the spleen is to carry out hemolysis of red cells and to liberate breakdown products, including particles of free iron. Saturation of the red pulp of the spleen with free iron thus points to more intensive hemolysis of red cells in germ-free than in ordinary rats.

The adrenal cortex of the gnotobiotic rats was thicker (Fig. 3) than that of ordinary animals. The sudanophilic zone was much wider. Lipid granules were found in the cells of the whole zona fasciculata and even in the zona reticularis of the cortex. These histological changes are evidence that the adrenal cortex of the germ-free animal is in a state of greater hormonal activity than that of the conventional animal.

In conclusion, the absence of a normal microflora in the body leads to underdevelopment of not every part of the lymphoid tissue. In the underdeveloped lymph nodes, chiefly the peripheral zone of B lymphocytes, precursors or plasma cells which produce humoral antibodies, does not develop properly. Meanwhile, in the lymph nodes of gnotobiotic rats the paracortical zone of thymus-dependent T lymphocytes is preserved. Preservation of this zone is evidently connected with preservation of the thymus, which does not disappear in germ-free animals but undergoes age involution [3], just as it does in ordinary animals. Underdevelopment of the cortical zone of the lymph node is paralleled by hypertrophy of the adrenal cortex and increased formation of lipid inclusions in its cells. In the writer's view, these changes in the lymph node of gnotobiotic rats, just as in guinea pigs [2], take place because of the absence of direct microbial stimulation, and a role in their mechanism is played by hormones of the adrenal cortex, which in germ-free rats is thickened, and the cells of all its layers accumulate substances rich in lipids. These factors have no significant effect on the histophysiology of the white pulp of the spleen of the germ-free rat. Not only the zone of T lymphocytes but also that of B-lymphocytes is clearly defined and of adequate size. The increased content of free iron particles in the organ discovered in these experiments points to more intensive destruction of red cells in the spleen of gnotobiotic animals, with liberation of protein products. This process can be regarded as a true congenital mechanism of stimulation of lymphopoiesis and the phagocytic activity of the red pulp cells. This mechanism acquires greater or lesser activity depending on the ecological factor. Gnotobiotic conditions stimulate the breakdown of red cells, and in this way (in the absence of microbial stimulation and in the presence of hypercorticism) normal lymphopoiesis is maintained in the spleen with the formation of reactive centers in the peripheral zone of the lymphatic follicle, evidence of the active state of the B lymphocytes. The conditions of inborn, natural stimulation of lymphopoiesis ensure the constant presence of considerable numbers of macrophages and plasma cells in the spleen. The absence of any marked cellular destruction in the lymph nodes and Peyer's patches leads to processes of lymphopoiesis and storage of lymphocytes which differ from those in the spleen.

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