EFFECT OF GNOTOBIOTIC CONDITIONS ON THE HISTOPHYSIOLOGY OF LIVER AND SPLEEN TISSUES

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The liver and spleen of gnotobiotic Wistar rats were studied by histochemical methods and the liver electron-microscopically. Under germfree conditions of existence of the animal the succinate dehydrogenase and nonspecific esterase activity in the liver decreased, fatty infiltration of the cytoplasm of the hepatocytes and Kupffer cells increased, and some of the cells developed fatty degeneration. Meanwhile acid phosphatase activity and the number of lysosomes increased in the biliary poles of the hepatocytes, whereas in the spleen destruction of erythrocytes and the liberation of free iron and pigments, which stimulate the excretion of bile in germfree animals, were increased.

KEY WORDS: germfree animals; liver; spleen; histochemical changes.

Gnotobiotic conditions have been shown to have a marked action on animals [1, 4, 9, 11]. The structure of those organs of germfree animals which, under normal conditions, are in contact with the microflora and its metabolic products, show the greatest changes. Significant disturbances have been observed, above all, in organs of the lymphoid system [3, 5, 8, 10]. However, the fine structural changes in other organs, notably parenchymatous organs, have not been described.

As an organ with many vitally important functions, there is no question that the liver plays an important role in adapatation of the animal to germfree conditions of life. There are only isolated details in the literature on the response of the liver to the conditions of germfree existence. The liver of gnotobiotic animals has been shown to have a more uniform histological picture and to contain fewer plasma cells in the connective tissue of the portal tracts [13] and also to have lower activity of its oxidative enzymes [12]. There is very little information on the spleen of germfree animals, and it is contradictory. Some workers consider that in the spleen, just as in the lymph node, lymphopoiesis is depressed [5, 8], whereas others found no changes in the structure of the pulp of this organ in germfree animals [3].

The object of this investigation was to study the character of histophysiological changes in the liver and spleen as organs without direct contact with the microflora and linked with each other through their participation in the production of bile pigments.

EXPERIMENTAL METHOD

Germfree Wistar rats were obtained in a special isolator at the Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, and kept on a sterile diet. Control Wistar rats were kept under ordinary conditions, but also received the sterile diet. The liver of female rats aged 15, 30, and 90 days (five animals of each age group) was studied. Material was fixed in Carnoy's fluid and 10% neutral formalin. Besides the ordinary histological methods, sections were stained with Sudan III and IV for neutral lipids, by Nachlas's method for succinate dehydrogenase (SD) activity, by Gomori's method for acid phosphatase (AP), and by azocoupling with α-naphthyl acetate for nonspecific esterase (NE) [2]. Control reactions also were carried out. The liver of the gnotobiotic and conventional rats at the age of 90 days was examined electron-microscopically. Material for this purpose was fixed in 1% osmium tetroxide solution in cacodylate buffer, pH 7.4, and embedded in Araldite. Sections were stained with uranyl acetate and examined in the Hitachi-7 electron microscope.

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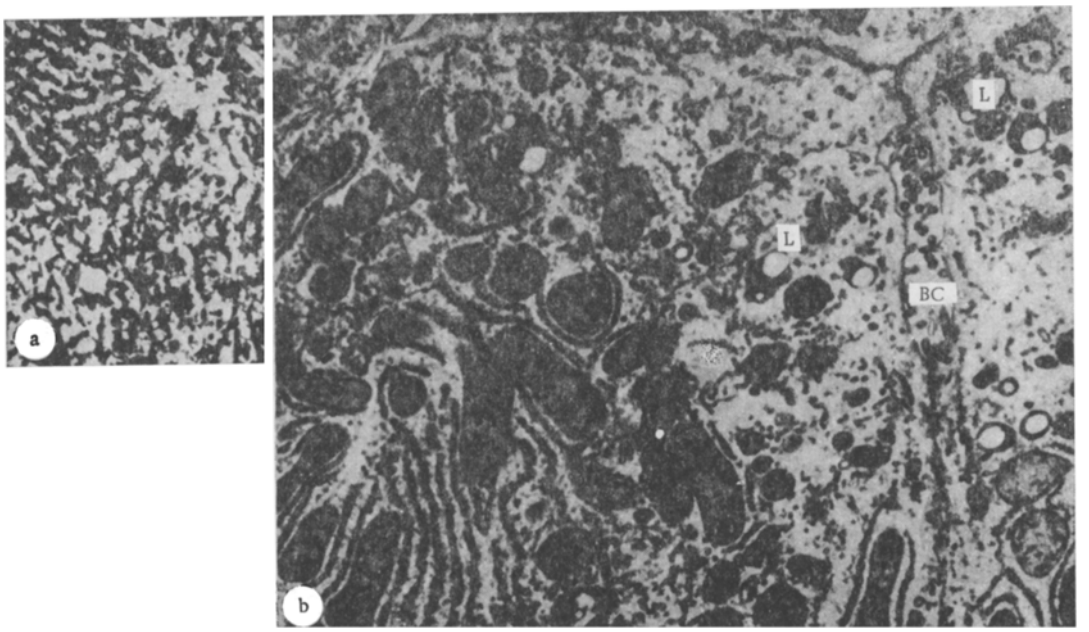

Fig. 1. Liver of gnotobiotic rat aged 90 days: a) high AP activity. Recent frozen section, stained by Gomori's method, 100×; b) many lysosomes (L) in region of bile capillaries (BC), 8200×.

The spleen of the same rats was investigated by Perls's method for its content of free iron. There are indications that in germfree animals the erythrocyte count in the peripheral blood is increased [7]. Erythropoiesis and the production of bile pigments are known to be stimulated by the accumulation of free iron in the spleen and of blood pigments formed during hemolysis of erythrocytes.

EXPERIMENTAL RESULTS

SD activity in the liver hepatocytes of germfree rats of all age groups was reduced. This was shown by a decrease in size of the diformazan granules deposited at sites of activity of the enzyme and by the lower density of filling of the cytoplasm of the hepatocytes with these granules compared with the liver of conventional rats. The highest activity of this enzyme in the liver of the experimental and control rats aged 15 days was found in a small group of hepatocytes surrounding the triads. With an increase in age of the animals this zone of cells with higher SD activity became wider. In conventional rats aged 30 and 90 days, as a result of an increase in enzyme activity in the hepatocytes at the periphery of the lobule, the zonal distribution of SD within the hepatic lobule was clearly defined. In the liver of the germfree animals, because of the very small increase in activity of the enzyme in the hepatocytes in the peripheral zone of the lobule compared with SD activity in the centrally situated cells, the zonal pattern of distribution of the enzyme was ill-defined.

In hepatocytes in the liver of the animals of all age groups differences were found between the germfree and conventional animals in the dynamics of accumulation of fat and NE activity. In germfree rats aged 15 days fat appeared in the cytoplasm of single hepatocytes as tiny droplets. In conventional animals of this age, most cells of the peripheral and middle zones of the lobule were infiltrated with fat. In germfree animals aged 30 and 90 days, infiltration of the cytoplasm of hepatocytes in all zones of the lobule with large droplets of fat was sharply increased. Furthermore, in the 90-day-old animals droplets of fat were observed in the cytoplasm of the Kupffer cells. Many hepatocytes and Kupffer cells of the germfree animals were in a state of fatty degeneration. In conventional animals of these age groups only slight infiltration of the cytoplasm of the peripheral hepatocytes with tiny droplets of fat could be seen, and accumulation of fat in the cytoplasm of the Kupffer cells and fatty degeneration of the hepatocytes were absent. The study of the age dynamics of NE activity showed that the density of filling of the cytoplasm of the hepatocytes with the end products of the reaction increased with age of the animals in both groups, reflecting a gradual increase in enzyme activity. However, NE activity was higher in the conventional than in the gnotobiotic rats.

According to the observations of Reddy et al. [2], activity of fatty acid synthetases is increased in the liver of germfree rats. The lower activity of lipolytic enzymes and increased activity of synthetases of fatty

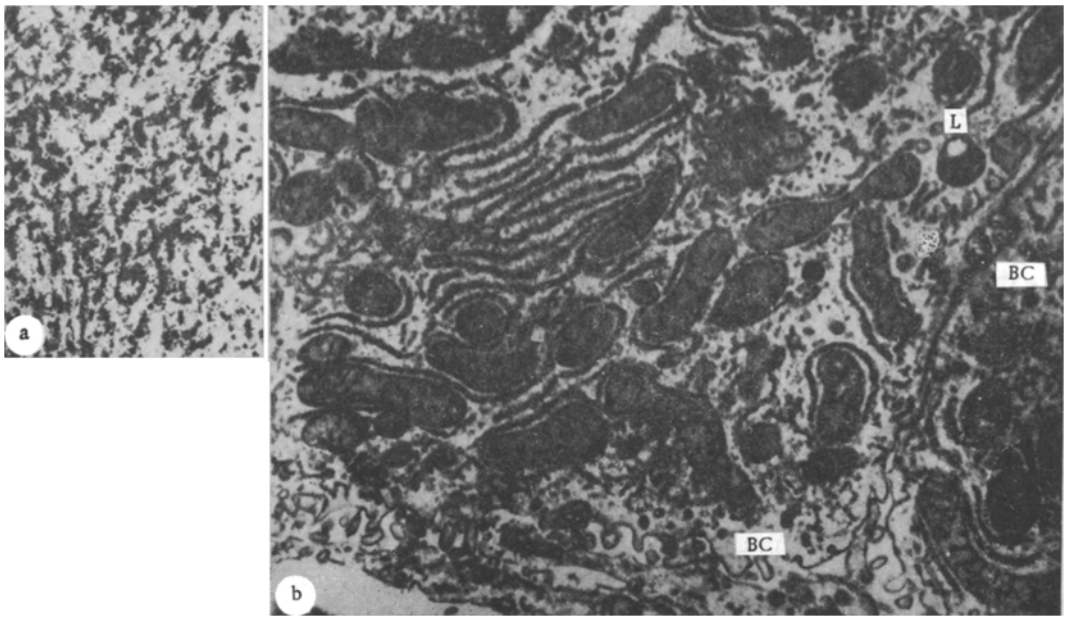

Fig. 2. Liver of conventional rat aged 90 days: a) low AP activity. Staining and magnification as in Fig. 1; b) solitary lysosomes (L) in region of bile capillary (BC), 8200×.

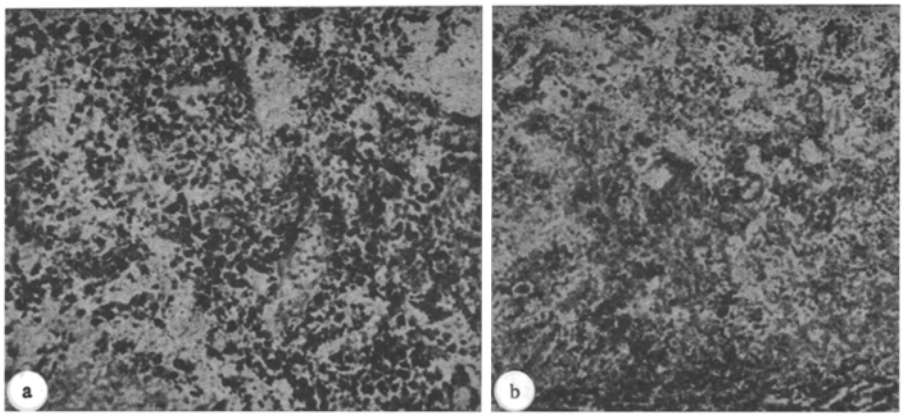

Fig. 3. Spleen of rat aged 90 days: a) spleen of germfree rat. High content of free iron. Fixation with 10% formalin solution, stained by Perls' method, 120×; b) spleen of conventional rat. Single crystals of free iron. Fixation, staining, and magnification the same.

acids are evidently the cause of the intensive accumulation of fat and the marked fatty degeneration of the hepatocytes and Kupffer cells in germfree rats aged 30 and 90 days.

AP was located mainly in the biliary pole of the hepatocyte. With age, its activity increased in both the germfree and the conventional rats. However, AP activity in the hepatocytes of gnotobiotic rats of all age groups was higher than in the conventional animals, and this was especially clear in rats aged 90 days (Figs. 1a and 2a). Electron-microscopic investigation revealed many lysosomes at the biliary pole of the hepatocytes of the germfree rats, whereas in the corresponding areas of the hepatocytes of conventional animals only solitary lysosomes were found. The biliary poles of the hepatocytes over a considerable extent of the bile capillaries are illustrated in Figs. 1b and 2b. The difference in the number of lysosomes in these regions of the hepatocytes in the gnotobiotic (Fig. 1b) and conventional animals (Fig. 2b) will be obvious.

A high concentration of free iron was found in the spleen of the 90-day-old gnotobiotic rats (Fig. 3a), on account of the intensive destruction of erythrocytes in it, because of the high erythrocyte count in the peripheral blood [7]. The iron level in the spleen of conventional animals of this age was low (Fig. 3b).

The absence of the normal microflora in the body thus affects histochemical processes and subcellular structures of the liver and spleen. This is particularly clearly seen in adult animals. Activity of oxidative and lipolytic enzymes in the hepatocytes of germfree rats is reduced and the assimulation of fat is increased not only in the hepatocytes, but also in the Kupffer cells. Parallel with this, there is a marked increase in the number of crystals of free iron in the spleen of the 90-day-old germfree rats, evidence of increased hemolysis of erythrocytes in the organ. This, in turn, causes increased excretion of bile, accompanied by an increase in AP activity and in the mass of lysosomes in the biliary poles of the hepatocytes [6].

The histophysiological features of the liver and spleen described above must be taken into account when the results of experiments on germfree animals are interpreted.

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