MORPHOLOGICAL CHANGES IN THE INTERNAL ORGANS IN CHRONIC STREPTOCOCCAL INFECTION

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A histological and histochemical study was made of the internal organs of albino mice at various times (from 24 h to 27 weeks) after a single intraperitoneal injection of L-forms of β-hemolytic group A streptococci. A progressive pathological process (myocarditis, hepatitis, glomerulonephritis) against a marked allergic background and leading to systemic lesions of the tissues was discovered.

KEY WORDS: L-forms of β-hemolytic streptococcus; infection; morphological changes.

Facts indicating that L-forms of streptococci can persist for a long time in an infected organism have recently been obtained [3]. There are some reports to indicate that L-forms of streptococci may have a harmful action on the tissues of the host [2, 6, 8, 9].

The object of this investigation was to study the dynamics of morphological changes in the internal organs of infected animals during prolonged (27 weeks) persistance of L-forms of streptococci.

EXPERIMENTAL METHOD

Histological (staining with hematoxylin–eosin and azure II–eosin) and histochemical (Brachet's and Feulgen's reactions, PAS) investigations were made of the heart, liver, spleen, and kidenys of 40 noninbred albino mice, weighing 16-20 g and infected intraperitoneally with L-forms of group A β-hemolytic streptococci (strain L-406) in a dose of $2 \cdot 10^6$ colony-forming units (CFUs). The method of preparing the antigen of the L-forms was described previously [1]. The animals were killed at various times after infection: 24 h, 1, 2, 3, 13, and 27 weeks. The control group consisted of 12 mice which were not infected but were inoculated with the culture medium of the L-forms of streptococci. Detection of the L-forms in the histological sections of the organs was carried out by the indirect immunofluorescence method [7] after staining with azure II–eosin.

EXPERIMENTAL RESULTS

A single injection of L-forms of group A hemolytic streptococci into the experimental animals caused morphological changes in their internal organs which reached maximal intensity by the end of the period of observation (27 weeks).

Histological investigation of the heart of the infected mice showed mucoid swelling of the cusps of the valves, edema and lymphohistiocytic (mainly perivascular) infiltration of the interstitial tissue, swelling, amounting sometimes to fibrinoid, of the walls of the blood vessels, circulatory disturbances sometimes producing discrete hemorrhages, and degenerative changes in the muscle fibers. The latter were expressed as swelling and vacuolation of the cytoplasm of the muscle cells, fragmentation of the fibers, and the formation of foci of necrobiosis. The nuclei of the muscle cells were weakly stained in some places, and completely lysed in others. These changes appeared for the first time 2 weeks after infection, after which they

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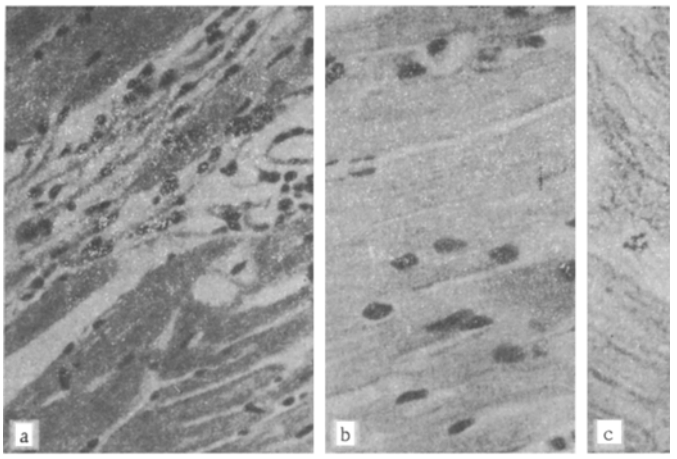

Fig. 1. Changes in heart of mice infected with L-form of streptococcus: a) perivascular edema and lymphohistiocytic infiltration of interstitial tissue 13 weeks after infection (hematoxylin–eosin, 360×); b) foci of necrobiosis of muscle fibers, perinuclear edema 27 weeks after infection (hematoxylin–eosin, 560×); c) L-forms of streptococcus in myocardium (azure II–eosin, 500 ×).

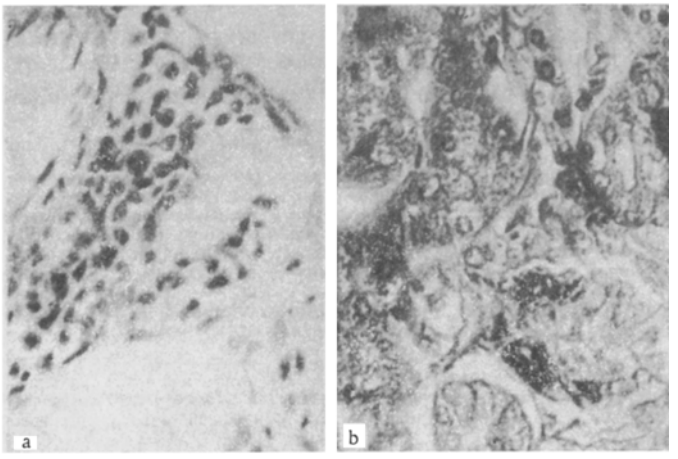

Fig. 2. Kidney 27 weeks after infection of mice with L-form of streptococcus: a) perivascular plasma cell infiltration (Brachet's reaction, 590 ×); b) L-forms of streptococcus in malpighian golmerulus (azure II–eosin, 590 ×).

increased in intensity, so that by 13 weeks changes in the interstitial tissue were the most conspicuous (Fig. 1a) whereas later in the experiment (27 weeks) the most severe changes were found in the myocardium (Fig. 1b). L-Forms of streptococci were discovered in the interstitial tissue, in the myofibrils, and beneath the epicardium and endocardium; they increased in number during the experiment (Fig. 1c). A clearly defined cellular reaction, mainly histiocytic, could be observed around the foci of persistence of the L-forms.

In the kidney, degenerative changes in the epithelium of the tubules and glomeruli and slight circulatory disturbances appeared 1 week after infection, progressed gradually, and in the 3rd week they were joined by a proliferative reaction of the mesangial cells. By the 13th week degenerative changes were predominant and a picture of membranous glomerulonephritis could be observed. In the last stage of the investigation the most conspicuous feature was disturbances of permeability, especially of the microcirculation. The walls of the blood vessels were severely altered, with fibrinoid swelling in some cases. A plasma-cell reaction was found in the later stages of the experiment (13-27 weeks, Fig. 2a). The L-forms were found in the kidney tissues at all times of observation, more often in the immediate vicinity of the blood vessels (Fig. 2b).

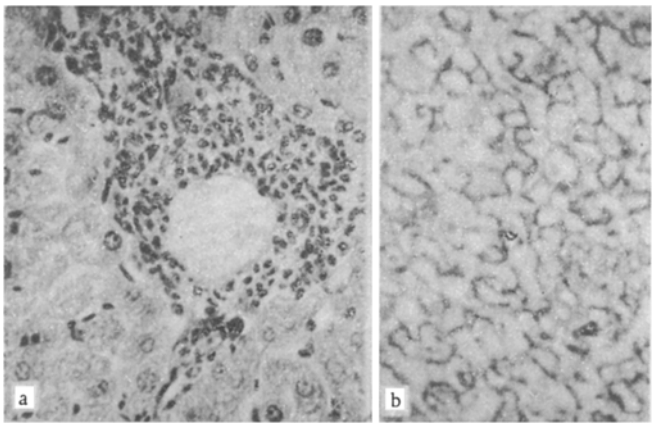

Fig. 3. Liver 27 weeks after infection of mice with L-form of streptococcus: a) lymphohistiocytic infiltration of portal tract, severe degenerative changes in hepatocytes, swelling of Kupffer cells (hematoxylin–eosin, 360 ×); b) L-forms of streptococcus (azure II–eosin, 1500 ×).

Circulatory disorders with hemorrhagic foci, degenerative changes in the hepatocytes extending to necrosis, lymphohistiocytic intralobular and perivascular infiltration, and a marked reaction of the Kupffer cells, with conversion into monocyte-like cells were observed in the liver (Fig. 3a). These changes were seen as early as 24 h after infection of the mice; they increased in severity to the end of the first week, after which they gradually diminished, only to increase again in severity from the 13th to the 27th week, when the picture was one of hepatitis with necrosis of individual hepatocytes. Many binuclear hepatocytes with giant and vesicular nuclei, accompanied by manifestations of karyorrhexis, were observed.

L-forms were found at all times of investigation, often in the lumen of the blood vessels (Fig. 3b).

In a morphological study of the spleen tissue a marked reaction of the organ was observed 24 h after infection of the animals, consisting of a vascular reaction (vasodilation, swelling of the walls and endothelium of the vessels), reticular hyperplasia of the pale centers of the follicles, widening of the sinuses with desquamation of cells into them, a macrophagal reaction, plasmatization of the cells of the follicles and medullary cords, and a reaction of the megakaryocytes. All these changes, initially mild in severity, increased gradually in the course of 2 weeks and sharply by the end of the 3rd week. At this time the last-cell reaction was particularly intensive. Later (13-27 weeks) the immunomorphological reactions diminished and morphological signs of increased permeability of the tissue of the organ appeared. These were particularly marked at the end of the experiment (27 weeks), when the dilated sinuses packed with erythrocytes resembled the picture of "legs of blood." At this period the cellular reactions were again of high intensity. L-forms were detected in the blast cells 24 h after infection and their number increased sharply until the end of the 3rd week. Starting from the 5th week of the experiment and until the 13th week the number of L-forms located extracellularly increased, but toward the 27th week L-forms began to appear intracellularly also.

The experiments thus showed that persistence of L-forms of group A β-hemolytic streptococci in mice gives rise to a severe pathological process leading to systemic lesions of the tissues. In all the organs studied, for instance, a vascular reaction developed and progressed; its manifestations included changes in the vessel walls, amounting in some cases to fibrinoid swelling and necrosis (in the heart), together with hemorrhages, sometimes extensive, into the tissue of the organs.

The connective-tissue stroma of the organs became involved in the pathological process. In the early period edema and swelling of the connective-tissue fibers together with lymphohistiocytic, chiefly perivascular, infiltration were observed. Later in the course of the experiments the changes in the connective tissue became diffuse in character: Intralobular foci of infiltration appeared in the liver and subendocardial foci and cellular infiltration between the muscle fibers were observed in the myocardium.

Degenerative changes in the parenchymatous cells were seen in the organs studied, and they were particularly clear in the liver, kidneys, and heart.

The morphological picture discovered in the heart, kidneys, and liver of the mice in the 13th-27th weeks of the experiment can be described as interstitial myocarditis, glomerulonephritis, and hepatitis, developing

as a result of the pathogenic action of L-forms of group A β-hemolytic streptococci against a well-marked allergic background.

It can be concluded from a comparison of the immunomorphological changes discovered in organs with manifestations of hypersensitivity of delayed type [4] that the pathological process developing in the present experiments in fact belonged to this type.

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