PATHOLOGICAL ANATOMY OF AN EXPERIMENTAL DISEASE OF SMALL LABORATORY ANIMALS CAUSED BY ENCEPHALOMYOCARDITIS VIRUS STRAIN EMK-70

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The results of a pathomorphological investigation of an experimental disease of small laboratory animals caused by strain EMK-70 of encephalomyocarditis virus isolated from monkeys are described. Regardless of the method of infection of the newborn and juvenile mice with the virus, they developed lesions of the brown fat, striated muscle, brain, and heart. The changes in guinea pigs were characterized by the development of severe myocarditis and encephalitis, accompanied by accumulation of virus antigen. The disease caused by strain EMK-70 could not be differentiated from Coxsackie infection on the basis of the pathomorphological data. This fact must be taken into account when problems concerning the diagnosis of virus diseases at autopsy are examined.

KEY WORDS: *encephalomyocarditis virus; Coxsackie viruses; encephalitis; myocarditis; myositis; brown fat.*

Problems to do with the morbid anatomical diagnosis of virus infections are assuming an ever-increasing importance which corresponds to their increasing frequency. Viruses of the encephalomyocarditis group have been isolated from various sources in different geographical zones [1, 6, 9, 11-13]. They are used to obtain a classical model of virus-induced cardiac pathology [3]. However, the differential diagnosis of the diseases they cause can be difficult.

The object of this investigation was to study the pathological anatomy of an experimental disease caused by a new strain of encephalomyocarditis virus isolated from sick monkeys in the Sukhumi nursery.

EXPERIMENTAL METHOD

Albino mice of different ages (315 newborn, 185 juvenile, 45 adult mice), guinea pigs (6 newborn and 60 juvenile), and rabbits (42 newborn and 6 juvenile) were used. The material for infection consisted of virus-containing fluid in a titer of 10^7 TCD_{50}/ml and a 10% suspension of the organs of dying or killed animals. The virus, identified as encephalomyocarditis virus strain EMK-70, was isolated in 1970 in Sukhumi from monkeys with encephalomyocarditis and polyserositis [5]. Newborn albino mice were injected intracerebrally, subcutaneously, and also simultaneously intracerebrally and subcutaneously (0.02 and 0.05 ml) with the virus-containing material, juvenile and adult mice intracerebrally (0.025 ml), intraperitoneally, or intramuscularly (0.15-0.3 ml). The newborn guinea pigs were inoculated both intracerebrally (0.05 ml) and intramuscularly (0.3 ml), and the juvenile guinea pigs intramuscularly or intraperitoneally (0.5 ml). The newborn rabbits were injected intracerebrally (0.05 ml) and intraperitoneally (1.0 ml) with the material, juvenile rabbits intraperitoneally and intramuscularly (1.0 ml).

Histological sections were stained with hematoxylin—eosin and by Nissl's and Goldman's methods. Pieces from the organs were studied simultaneously for their content of virus in a culture of MA-104 cells and by the fluorescent antibodies method.

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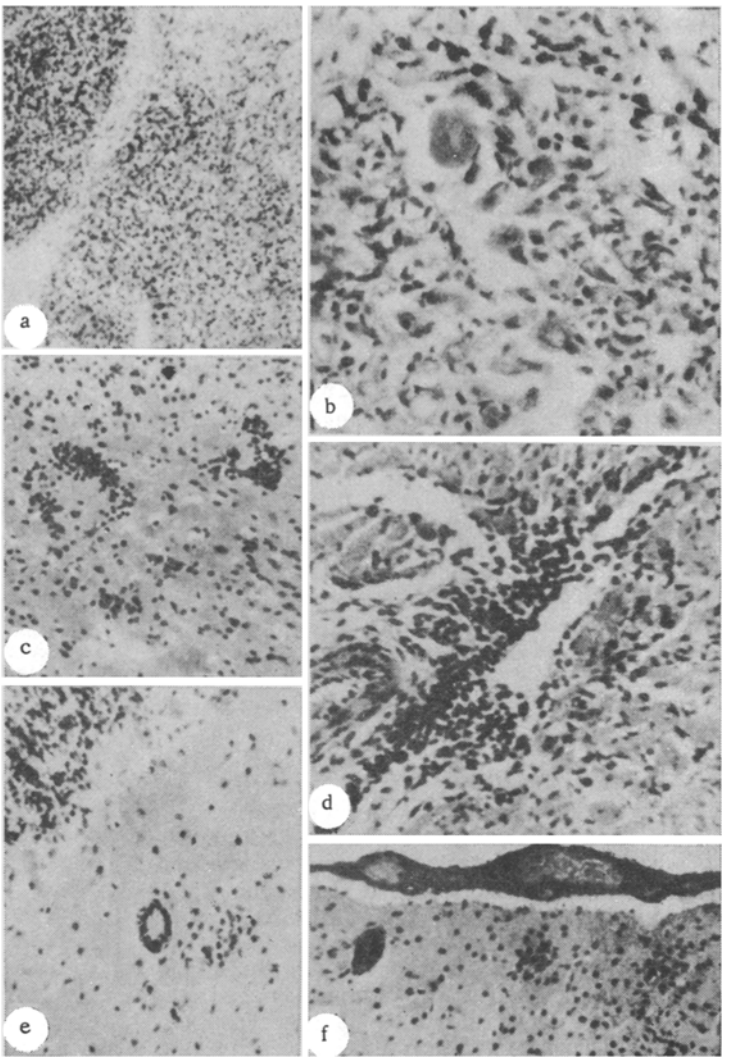

Fig. 1. Experimental encephalomyocarditis. a) Inflammation in brown fat of a newborn mouse. Hematoxylin–eosin, 40 ×; b) interstitial myositis and lysis of muscle fibers in a newborn mouse. Hematoxylin–eosin, 200 ×; c) edema and glial nodules in brain of a juvenile mouse. Hematoxylin–eosin, 80 ×; d) myocarditis in a guinea pig. Hematoxylin–eosin, 200 ×; e) vasculitis and perivascular glial-mesenchymal nodule in cerebellum of a guinea pig. Lysis of Purkinje cells. Stained with thionine by Nissl's method, 80 ×; f) infiltration of meninges of cerebellum in a guinea pig, glial reaction in marginal layer of cortex. Hematoxylin–eosin, 80 ×.

EXPERIMENTAL RESULTS

The experimental animals of all species developed the disease regardless of the method of inoculation of the virus-containing material. All newborn mice died 24-72 h after inoculation with no visible clinical manifestations of the disease. Juvenile mice developed the disease on the 2nd day, with paralysis of the hind limbs and also sometimes of the forelimbs. More than half of the animals died on the 3rd-5th days. Paralysis of the hind limbs was observed in only half of the adult mice used in the experiments. Of the 22 mice which developed the disease, 11 died on the 4th-6th day after infection. All the newborn guinea pigs died on the 4th-6th days. Of the 60 juvenile guinea pigs, 49 which developed the disease were killed on the 5th-7th day, when their condition was serious. The juvenile rabbits were resistant to virus of strain EMK-70. Its pathogenicity also was low for newborn rabbits: Of 42 animals two died on the 3rd day and another two died 3 months after infec-

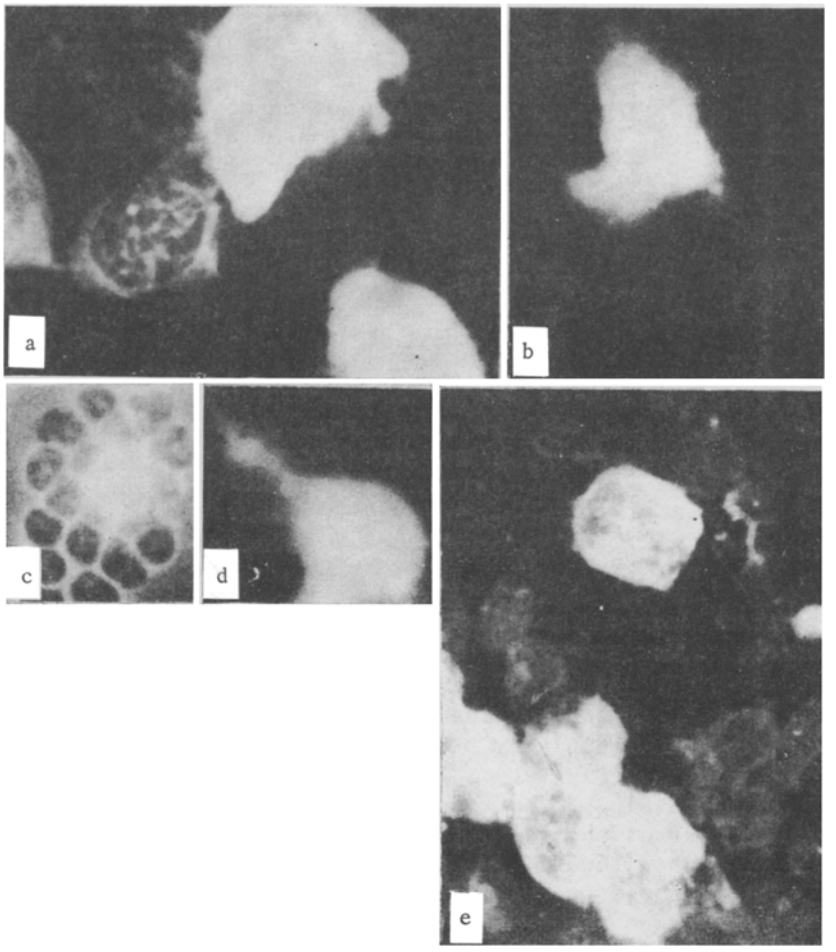

Fig. 2. Fluorescence of virus antigen in organs of guinea pigs (indirect fluorescent antibodies method). a) Fluorescence of antigen in kidney, 900 ×; b) fluorescent cell in medulla, 900 ×; c) presence of antigen in brown fat, 400 ×; d) fluorescence of neuron in brain, 900 ×; e) fluorescent antigen in infiltrating cells in heart, 900 ×.

tion with manifestations of paralysis of the hind limbs. The morbid anatomical picture likewise was independent of the method of infection. In newborn mice dying on the first day after infection only circulatory disturbances were observed, with tiny foci of necrosis in the CNS, liver, and myocardium. On death of the mice of the other age groups, in the later stages, changes in the brown fat and striated muscles were constantly found. Sometimes on macroscopic examination the mass of fatty areolar tissue in the interscapular region was appreciably increased, and the tissue itself was edematous and hyperemic. Severe forms of degeneration were observed in the cells of the lobules. Some cells had undergone lysis and diffuse inflammatory zones of infiltration appeared (Fig. 1a). Focal myositis was observed in the striated muscle of the dorsum, neck, and limbs (Fig. 1b). These lesions in newborn mice were more severe and extensive. In one-third of the mice of all age groups myocarditis was observed. Mild encephalitis (Fig. 1c) and sometimes meningoencephalomyelitis were present in more than half of the adult mice.

The highest titers of virus in the internal organs of the mice of the juvenile group (10^3 TCD_{50}/ml) were found in the brain and spleen, but in the newborn mice the titers were higher: in the brain 10^6 TCD_{50}/ml, in the liver 10^4 TCD_{50}/ml, and in the spleen 10^3 TCD_{50}/ml.

In all the guinea pigs which died macroscopic examination showed whitish areas in the myocardium and hyperemia of the meninges and brain tissue. Histological examination showed diffuse, mainly interstitial myocarditis with massive round-cell infiltration of the intermuscular and perivascular spaces (Fig. 1d). Mild encephalitis affecting mainly the brain stem and cerebellum was found in nearly all the animals. The walls of the vessels were in-

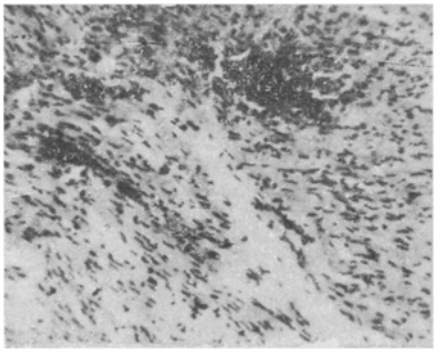

Fig. 3. Lesion of the heart in a rabbit. Calcification of area of necrosis. Hematoxylin-eosin, 80 ×.

filtrated with lymphocytes and histiocytes. Small glial-mesenchymal nodules (Fig. 1e), necrotic areas, and hemorrhages were present in the brain tissue. In some guinea pigs slight infiltration of the meninges of the cerebellum was observed (Fig. 1f).

Investigations of the material by the immunofluorescence method during the 1st day after infection revealed only solitary fluorescent cells in squash preparations from the brain and heart of the guinea pigs. No cells with the specifically fluorescent antigen could be found in the other organs. In the blood at this time the specific antigen was present in the perinuclear zone of cells of the monocyte type. On the 2nd-4th day the virus was widespread in the animals. Specific fluorescence of the virus antigen was found in the cytoplasm of cells infiltrating the heart, in cells of macrophage type in the lungs and spleen, and in cells of the renal epithelium (Fig. 2a). In the nervous system antigen was found both in the glial cells, especially perivascular, and in neurons. Most fluorescent cells were found in the medulla (Fig. 2b). Brightly fluorescent antigen was seen in some guinea pigs in the brown fat (Fig. 2c). Evidence of reproduction of the virus in the tissues was given by high titers of the virus in the brain, heart, and liver (10^4 TCD_{50}/ml). Titers of the virus were rather lower in the kidneys, spleen, and lungs (10^3 TCD_{50}/ml). On the 7th day virus continued to be constantly detectable in both glial cells and neurons of the brain (Fig. 2d), macrophages of the spleen, and cells infiltrating the heart (Fig. 2e), but they had almost disappeared from the lungs and liver (they were present only in individual cells of these organs).

In rabbits, whitish regions in the myocardium and hyperemia and edema of the brain and spinal cord were seen macroscopically. Histological investigation revealed areas of inflammation with death of muscle fibers in all parts of the heart. Characteristically, calcification was present nearly everywhere in the foci of necrosis (Fig. 3). Foci of necrosis also were found in the liver. Lesions in the nervous system were of encephalomyelitis type, involving chiefly the brain and the lumbar region of the spinal cord. Besides myocarditis and encephalomyocarditis, lesions of the brown fat similar to those in mice were observed in one rabbit which died after 3 months. On titration of the virus in MA-104 tissue culture the highest titers of virus in the rabbits were found in the mesenteric lymph nodes (10^3 TCD_{50}/ml).

The infection caused in small laboratory animals by strain EMK-70 of encephalomyocarditis virus was thus characterized by lesions in the heart (most marked in guinea pigs), changes in the nervous system (usually in the form of encephalitis, less frequently of meningoencephalitis, and also by constant lesions of the brown fat and striated muscle in mice.

High titers of the virus were found in the brain, heart, and liver of the animals, evidently indicating reproduction of the virus in those organs. The most intensive and prolonged specific fluorescence of the antigen was observed in the guinea pigs in organs with the highest concentration of virus (the brain and heart).

In most animals the virus caused an acute infection, and the disease was chronic in only two rabbits. The character of the myocarditis and encephalitis in the experimental animals was virtually indistinguishable from that of the similar processes described previously following infection with other strains of this group of viruses [9, 11]; no changes in the striated muscle and brown fat were observed previously in this infection. Hitherto such changes have been regarded as pathognomonic for Coxsackie infection and, in particular, for Coxsackie B infection [2, 4, 7, 8, 10]. Some workers [2] attach great importance to

lesions of striated muscle and consider that they are the most informative differential diagnostic sign of Coxsackie infection. The results described in this paper indicate that a representative of a different group of infectious enteroviruses possesses the same property. It is not yet clear whether this is a feature confined to strain EMK-70 of encephalomyocarditis virus (other members of this group have not been investigated in this direction).

Considering the difficulties of differential diagnosis of virus infections, mention must be made of the possible role of the encephalomyocarditis group of viruses in the etiology of myocarditis in man. Whereas a meningoencephalitis caused by these viruses in man is known [6, 13], their participation in diseases of the heart has not hitherto been discovered. However, there are descriptions in the literature of severe cases of myocarditis connected with encephalomyocarditis virus in the anthropoid apes [12]. We have observed lesions of the heart not only in small laboratory animals, but also in several species of lower monkeys: The clinical and electrocardiographic picture in some cases corresponded to that of macrofocal necrosis of heart muscle. It can tentatively be suggested that infection with encephalomyocarditis virus, with a clinical course of myocarditis or even of myocardial infarction, may occur in man also, especially in young patients. This possibility must be taken into account during the etiological diagnosis of human diseases.

LITERATURE CITED

1. Viral and Rickettsial Infections of Man (ed. by T. Rivers) [Russian translation], Moscow (1955), pp. 756-763.
2. N. A. Maksimovich and E. A. Suptel', Arch. Patol., No. 3, 3 (1973).
3. N. M. Mukharlyamov and R. A. Charchoglyan, Kardiologiya, No. 4, 141 (1974).
4. I. A. Prokhorova, in: Virus Infections and Antiviral Preparations (Transactions of the Moscow Institutes of Viral Preparations) [in Russian], Vol. 2, Moscow (1961), pp. 169-173.
5. Z. V. Shevtsova, E. K. Dzhikidze, M. K. Voroshilova, et al., Vopr. Virusol., No. 5, 531 (1976).
6. G. W. A. Dick, A. J. Haddow, A. M. Best, et al., Lancet, 2, 286 (1948).
7. R. Gifford and G. Dalldorf, Am. J. Pathol., 27, 1047 (1951).
8. G. C. Godman, H. Bunting, and J. L. Melnick, Am. J. Pathol., 28, 223 (1952).
9. F. C. Helwig and E. C. H. Schmidt, Science, 102, 31 (1945).
10. A. M. Pappenheimer, J. B. Daniels, F. S. Cheever, et al., J. Exp. Med., 92, 169 (1950).
11. M. Roca-Garcia et al., Am. J. Trop. Med. Hyg., 6, 84 (1957).
12. E. C. H. Schmidt, Am. J. Pathol., 24, 97 (1948).
13. J. E. Smadel and G. Warren, J. Clin. Invest., 2, 1197 (1947).