EXPERIMENTAL DATA ON THE CONNECTION BETWEEN VIRAL MYOCARDITIS AND DILATATION CARDIOMYOPATHY

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The problem of the connection between myocarditis and dilatation cardiomyopathy remains one of the most urgent in contemporary cardiology. According to data in the literature, the frequency of discovery of myocarditis during the investigation of endomyocardial biopsy specimens obtained from patients with the clinical diagnosis of dilatation cardiomyopathy (DCMP) may reach 67% [2-4, 6-9, 11, 12, 15]. There is evidence of a direct connection between myocarditis and viral infection and the transformation of myocarditis into DCMP [1, 5, 13]. For instance, in 50% of cases of patients with DCMP virus particles, in most cases Coxsackie B viruses, have been identified by the immunofluorescence method [7]. Other workers also observed Coxsackie viruses in patients with DCMP [10].

The aim of this investigation was to create an experimental model of viral myocarditis (VMC) and to obtain morphological proof of its transformation into DCMP.

EXPERIMENTAL METHOD

Experiments were carried out on 70 male Syrian golden hamsters aged 40 ± 5 days and weighing 60 ± 6.5 g, receiving an intraperitoneal injection of 1 ml of Coxsackie B_2 virus in a titer of 10^{-7} TCD/ml, obtained from the museum or viruses, Institute of Poliomyelitis and Virus Encephalitis, Academy of Medical Sciences of the USSR. The ECG was recorded for the duration of the experiment. The animals were killed (under general anesthesia) by decapitation and their body weight and the weight of their heart was determined. Material was taken for morphological investigation 5, 15, 25, 30, and 180 days after infection. Intact hamsters and hamsters receiving an injection of culture fluid only were used as the control. For light and electron microscopy, three animals were killed at each stage of the investigation and material was taken from the left ventricle. The subsequent processing of the material followed the usual techniques. The ratio of the weight of the heart to body weight (WH/BW) also was studied. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Of 40 infected animals six died on the 5th day, 3 on the 15th, and 6 on the 180th day of the experiment. None of the control animals died. Both infected and control hamsters appeared healthy during the continuation of the experiments.

Investigation of the ECG revealed no rhythm disturbances or pathological changes throughout the experiment (in either infected or control groups).

The ratio WH/BW was significantly increased in the infected animals on the 25th day of the experiment, for their WH exceeded that in the control group, whereas BW was a little lower than in the control. In the infected group, an increase in WH was observed in five animals on the 180th day, and biventricular dilatation was found macroscopically (Table 1).

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TABLE 1. Weight of Heart and Ratio WH/BW · 10^{-3} in Control and Infected Hamsters during Experiment ($M \pm m$)

Expt. days	WH, g		WH/BW × 10^{-3}	
	infected group	control group	infected group	control group
5	0,238±0,012	0,230±0,05	3,74±0,25	3,46±0,15
15	0,296±0,028	0,280±0,04	3,80±0,35	3,28±0,007
25	0,385±0,0038**	0,332±0,009	3,88±0,18*	3,21±0,14
30	0,436±0,042	0,362±0,016	3,82±0,25	3,29±0,15
180	0,542±0,0045**	0,484±0,022	3,94±0,19	2,82±0,18

Legend. Asterisks indicate parameters differing significantly from control: *$p < 0.05$, **$p < 0.001$.

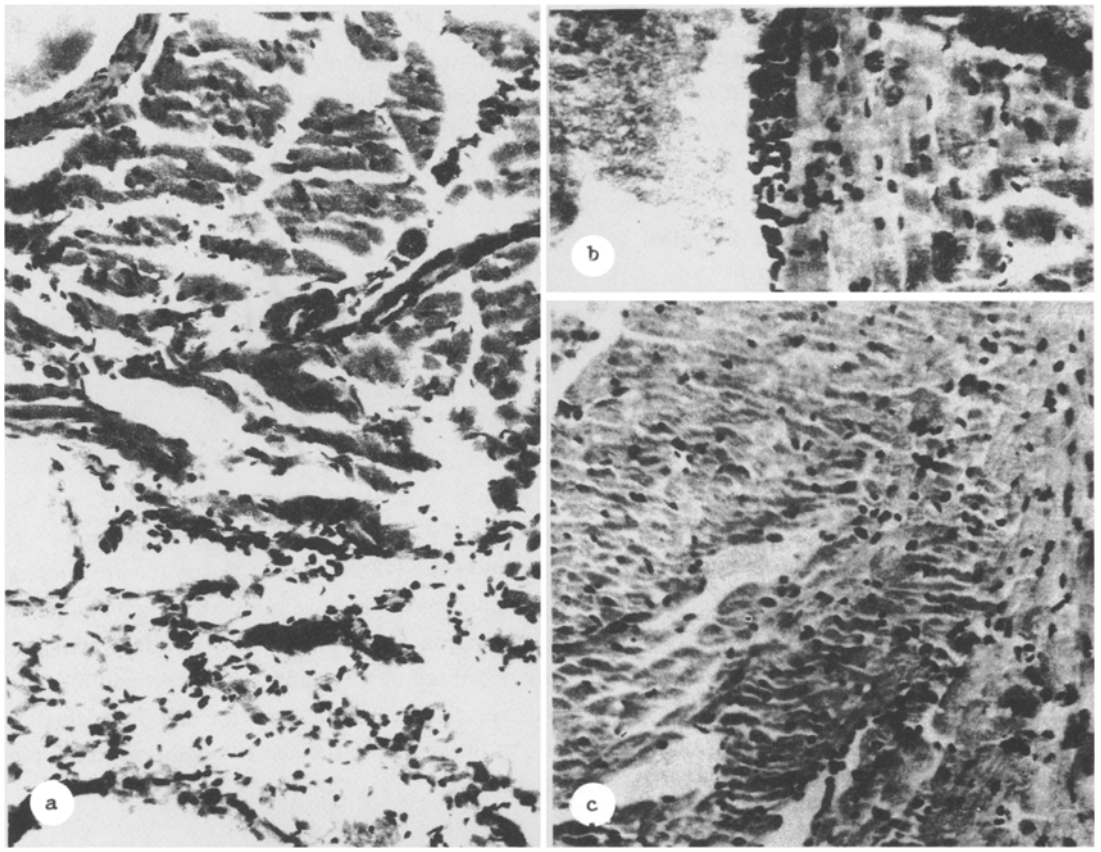

Fig. 1. Myocardium of left ventricle. a) Foci of necrosis, loose connective tissue, infiltration by lymphocytes and macrophages; b) lymphocytic infiltration; c) low-density infiltration of myocardium by lymphocytes. Hematoxylin and eosin. 200×.

Under the light microscope, on the 5th day of the experiment, foci of sclerosis, composed of loose connective tissue, were identified in the myocardium of the left ventricle, and they were infiltrated with lymphocytes and macrophages, mixed with a few eosinophils and plasma cells (Fig. 1a). Congestion of the venules, arterioles, and small arteries was observed, with swelling of their endothelium. As regards the cardiomyocytes (CMC) obliteration of their cross-striation was observed. Along the course of the small arteries and arterioles and in other parts of the myocardium, small foci of low-density infiltration, chiefly by lymphocytes, were seen. In the later stages individual small areas of sclerosis were found both in the endocardium and in the thickness of the myocardium, consisting of denser connective tissue, with very few cells. Outside the foci of sclerosis there were single foci of infiltration by lymphocytes and low-density infiltration of the myocardium by lymphocytes (Fig. 1b, c). In the microcirculatory bed the same changes were found as in the early periods (congestion of microvessels, swelling of the endotheli-

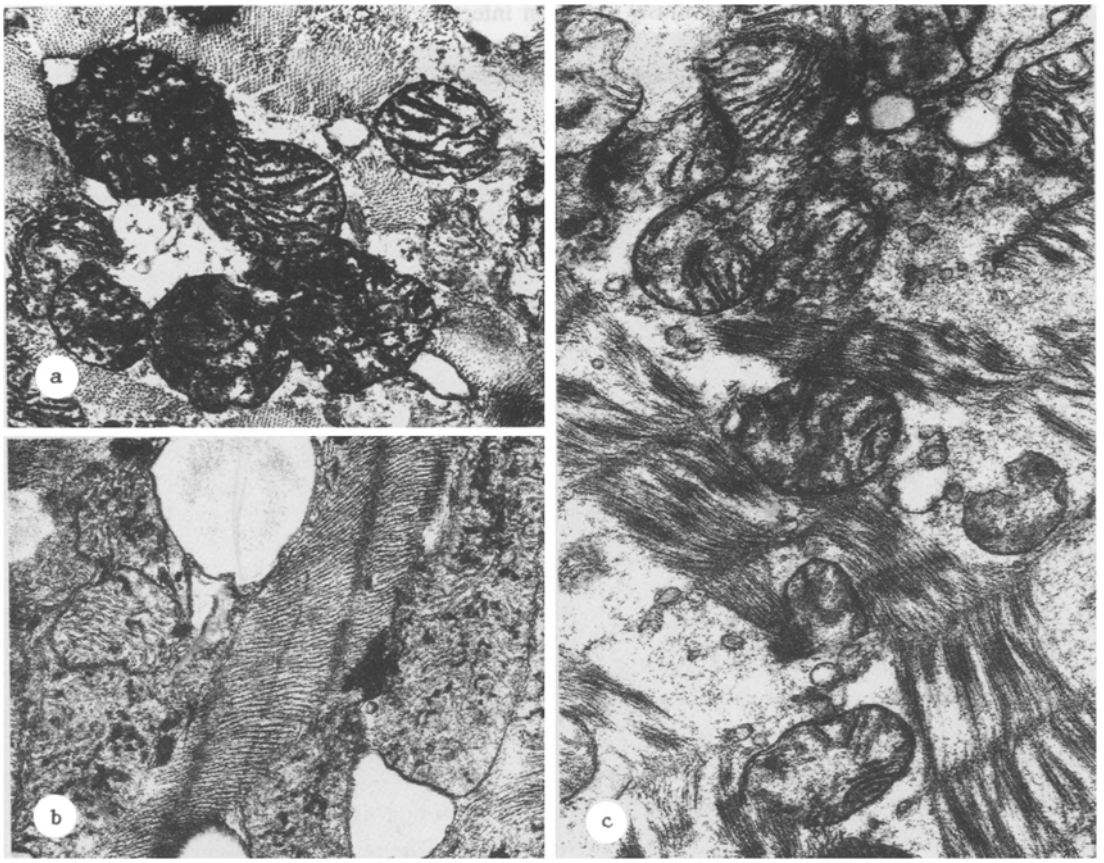

Fig. 2. Heterogeneity of mitochondria, dilatation of tubules of sarcoplasmic reticulum (a), dense inclusions in sarcoplasm and mitochondria, lipid infiltration of CMC (b), and marked lysis and disorientation of myofibrils (c). Magnification: a) 16,000; b, c) 20,000.

um). Cross-striation of CMC was difficult to distinguish in some places. On the 180th day solitary areas of sclerosis and small foci of lymphocytic infiltration were observed both along the course of the vessels and away from them.

As early as the 5th day after infection changes were observed electron-microscopically in the myocardium in CMC. Local lysis of myofibrils and the appearance of lipid inclusions were noted. Marked heterogeneity of the mitochondria was observed: some of them appeared pale and swollen whereas others had become strongly osmiophilic (Fig. 2a), and tubules of the sarcoplasmic reticulum were dilated sometimes with the appearance of small vacuoles (Fig. 2a). Necrosis of some CMC was noted, with the appearance of cell debris in the interstitial tissue and an increase in the number of active macrophages. These changes progressed and reached a peak by the 15th day after infection. This time after infection was interesting because electron-dense deposits began to appear at this time in CMC and in some of the mitochondria, whereas marked lipid infiltration was found in the same CMC (Fig. 2b). By the 25th day of infection the number of necrotic myocardial cells revealed under the light microscope decreased, but electron-microscopically changes indicating the presence of myocarditis were found in both CMC and the interstitial tissues: deposition of fibrin near the capillaries, and large numbers of active macrophages and lymphocytes. Considerable lysis and disturbance of the regular orientation of the myofibrils were noted in CMC (Fig. 2c). By the 180th day of infection, the alterative component in the myocardium was weakly defined, but there was an increase in the number of cells with electron-dense deposits in their sarcoplasm and mitochondria. Grossly hypertrophied CMC and also CMC with disturbance of the regular orientation of their contractile structures (myofibrils) were detected. Locally there was considerable interstitial sclerosis.

The investigation showed that infection of golden hamsters by Coxsackie B_2 virus leads to the development of the typical picture of infectious myocarditis in the animals' myocardium. In the early stages, changes of alterative character predominate with infiltration of mononuclear cells and macrophages. Later (after 15 days) the morphological picture of the myocardium begins to resemble that in DCMP, described on the basis of the study of heart biopsy specimens from patients with the clinical diagnosis of DCMP [4].

Material with high electron density, previously found on infection of mice with Coxsackie B_3 virus, was discovered in CMC and the mitochondria [8]. It is difficult to judge the nature of these formations, for *x*-ray microanalysis was not carried out; however, the similarity with deposits containing calcium, found previously, suggests the similarity of their nature.

Morphological changes in the myocardium detected by light-optical and electron-microscopy (disturbance of the regular orientation of CMC and myofibrils, fibrosis) suggests the possible transformation of infectious VMC into DCMP; first, this means that this particular experimental model can be used to study DCMP, and second, it provides experimental proof that a high proportion of patients with the clinical diagnosis of DCMP suffered previously from VMC.

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