Ultrastructural Lesions of the Myocardial Cell in Coxsackie B_4 Virus Infected Mice*

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Coxsackie B virus group is known to cause myocarditis (RABIN et al., 1964; SUN et al., 1967), valvulitis and endocarditis in experimental animals (Burch et al., 1966; Sun et al., 1967) and in man (Burch et al., 1967, 1968). Localization of Coxsackie B virus antigen in the damaged myocardial cells by immunofluorescent technics (Rabin et al., 1964; Sun et al., 1967) indicates that the myocardium, epicardium, mural endocardium and valves are target tissues for these viruses. Although a great deal of information is available on the cytopathic effects of enterovirus on the tissue culture cells, similar in vivo studies are incomplete (Godman, 1966). The purpose of this report is to describe the ultrastructural lesions of the myocyte produced in vivo by Coxsackie B_4 virus in the myocardium of young suckling mice.

Material and Methods
Coxsackie B_4 virus used in these experiments was originally recovered by KIRBRICK and BENIRSCHKE (1958) from a 10-day-old infant who died with encephalo-hepato-myocarditis. The virus was obtained as monkey kidney culture strain (MK) and prepared for inoculation in rhesus monkey kidney cultures. Control fluid from MK culture free of virus was also obtained. Virus and control fluid were stored at -65°C.

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Twenty-eight suckling five-day-old mice of a random strain of HaM/ICR breed were inoculated intraperitoneally with 0.1 ml of fluid containing $10^8$ TCID$_{50}$. A second group of twenty-eight animals of the same age and stock were injected with 0.1 ml of virus-free fluid from monkey kidney culture and a third group of animals was not inoculated.

**Microbiology.** Hearts of two animals from inoculated and uninoculated groups sacrificed at 24 hours, 3, 6, 10 and 21 days were immediately washed in Hanks solution supplemented with penicillin and neomycin and ground in 1 ml of the same solution. The suspension was centrifuged at 1,000 RPM for 10 minutes. The supernatant was saved and stored at $-65^\circ$C until the cultures were performed. 0.1 ml of the fluid was inoculated into each of 4 Rhesus monkey kidney (MK) cell tubes for every specimen. The tubes were examined daily for cytopathic effect (CP) and after one week the fluid was pooled and inoculated into new MK tubes. When definite CP effect was detected, the culture was considered positive. CP effect was produced in the initial cultures of specimens of the Coxsackie B$_4$ inoculated mice sacrificed at 24 hours and 3 days and in the second passage of those sacrificed at 24 hours, 3, 6 and 10 days. No change was observed in the specimens of the animals sacrificed on the twenty-first day after inoculation or of the controls. Coxsackie B$_4$ virus was identified in the positive cultures.

Neutralizing antibodies were determined in the sera of experimental and control mice sacrificed at 24 hours, 3, 6, 10 and 21 days. Coxsackie B$_4$ virus adapted to KB cells, $10^8$ TCID$_{50}$, and diluted serum were kept at 37°C for one hour, planted on stationary KB cultures and incubated for 48 hours at 35°C. Antibody titers were expressed as the initial dilution of serum inhibiting virus effect. Sera of the Coxsackie B$_4$ virus infected mice sacrificed on sixth, tenth and twenty-first days had neutralizing antibodies to a dilution of 1:16, whereas sera of the control mice did not show any antibodies.

**Electron Microscopy.** For electron microscopy, six animals from each group sacrificed at third, sixth and tenth day were used.

Small pieces of the ventricular myocardium were fixed in 4% phosphate buffered (Millonig, 1962) glutaraldehyde for 2 hours at 4°C, washed in phosphate buffer and refixed in 1% osmium tetroxide for 1 hour. Subsequent to the glutaraldehyde fixation and buffer wash but prior to the osmium fixation, some tissue blocks were subjected to glycogen digestion by incubation in 0.5% alpha-amylase in Millonig's buffer, pH 7, for 1—2 hours at 37°C (Coimbra and Leblond, 1966). Control tissues were kept in buffer solution without alpha-amylase. The tissues were dehydrated in an ascending series of ethanol and embedded in Maraglas. Thin sections were cut with an LKB “Ultrotome” microtome, stained with uranyl acetate and lead citrate. A Siemens “Elmiskop I” electron microscope was used for observations.

**Observations**

The normal ultrastructure of the mouse myocardial cell is similar to that described by Moore and Ruska (1957) for other mammalian hearts. The myofiber is limited laterally by a sarcolemma. At intervals plasma membranes of the two adjacent cells invaginate to form an intercalated disc. Myofibrils present the usual striated appearance. Mitochondria lie in the perinuclear region and between the myofibrils. Components of sarcoplasmic reticulum and glycogen particles are distributed between and within the myofibrils (Fig. 1).

Because there was considerable variation in the degree of myocardial cell damage within all the groups of infected animals and even within the same animal, an account of the temporal sequence of the cellular changes was not attempted. However, maximum damage was observed on the sixth day after inoculation. Myocardial damage by the virus was focal. Myocardial cells frequently showed the formation of blunt pseudopodia-like processes which extended from the surface of cells into the interstitial spaces (Figs. 2, 3). Some of these processes lacked a cytoplasmic continuity with the myofiber. These cytoplasmic