7. A. I. Strukov and V. S. Paukov, Kardiologiya, No. 9, 3 (1967).
8. Yu. S. Chechulin, Injury to the Heart (Combined Experimental Investigation at Different Levels of Integration of the Organism) [in Russian], Moscow (1975).
9. B. Fischer, G. Schlüter, C. P. Adler, et al., Beitr. Pathol., 141, 238 (1970).
10. F. Korner, Z. Mikr.-Anat. Forsch., 38, 441 (1935).
11. J. Linzbach, Verh. Dtsch. Ges. Path., 51, 124 (1967).
12. W. Sandritter and C. P. Adler, Experientia, 27, 1435 (1971).
13. W. Sandritter and G. Scomazzoni, Nature, 202, 100 (1964).
14. R. Sasaki, T. Morishita, and S. Yamagata, Tohoku J. Exp. Med., 101, 147 (1970).

ELECTRON-MICROSCOPIC STUDY OF THE MOUSE MYOCARDIUM IN EXPERIMENTAL COXSACKIE A13 VIRUS INFECTION

V. P. Kaznacheev, E. F. Bocharov,
V. A. Shkurupii, and O. B. Meleshina

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Diffuse degenerative-proliferative myocarditis is described in adult BALB/c mice infected with Coxsackie A13 virus. A marked tendency was observed for sclerotic processes to develop 30-60 days after infection; this may lie at the basis of the reduced functional activity of the myocardium and may lead to the development of cardiomyopathy.

KEY WORDS: *Coxsackie viruses; myocarditis; ultrastructural changes.*

The Coxsackie viruses play an important role in human pathology. They are the most cardiotropic of all known viruses. It was formerly considered that they are pathogenic only for newborn rodents. However, it has now been shown that adult mice can also develop Coxsackie infection, sometimes in chronic forms [8-10, 12].

The object of this investigation was to study ultrastructural changes accompanying the development of acute and chronic myocarditis in adult mice infected with Coxsackie A13 virus.

EXPERIMENTAL METHOD

Male BALB/c mice aged 2 months were infected intraperitoneally with 0.3 ml of a culture of Coxsackie A13 virus in a titer of $10^{-5.5}$-$10^{-7.2}$ TCD_{50}/ml. Material was taken 1, 7, 30, and 60 days after infection. Intact mice and animals receiving an injection of culture fluid served as the control. At each stage of the investigation material was taken from the left ventricle of five mice, in the region of its apex, for light and electron microscopy. The subsequent processing of the material was carried out in the usual way.

EXPERIMENTAL RESULTS

Congestion of the vessels and foci of recent hemorrhages, and perivascular and irregularly distributed edema of the stroma were discovered in the hearts of the mice 24 h after infection; histiocytes, fibroblasts, and lymphocytes appeared in the intermuscular bands of connective tissue. This diffuse response of the stroma was found in all the mice. Small perivascular collections of histiocytes and lymphocytes were seen. The myocardium of the mice contained many foci of necrobiotic myocytes and solitary muscle fibers which stained more intensely with acid dyes.

Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR. Novosibirsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 489-491, April, 1977. Original article submitted June 1, 1976.

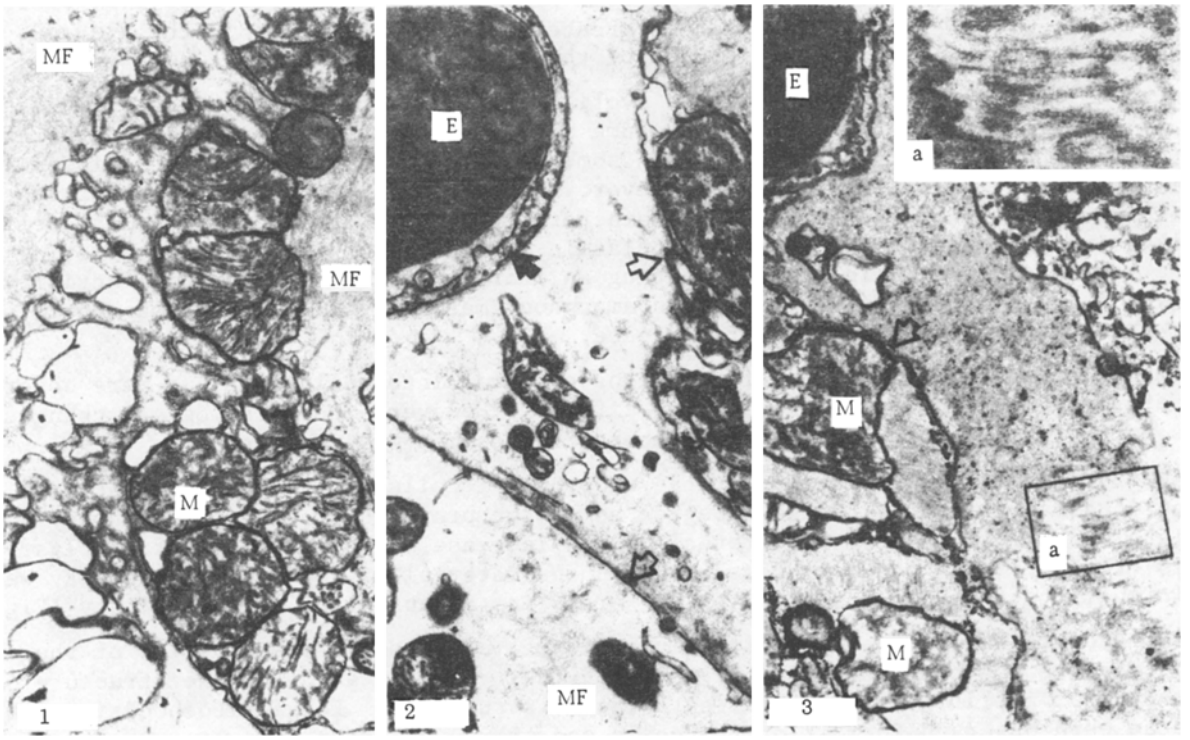

Fig. 1 Fig. 2 Fig. 3

Fig. 1. One day after infection with Coxsackie A13 virus. Part of myocyte of mouse heart, moderate degenerative changes. MF) Myofibril, M) mitochondrion. Epon, stained with saturated uranyl acetate and lead citrate solution, 30,000×.

Fig. 2. Seven days after infection. Space between cells filled with amorphous low-contrast material. Light arrows indicate areas of plasmalemma of myocytes; dark arrows indicate capillary endothelial cells. E) Part of erythrocyte in capillary lumen; MF) myofibril, 30,000×.

Fig. 3. Thirty days after infection. Space between cells filled with material of low electron density. Pale arrow indicates plasmalemma of myocytes. Remainder of legend as in Figs. 1 and 2, 22,500×. Inset a): 45,000× magnification of box (a) showing fibrillary structure of material filling intercellular space.

Electron-microscopic investigation revealed a picture of diffuse, mainly degenerative changes. Their intensity varied from cell to cell, from minimal to prenecrotic (Fig. 1). The greatest changes, varying in severity within the same cell, were observed in the mitochondria. They consisted of increased translucency of the matrix of the mitochondria and complete or partial destruction of the cristae. The sarcoplasmic reticulum was visible as vesicular structures. The contents of the attached and free ribosomes and also the glycogen content were considerably reduced. The Golgi complex and lysosomes were few in number, and in cells with marked changes the lysosomes were mainly secondary. The nuclei were elongated in shape and the outlines of the nuclear membrane slightly undulating. Minimal changes in the myofibrils consisted of a decrease in the clarity of the boundaries of the sarcomeres and also in the distinctness of the isotropic and anisotropic disks, whereas more severe changes could amount to local destruction of the myofibrils. The cisterns of the T system were increased in volume and often contained lipid- and myelin-like material.

Irregularly distributed connective tissue fibers were frequently observed in the interstitial spaces. The ultrastructure of the endothelial cells on the whole showed no visible changes. In some of them large phagosomes filled with granules of high electron density and measuring about 20 and 30 nm, together with numerous micropinocytotic vesicles, were observed.

After 7 days the changes described above on the whole were still present. The perivascular pattern was diffusely intensified in the myocardium; fibroblasts with pyroninophilic granules in their cytoplasm were clearly visible in the intermuscular bands of con-

nective tissue, with thin collagen fibers in some places. The number of myocytes, which stained intensely with acid dyes, was rather greater at this stage than in the previous stage and they were arranged in small groups of three to five cells.

Changes in the relative volumes of the myofibrils and mitochondria in the cell were observed in the electron microscope: The volume of the mitochondria increased appreciably, whereas that of the myofibrils decreased. In some cells the number of mitochondria on average was increased to two per sarcomere. However, they were smaller. The number of lysosomes, mainly secondary, was a little larger. A characteristic feature of the endothelial cells was a marked increase in the content of endoplasmic reticulum, ribosomes, mitochondria, and micropinocytotic vacuoles, evidently indicating an increase in their functional activity [3, 4, 11]. A few fibroblast-like cells and amorphous material of low electron density (Fig. 2) were observed in the interstitial space.

One month after infection, as before, signs of focal intercellular edema were observed. Just as before, damage to the myocardium was diffuse. However, the pathological changes were less marked and the decrease in the number of ribosomes was smaller.

The ultrastructural organization of the endothelial cells pointed to some increase in their functional activity, to the same degree as at the previous stages of the investigation. Light and electron microscopy revealed some increase in the number of fibroblast-like cells, together with which masses of amorphous material of low electron density were present in the intercellular spaces. In some areas its organization was fibrillary (Fig. 3).

At the end of the second month after infection, a decrease in the severity of the degenerative changes was characteristic of the myocytes: normalization of the structure of the mitochondria, myofibrils, and other cytoplasmic structures. Large mitochondria with many cristae were observed in most of the cells and there were more ribosomes than in previous periods. The normal ultrastructure of the myofibrils was restored. The nuclear membrane in the sections outlined large lobules on account of deep invaginations. All these changes, according to other workers [3-5, 11], may be evidence of restoration of the structure and function of the damaged cells during recovery of the structure of injured organoids in several cells and restoration of the functional capacity of the organ through hyperplasia of specific ultrastructures in other cells. However, it must be pointed out that all these changes observed at this stage of the investigation must be regarded purely as a tendency toward the development of regenerative processes in the organ.

As well as the changes described above, light and electron microscopy showed that the number of cells of the stroma was virtually the same as in the control. A diffuse distribution of collagen fibers was observed in the intermuscular zones. Electron microscopically they sometimes appeared as aggregations of amorphous material, which at this stage had a more clearly defined fibrillary structure.

The results thus indicate that Coxsackie A13 virus can give rise to changes of diffuse degenerative and proliferative myocarditis.

The mechanisms of the harmful action of the virus on the heart may be various. In the acute period the possibility of a direct action of the virus on the myocardial cells cannot be ruled out. No virus particles could be found as crystals in the material studied. However, in the phagosomes of the endothelial cells during the first day after infection clusters of granular formations measuring about 30 nm could be observed. The writers observed similar structures previously in human embryonic fibroblasts infected with Coxsackie A13 virus [1] and in HEp-2 cells infected with Coxsackie B3 virus [2].

The clear tendency for the condition to pursue a chronic course 30-60 days after infection will be noted. The interstitial sclerosis observed in the myocardium probably led to a decrease in its functional capacity and was a significant factor in the pathogenesis of the cardiomyopathy [6], confirming observations by other workers in experiments on mice with Coxsackie B4 and encephalomyocarditis viruses [8, 10]. At the same time, the ultrastructural changes in the late stages resemble those in the human myocardium in chronic cardiomyopathy connected with Coxsackie B4 virus [7, 8].

LITERATURE CITED

1. E. F. Bocharov, V. E. Yavorovskaya, V. A. Shkurupii, et al., Izv. Sib. Otd. Akad. Nauk SSSR, No. 10, 131 (1971).

2. T. I. Lanskaya and B. A. Erman, in: Enterovirus Infections [in Russian], Sverdlovsk (1967), p. 53.
3. D. S. Sarkisov and B. V. Vtyurin, Electron Microscopy of Destructive and Regenerative Intracellular Processes [in Russian], Moscow (1967).
4. A. M. Strukov and K. S. Mitin, in: Mitochondria. Structure and Functions under Normal and Pathological Conditions [in Russian], Moscow (1971), p. 18.
5. Yu. G. Tsellarius, in: Mechanisms of Sclerotic Processes and Scar Formation [in Russian], Novosibirsk (1964), p. 35.
6. M. D. Abelmann, Ann. Rev. Med., 16, 145 (1973).
7. G. E. Burch and T. Giles, Am. J. Cardiol., 29, 230 (1972).
8. G. E. Burch, J. M. Harb, and G. Hiramoto, Cardiology, 59, 83 (1974).
9. G. E. Burch and C. G. Tsui, Br. J. Exp. Path., 52, 360 (1971).
10. G. E. Burch, C. G. Tsui, J. M. Harb, et al., Exp. Mol. Pathol., 14, 327 (1971).
11. P. N. U. Campbell, C. Cooper, and M. Ricks, Biochem. J., 92, 225 (1964).
12. Q. R. Miranda, R. S. Kirk, T. S. Beswick, et al., J. Pathol., 109, 175 (1973).

DECREASE IN TISSUE-SPECIFIC RESISTANCE OF THE GASTRIC EPITHELIUM TO ACUTE CELL DEATH INDUCED BY INHIBITION OF DNA SYNTHESIS

O. S. Frankfurt

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Administration of hydroxyurea to mice caused acute death of a few cells synthesizing DNA in the epithelium of the glandular stomach. If actinomycin D was given 8 h before the hydroxyurea, cell death was sharply intensified and about 80% of cells synthesizing DNA died during administration of the hydroxyurea. Administration of actinomycin D simultaneously with hydroxyurea had no potentiating effect on cell death. It is postulated that actinomycin D stimulates protein synthesis in stomach cells (the "superinduction" effect), thus increasing their sensitivity to inhibition of DNA synthesis.

KEY WORDS: *stomach; DNA synthesis; hydroxyurea; actinomycin D; cell death.*

Inhibition of DNA synthesis by hydroxyurea causes acute death of nearly all cells in the S phase in the epithelium of the small intestine but of only solitary cells in the epithelium of the stomach [1]. The ability of gastric cells to withstand inhibition of DNA synthesis without dying reflects the tissue-specific features of the organization of these cells which, since they persist after malignant degeneration, determine the resistance of tumors of the stomach to chemotherapy [2].

The object of this investigation was to study the action of substances which reduce the resistance of the gastric epithelium to hydroxyurea, an inhibitor of DNA synthesis, which is a matter of considerable interest.

EXPERIMENTAL METHOD

To determine acute death of cells synthesizing DNA, thymidine-^{3}H was injected intraperitoneally in a dose of 1 μCi/g body weight into noninbred mice, and 30 min later hydroxyurea was injected in a dose of 500 mg/kg body weight. At 8 h before injection of hydroxyurea or simultaneously with it, actinomycin D was administred in a dose of 0.25-0.5 mg/kg body weight. In some experiments hydroxyurea was injected twice at an interval of 4 h. The vari-

Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 491-492, April, 1977. Original article submitted October 25, 1976.