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SOME FEATURES OF GROWTH OF AN ORGAN CULTURE OF THE LIVER FROM MICE INFECTED WITH COXSACKIE A13 VIRUS

V. E. Yavorovskaya, Yu. P. Gichev, L. F. Bakulina, and T. A. Gicheva

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Features of growth and proliferation of organ cultures of the liver from noninbred albino mice infected with a single dose of Coxsackie A13 virus were investigated. A marked zone of growth mainly of epithelial cells was found early in explants of the liver of the experimental group of mice, whereas growth of cells around the liver explants of the control mice either was absent or was very weak. Moreover, many lymphocytes uniformly distributed in the zone of growth of the liver cells were found in preparations of the liver of the experimental mice. In some explants the picture of adhesion of lymphocytes to the hepatocytes of the culture was seen, and in places where lymphocytes accumulated death of the liver cells and marked thinning of the cellular layer were observed on the 21st and 28th days of growth of the culture.

KEY WORDS: Coxsackie A13 virus; organ culture; proliferation; liver.

In recent years investigators have paid considerable attention to the ability of some viruses to stimulate cell division [4, 5, 8]. This phenomenon, known as the cytoproliferative activity of viruses, plays an important role in the pathogenesis of certain acute, chronic, and slow virus infections [2].

In this investigation an attempt was made to study the morphology of organ cultures of the liver of mice infected with Coxsackie A13 virus. Previous investigations showed increased mitotic activity of the liver cells of mice infected in the neonatal period with Coxsackie A13 virus [1].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female mice weighing 20-22 g. The mice were infected with virus-containing culture fluid, undiluted, containing $10^{-5.35}$ TCD_{50}/ml. Virus was injected intramuscularly into the animals as a single dose of 1 ml on the 7th day of pregnancy. The day of discovery of a vaginal plug was taken as the 1st day of pregnancy [9]. The mother rats were autopsied on the 13th, 15th, and 36th days after infection. Virus was isolated from the liver tissue of the experimental mice by the usual method.

Organ culture of the liver was carried out by Grobstein's method in the modification of Luria and P'yanchenko [3] in Conway dishes at the boundary between two media. Pieces of mouse liver were cultured on millipore filters (RUFS brand, Czechoslovakia) with a pore diameter of 1-2 μ. The liver explants were cultured in medium No. 199 with the addition of bovine serum, 40% glucose solution, 5% ascorbic acid solution, penicillin, and streptomycin. The gas mixture used for culture consisted of 60% O_2, 5% CO_2, and 35% atmospheric air.

Department of Microbiology, Novosibirsk Medical Institute. Group for Clinical Pathology, Department of General Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk.

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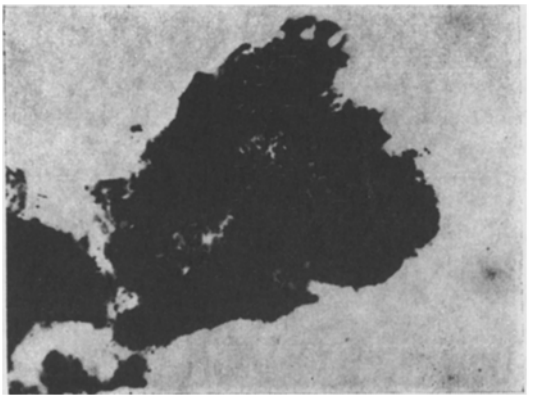

Fig. 1

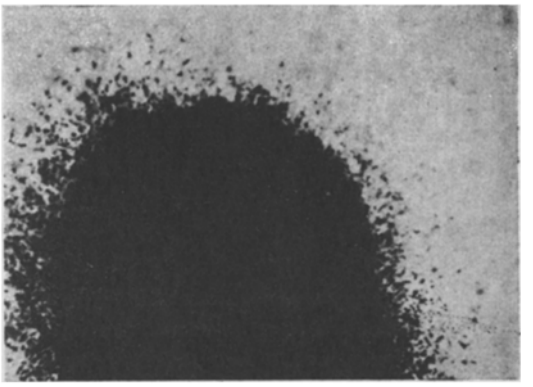

Fig. 2

Fig. 1. Weak zone of growth around liver explant on 14th day of culture (control). Here and in Fig. 2, stained with hematoxylin, 100×.

Fig. 2. Distinct cell proliferation around liver explant on 7th day of culture (experiment).

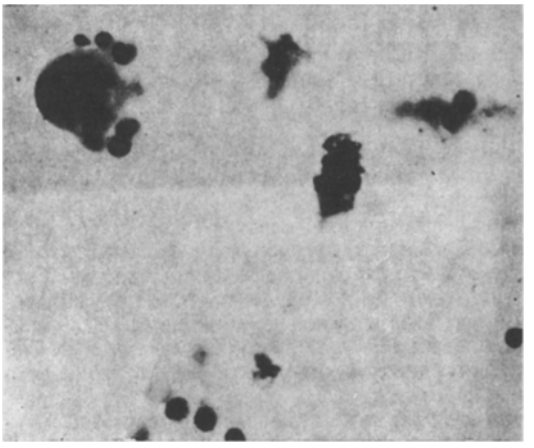

Fig. 3. Picture of adhesion of lymphocytes to hepatocytes and their destruction on 21st day of culture (experiment). Hematoxylin, 400×.

The nutrient medium was changed twice a week. Every 7 days individual filters with 2 or 3 explants were removed from the dishes, fixed in 96° ethanol, and stained with Mayer's hematoxylin. After dehydration and clearing, the filters with the explants were placed on a slide and mounted in Canada balsam. Altogether 233 explants obtained from 6 mice were studied. Three mice served as the control: No. 1 – 20th day of pregnancy; No. 2 – nonpregnant; No. 3 – 3rd day after parturition. The experimental group also consisted of three mice: No. 4 – infected on the 7th day of pregnancy (autopsied 13 days after injection of the virus); No. 5 – nonpregnant (autopsied 15 days after injection of the virus); No. 6 – infected on the 7th day of pregnancy (autopsied 36 days after injection of the virus). The duration of culture of the explants was 28 days.

EXPERIMENTAL RESULTS

Virus could not be isolated by the usual methods from the liver tissue of the infected mice 13, 15, and 36 days after infection.

Analysis of the features of growth of the organ cultures of the liver showed that growth of the cells around the explants in specimens from the control group of mice either was absent altogether (mice Nos. 1 and 2) or it was very weak in 50% of the explants, and consisted chiefly of fibroblasts (mouse No. 3) (Fig. 1). In explants of the liver of mice of the experimental group early and marked proliferation mainly of epithelial cells was observed (mice Nos. 4 and 5) (Fig. 2). In 26% of explants obtained from mouse No. 6, weak cell pro-

liferation was present. It was in this case that the longest time had elapsed after injection of the virus (36 days), and this may have accounted for the decrease in proliferative activity of the liver tissues.

In the zone of growth of the control explants only a few lymphocytes were seen; their number decreased after the 14th day of culture and by the 28th day they had almost completely disappeared. Meanwhile, in specimens obtained from the experimental mice (Nos. 4 and 5) lymphocytes were very numerous and were uniformly distributed in the zone of growth of the liver cells. Only in explants of the liver of experimental mouse No. 6 was infiltration by lymphocytes much less marked.

It is important to mention that in all specimens obtained from experimental mouse No. 5, lymphocytes were present in very large numbers at all stages of culture. Pictures of adhesion of lymphocytes to the hepatocytes of the culture were seen (Fig. 3). In places where lymphocytes accumulated death of the liver cells and marked thinning of the cell layer were observed on the 21st and 28th days of growth of the culture. Presumably the changes observed in the culture corresponded to the pattern of the cytopathic effect of the sensitized lymphocytes. These changes were distinctly observed only in nonpregnant mouse No. 5 on the 15th day after infection with the virus. In other mice of the experimental group (Nos. 4 and 6) no noticeable degenerative changes could be detected in the cells of the culture where there were groups of lymphocytes. It can tentatively be suggested that the reason for this difference was the presence of special immunosuppressor substances in the blood serum of the pregnant animals [6, 7, 10].

This investigation thus revealed the marked cytoproliferative action of Coxsackie A13 virus during organ culture of the liver from mice infected with a single dose of the virus.

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