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LIVER ORGAN CULTURE FROM SUCKLINGS BORN TO MICE INFECTED WITH COXSACKIE VIRUS DURING PREGNANCY

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The writers previously established the marked cytoproliferative action of Coxsackie A13 virus during organ culture of liver obtained from females infected once with the virus during pregnancy [4]. Since manifestations of the cytoproliferative effect in experiments in vivo and in vitro are characteristic of chronic virus infection [1], the object of this part of the investigation was to study growth of an organ culture of liver taken from the young mice born to these mothers.

Experiments were carried out on noninbred albino mice weighing 20-22 g. The mothers were infected with undiluted virus-containing culture fluid, containing $10^{5.35}$ TCD_{50}/ml. The virus was injected intramuscularly into the animals once, on the 7th day of pregnancy, in a dose of 1 ml. The day of discovery of a vaginal plug was taken as the first day of pregnancy.

Eight sucklings divided into two groups were used in the experiment: group 1 (experimental) consisted of five newborn mice autopsied 15 days after injection of virus into the mothers; group 2 (control) consisted of three sucklings obtained from uninfected healthy mothers. Virus was isolated from the liver tissue of the experimental newborn mice by the usual methods.

Organ culture of the liver was carried out by Grobstein's method in the modification of Luriya and P'yanchenko [3], in Conway dishes at the partition boundary between two media. Details of the method were described previously [4].

Altogether 88 explants were studied. To compare growth of organ cultures of the liver from mice of the above groups, features reflecting the character of growth of cells around the explant (absence of growth, small zone of cell growth, good growth — a wide zone of cell growth), the presence of lymphocytes in the zone of

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TABLE 1. Intensity of Growth of Liver Organ Cultures from Newborn Mice (number of explants investigated)

Feature	Experiment (I)			Control (II)		
	period of culture, days					
	7	14	21	7	14	21
Good growth	15	3	3	—	—	—
Small growth zone	1	—	—	—	4	—
Growth absent	—	—	—	6	2	5
Total	16	3	3	6	6	5
P_{I-II}	<0,001	<0,05	<0,025			

TABLE 2. Viability of Liver Organ Cultures from Newborn Mice (number of explants investigated)

Feature	Experiment (I)		Control (II)	
	period of culture, days			
	21	28	21	28
Reduced density in growth zone	6	—	2	—
Degeneration	—	9	4	24
Necrosis	—	—	—	4
Total	6	9	6	28
P_{I-II}	<0,05	>0,05		

Legend. Here and in Table 2: I) newborn mice obtained from mothers infected on 7th day of pregnancy; II) newborn mice obtained from uninfected (control) mothers.

growth, degeneration of cells in the zone of growth, and necrosis of the explants, were analyzed [2]. The features enumerated above were assessed on the 7th, 14th, 21st, and 28th days of culture.

Virus could not be isolated from the tissues of the newborn mice.

Analysis of the character of growth of the liver organ cultures from the suckling mice showed that a clearly marked zone of growth around the explants (good cell growth) was characteristic only of mice of the experimental group; this feature was observed in nearly all explants studied (Table 1). The liver preparations from the same group of mice were characterized by later appearance of signs of degeneration than in the control animals and absence of necrosis of the explants at all times of culture (Table 2).

Analysis of liver preparations from suckling mice born to control mothers showed either absence of cell growth around the explants or the presence of a small zone of growth (Table 1), with the appearance of signs of necrosis of individual explants on the 28th day of culture.

In both the experimental and the control preparations lymphocytes were present. In the zone of growth of some experimental explants a picture of "adhesion" of lymphocytes to cells of the culture was observed, followed by death of the liver cells and a marked decrease in density of the cell layer. Similar changes were observed in organ culture of the liver of infected adult mice [4].

Consequently, the facts described above are evidence of possible transplacental transmission of Coxsackie A13 virus and of the formation of chronic virus infection in the liver cells of experimental animals whose mothers were infected during pregnancy.

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