GROWTH OF THE ADULT HUMAN LIVER IN ORGAN CULTURE

A. F. Blyuger, Kh. M. Veksler, Zh. P. Timoshenko, I. A. Treiya, and I. S. Golubov

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During organ cultivation of pieces of the liver obtained from 49 patients by punch biopsy active growth with the formation of extensive zones of epithelial and connective-tissue cells was observed with materials from the liver of 20 patients. Successful growth was obtained with material from 6 of the 10 patients with virus hepatitis, 8 of the 16 patients with chronic hepatitis, 2 of the 4 with chronic cholecystitis, 3 of the 8 patients with benign pigmentary hepatosis, and the 1 patient with mild acute dysentery in the convalescent stage. The character and rate of growth of the liver depended on the type and stage of the pathological process in the patient.

Several investigations have been made of growth of the pathologically changed human and animal liver in tissue culture [1, 2, 6]. The character of growth of the liver in culture was found [2, 6] to depend on the type of the pathological process in the donor organism. In the accessible literature there were only isolated accounts of cultivation of the healthy human and animal liver. Some workers [1, 2] were unable to obtain growth of the liver from healthy rabbits, mice, or man in vitro. However, Laufs and Walker [8], by improving the method of Kalus et al. [7], obtained an organ culture of the liver from healthy adult monkeys. As matrix they used hemostatic sponge, and the atmosphere in which the cultivation took place consisted of 60% oxygen, 5% CO_2, and 35% air. These workers concluded that these are the optimal conditions for survival and activity of the monkey liver in organ culture.

The object of the investigation described below was to study the character and intensity of growth of organ cultures of the liver from patients with virus hepatitis.

EXPERIMENTAL METHOD

Pieces of the liver of 49 patients obtained by punch biopsy were used for organ cultivation. Cultivation was carried out on Aufs (Czechoslovakia) and HU (USA) Millipore filteres in Conway dishes by a modified Grobstein's method [3]. The liquid phase consisted of medium No. 199 with the addition of 20% healthy donor's serum, kept for 30 min at 56°C; 1% glucose, 0.1% vitamin C, and 100 unit/ml each of penicillin and streptomycin. The atmosphere used for cultivation consisted of 60% oxygen, 5% CO_2, and 35% air. Cultivation continued for 3 weeks and the medium was changed every 48-72 h. The filteres were fixed on the 4th-20th day with 96° alcohol and stained with hematoxylin.

EXPERIMENTAL RESULTS

Active growth was obtained in 20 of the 49 biopsy specimens cultivated, in 13 there was sluggish proliferation of the cells, while in 16 the explant underwent necrosis (Table 1).

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TABLE 1. Characteristics of Liver Growth in Organ Culture Depending on Pathological Condition

Diagnosis	Total number of biopsies	Active growth		Sluggish proliferation of cells	Necrosis
		mixed zones of growth	predominantly epithelial zones		
Virus hepatitis	10	6	0	3	1
Chronic hepatitis	16	8	0	5	3
Fatty degeneration	1	0	0	1	0
Cirrhosis	4	0	0	2	2
Chronic cholecystitis	4	0	2	1	1
Benign pigmentary hepatosis	8	0	3	0	5
Other diseases	6	0	1	1	4
Total	49	14	6	13	16

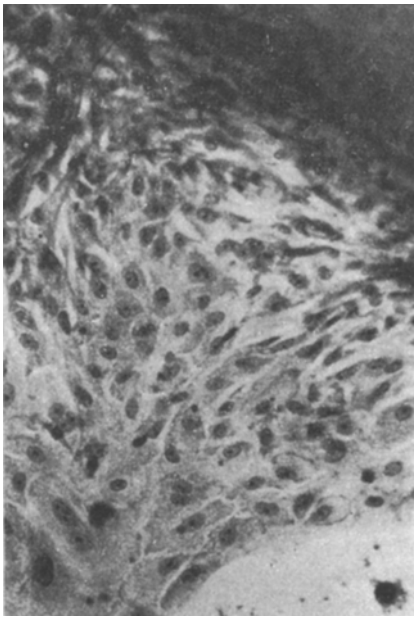

Fig. 1. Active proliferation of all cells during cultivation of liver material from patient with virus hepatitis in the convalescent stage. Hematoxylin-eosin, 63 ×.

Of the 10 biopsy specimens obtained from patients with virus hepatitis, active proliferation of all cells, both epithelial and connective-tissue, with the formation of extensive zones of growth in the early periods of cultivation (by the 6th-7th days), was observed in liver explants from 6 patients in the convalescent stage. All cells, both epithelial and connective-tissue, soon began to proliferate inside the liver fragments (Fig. 1), and their viability was maintained throughout the period of cultivation (up to 3 weeks). Liver explants of 2 patients with virus hepatitis undergoing biopsy at the height of the disease showed very sluggish proliferation of both epithelial and connective-tissue cells, although the liver fragments survived until the 20th day of cultivation.

In 8 of the 6 biopsy specimens obtained from patients with chronic hepatitis active growth was observed. Seven of these specimens were taken from patients with active chronic hepatitis, and 1 from a patient with alcoholic hepatitis. Of the 5 specimens giving only sluggish proliferation 3 were obtained from patients whose chronic hepatitis was accompanied by cholestasis, and 2 from patients in an inactive stage of the disease. Liver biopsy specimens from 4 patients with active decompensated or subcompensated cirrhosis showed no marked evidence of growth.

The character of growth in 2 of the 4 cases of chronic cholecystitis was different from that of the material obtained from patients with virus and chronic hepatitis. Around the explant, wide stratified epithelial zones with a few connective tissue cells were formed by the 8th day of cultivation (Fig. 2). In the explant itself macrophages with yellow pigment in their cytoplasm began to accumulate early, and degeneration ensued. In all 4 cases morphological examination revealed loading of the hepatocytes with fat.

When liver biopsy material from 3 of the 8 patients with benign pigmentary hepatosis was cultivated active growth was obtained with the formation of wide epithelial zones containing a number of connective-tissue cells.

With material from patients with acute dysentery (2 cases), acute gastroenterocolitis of unknown etiology (1 case), duodenitis (1 case), biliary dyskinesia (1 case), and microspherocytic hemolytic jaundice (1 case) growth (with the formation of extensive, predominantly epithelial, zones) was obtained only by the use of liver from the dysentery patient, while sluggish proliferation was obtained with pieces of liver from the patient with gastroenterocolitis.

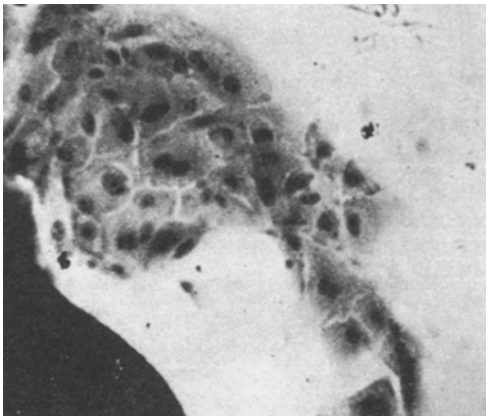

Fig. 2. Sluggish proliferation of epithelial cells during cultivation of liver fragment from patient with chronic cholecystitis. Hematoxylin-eosin, 63 ×.

From the theoretical aspect the fact that organ cultivation of the liver is possible in vitro is itself evidence of the ability not only of cells derived from the mesenchyme, but also of the epithelial cells of the adult human liver to proliferate. The results show that under certain pathological conditions metabolites formed in the liver tissue act as inducers or depressors of regeneration. For instance, during organ cultivation of the liver from patients with acute and chronic lesions of the organ, cell proliferation can be induced by metabolites of hepatocytes in a state of degeneration and, in particular, of necrosis and by metabolites of inflammatory cells. In the light of these findings it seems probable that in cholestasis (syndromes associated with disturbance of bilirubin and bile acid metabolism) accumulation of metabolites of these substances in the hepatocyte has a depressor or toxic effect on cell proliferation.

In cirrhosis the inability of the liver cells to undergo active growth in culture is evidently due to the chronic disorder of the circulation in the liver and the tissue hypoxia characteristic of this disease.

Having regard to the modern views on the immunological character of interaction between proliferating epithelial cells and derivatives of the mesenchyme [4, 5] it can be postulated that the mechanism of these different patterns of regenerative activity of liver cultures under different pathological conditions is immunological in nature and follows the feedback principle. This is seen particularly demonstratively in the case of organ cultures of liver tissue from patients with virus hepatitis in the convalescent stage. Replication of the virus of infectious hepatitis in the lymphocytes and hepatocytes probably acts as an inducing factor stimulating proliferative activity of these cells. The destruction due to the virus, moreover, induces sensitization of the lymphocytes and macrophages. Activated connective tissue cells, in turn, intensify the regenerative activity of the epithelial cells. In active forms of chronic hepatitis and cirrhosis, the observed effect of the virus takes place only in rare cases of its persistence in the liver cells after infectious hepatitis, and even then it is exhibited to a reduced degree. The results showing the different types and rates of growth of the adult human liver in organ culture when obtained from patients with different pathological conditions encouraged the hope that this phenomenon will become an additional diagnostic criterion.

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