IN VITRO CULTIVATION OF PATHOLOGICALLY CHANGED HUMAN AND RABBIT LIVER TISSUE

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Cells from liver explants from patients with chronic liver diseases and of liver tissue from rabbits receiving injections of carbon tetrachloride, are capable of active proliferation in vitro with the formation of an epithelial zone of growth, the structure of which differs depending on the character of the pathological process.

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Despite the well marked ability of the liver to regenerate, cultivation of adult human and animal liver in vitro as a rule gives negative results. Proliferation of epithelial liver cells in culture has been obtained only in the case of embryos and newborn animals [4-9].

Studies of cultivation of embryonic liver cells have revealed that these cells in vitro retain the structure and metabolic features of cells of the original tissue [3, 10, 11]. Since chronic diseases of the liver are accompanied as a rule by a marked increase in the regenerative activity of the organ, it is to be expected that this activation will also be manifested in vitro.

This paper describes the results of in vitro cultivation of pieces of liver tissue obtained by diagnostic biopsy on patients with chronic diseases of this organ, and also the results of experiments to cultivate the liver of adult rabbits before and after poisoning with carbon tetrachloride.

EXPERIMENTAL METHOD

Liver tissue from 6 patients (3 with chronic hepatitis developing after infective jaundice, 3 with postnecrotic cirrhosis of the liver) aged 18-23 years was obtained by biopsy under direct vision during during laparoscopy. In control experiments pieces of liver obtained not more than 2 h after death from a group of 3 patients free from liver disease during life were used. The liver was explanted in pieces not more than 0.5 mm in diameter in Carrel's flasks. A mixture of human and cock plasma was used as solid phase. The nutrient medium, consisting of 70% medium No. 199, 25% human serum, 5% chick embryonic extract (50%), and antibiotics (100 units each of penicillin and streptomycin per ml medium) was added 20-30 min after explantation of the tissue. A gas mixture containining 5% CO_2 was then blown into the flasks (pH of medium 7.2-7.4) and the cultures were incubated at 37°, the nutrient medium being changed every 3-4 days for 4 weeks. The cultures were stained with hematoxylin-eosin or Sudan black. Altogether more than 100 human liver cultures were obtained.

In another series of experiments the liver of chinchilla rabbits aged 6-7 months was used. Serum and heparinized plasma were prepared from blood obtained by cardiac puncture. Laparotomy was performed on the rabbits 1-2 days later, films were made of the liver, and using a human kidney puncture needle, two identical cylinders of tissue were removed from the right lobe of the liver, after with the operation wound was closed. One of the pieces of liver tissue thus obtained was explanted into Carrel's flasks, while from the second a 50% extract of liver tissue was obtained by homogenization in two volumes of nutrient medium, keeping the homogenate for 24 h at 4°, and separation of the supernatant after centrifugation for 10 min at 2500 rpm. Pieces of liver not larger than 0.5 mm were placed in a thin layer of donor's plasma. Nutrient medium consisting of 20% serum of the same rabbits and 80% Eagle's medium was added 30 min later. In some series of cultures, 10-20% of Eagle's medium was replaced by an equal volume of extract of donor's liver. Cultivation was carried out at 37° with change of nutrient medium every 2-3 days for 3-4 weeks.

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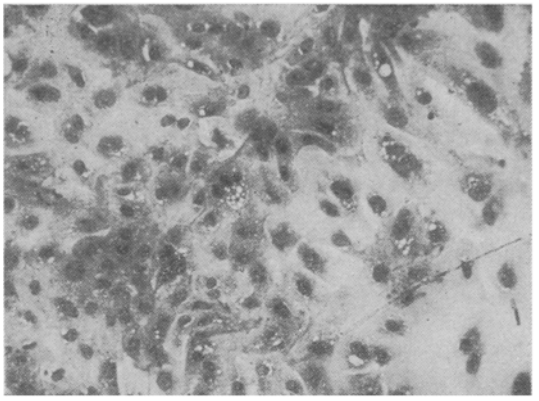

Fig. 1. Epithelial zone of growth in liver culture of patient P., aged 29 years, with chronic hepatitis (18th day of cultivation). Sudan black–hematoxylin. 100 ×.

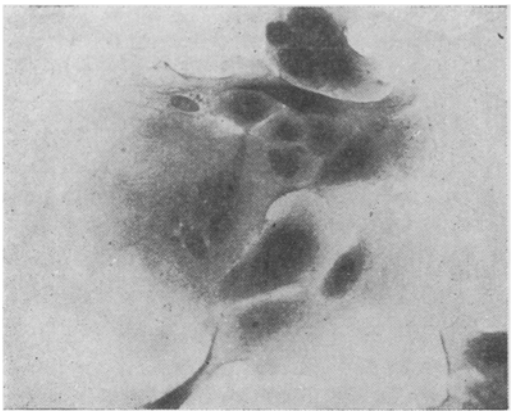

Fig. 2. Liver culture of patient K., aged 32 years, with postnecrotic cirrhosis of the liver (19th day of cultivation). Hematoxylin-eosin, 400 ×.

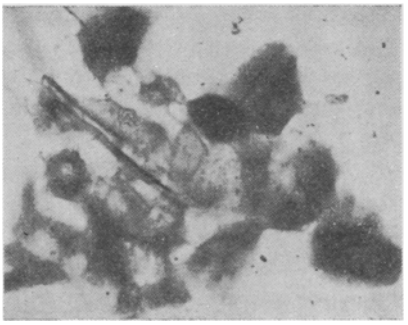

Fig. 3. Hepatocytes in a culture of rabbit liver obtained after administration of carbon tetrachloride (4th day of cultivation in medium with autologous serum). May–Gruenwald–Giemsa, 400 ×.

After laparotomy, the rabbit received a subcutaneous injection of carbon tetrachloride in a dose of 0.3 ml/kg twice a week for 4 weeks. A second laparotomy was then performed, two pieces of liver were again removed, and the animal was sacrificed by bleeding from the carotid artery, the blood obtained being used to prepare serum and plasma. A further series of experiments was then carried out to cultivate the newly obtained liver tissue. The cultures were stained by the May–Gruenwald–Ciemsa method or with hematoxylin-eosin. In each series of experiments 16-20 cultures of donor's liver, serum, and liver extract were prepared. Experiments were carried out on 4 rabbits.

In a series of experiments to test the toxicity of the nutrient medium in the liver cultures, a trypsinized subculture of rapidly proliferating fibroblasts, obtained from the rabbit acting as donor of liver tissue by cultivation of pieces of perihepatic cellular tissue of the same rabbits in a medium of corresponding composition, was used.

EXPERIMENTAL RESULTS

In the experiments to cultivate liver tissue from cadavers of persons free from liver disease during life and also liver tissue from adult rabbits obtained at biopsy before the beginning of carbon tetrachloride administration, negative results were obtained. Negative results were also obtained during cultivation of the liver of healthy rabbits with 10-20% liver extract of the tissue and serum donor when 10-30% nutrient medium from cultures of rapidly proliferating fibroblasts were added to the flasks containing rabbit liver explants at various stages of cultivation, and when a suspension of fibroblasts of the same rabbit was introduced into the flasks. Absence of proliferation of intact liver tissue in vitro was not connected with the toxicity of the nutrient medium used, because the fibroblasts in the flasks with liver explants proliferated rapidly, forming a monolayer suitable for subcultivation after 5-6 days.

Different results were obtained by cultivation of liver tissue from patients and from rabbits poisoned with carbon tetrachloride. Initial migration of polygonal cells of epithelial type in the liver cultures of patients with chronic hepatitis was observed on the 4th-5th day, and in cultures of cirrhotic liver on the 8th-10th day after the beginning of cultivation. Toward the end of the 2nd-3rd week, cells which initially had been present singly, in pairs, or in small groups, formed an epithelial monolayer in contact with the explants (Fig. 1). In some parts of the zone of growth the epithelial layer was intersected by narrow bands of interwoven, highly elongated fibroblast-like cells. In the zone of epithelial membrane, areas were found with

organotypic growth: in these regions the bands of epithelial cells were columnar in structure, i.e., they were arranged in chains of 3-9 cells in contact with each other by one side. Solitary mitotic figures were visible in liver cultures from patients with chronic hepatitis. Marked polymorphism of the epithelial cells was a noteworthy feature (Figs. 1 and 2). Nuclei of epithelial cells were usually located centrally, and they were small in size compared with the area of the cytoplasm. The nuclei were homogeneous in structure and contained from 1 to 7 nucleoli, which were round or, sometimes, rod-shaped or irregular. Occasionally giant cells were even, having 3-8 small nuclei, in contact with each other, as well as one or two ordinary nuclei. The region of the cytoplasm of the epithelial cells was nonhomogeneous in structure close to the nucleus. This region often contained large vacuoles unstained by eosin and Sudan black, granules of dark pigment, and also sudanophilic inclusions. The signs of cell degeneration in liver cultures of patients with hepatitis were ill defined.

The rate of growth of cultures of cirrhotic liver was slower, and their zones of growth consisted chiefly of islets of epithelial cells with a few fibroblasts (Fig. 2). Still more marked polymorphism of the epithelial cells was observed in these cultures, and atypical, giant and multinuclear cells, or cells with a collection of 4-8 small nuclei and irregularly shaped nuclei, were more frequently seen. The cell nuclei usually contained not more than one or two large nucleoli. Cultures of adult human cirrhotic liver also were distinguished by their much more marked evidence of cell degeneration: marked eosinophilia and vacuolation of the cytoplasm, especially of its inner portions, and pycnosis of the nuclei were frequently observed. However, the degree of cell lipoidosis was moderate in these cultures also.

In liver cultures of rabbits poisoned with carbon tetrachloride, migration of hepatocytes, initially forming epithelial islets near to or some distance from the explant (Fig. 3), and subsequently an epithelial zone of growth, was observed by the 3rd-4th day of cultivation. The best results were obtained by cultivation of liver tissue with serum of the same rabbit obtained after administration of carbon tetrachloride. Liver extract of the same rabbits slightly inhibited proliferation of the liver cells. Serum obtained before administration of carbon tetrachloride stimulated proliferation of hepatocytes to a far less degree than serum obtained after a course of injections of carbon tetrachloride. After staining by the May–Gruenwald–Giemsa method, the small round nucleus with a coarse chromatin structure appeared red with a violet tinge, while the cytoplasm appeared light or dark blue, and when stained with hematoxylin-eosin, considerable eosinophilia, granulation,and vacuolation of the cytoplasm were revealed.

It is clear from this description and illustration that cells in cultures of human liver affected by a pathological process possess considerable morphological similarity with cells of the hepatic epithelium in preparations obtained by punch biopsy of the liver [1, 2]. The results are evidence that epithelial cells from the liver of adult patients with chronic liver diseases and of the liver of rabbits with experimental toxic hepatitis can be cultivated in vitro for a long time.

LITERATURE CITED

1. M. G. Abramov, Clinical Cytology [in Russian], Moscow (1962).
2. E. V. Chernysheva, in: Instrumental Diagnosis of Liver Diseases [in Russian], Moscow (1965), p. 165.
3. R. Angelucci and F. Clementi, Boll. Roc. Ital. Biol. Sper., 38, 1194 (1962).
4. S. W. Benewolenskaja, Arch. Exp. Zellforsch., 9, 128 (1929).
5. W. D. Hillis and F. B. Bang, Exp. Cell Res., 26, 9 (1962).
6. M. Iunkin, Arch. Exp. Zellforsch., 3, 166 (1926).
7. M. Wordmann, Arch. Exp. Zellforsch., 8, 371 (1929).
8. M. Soukupova, Physiol. Bohemoslov., 14, 107 (1965).
9. A. I. Thomas, Arch. Exp. Zellforsch., 22, 16 (1938).
10. I. Verne and S. Hébert, Bull. Ass. Anat. (Nancy), No. 133, 911 (1966).
11. I. Verne and S. Hébert, Diabéte, 13, 2 (1965).