PREPARATION OF HUMAN EMBRYONIC LIVER CULTURES

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Transplantation of isolated hepatocytes or microfragments of liver is a possible method of treatment of patients with hepatic failure [4]. Cases have been described in the literature [4] in which a therapeutic effect has been obtained by the use of transplantation of isolated hepatocytes [9]. At the same time, it has been observed [8] that isolated hepatocytes do not remain viable for long. Survival of hepatocytes can be increased as a result of their culture in vitro. The writers previously developed ways of obtaining monolayer cytotypic and floating organotypic cultures from the pancreas of intrauterine human and animal fetuses [1, 3], capable of producing insulin over long periods of time [2, 3]. These cultures have been used in the Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR for clinical allo- and xenografting into diabetic prolonged therapeutic effect [5-7].

We have used these methods to obtain cultures of other organs of human and animal fetuses. This paper describes the method of obtaining cultures of human embryonic liver.

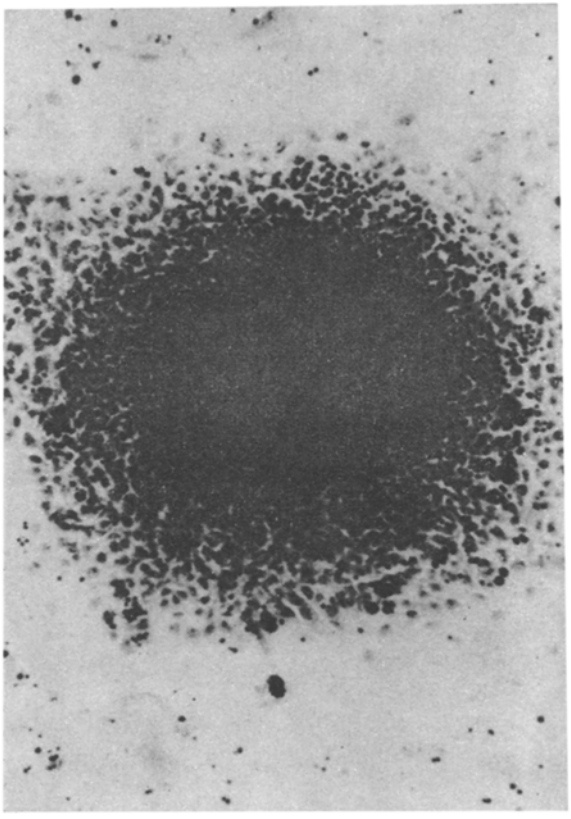

Fig. 1. Three-day culture obtained from human embryonic liver (40 ×). Central focus of attachment and monolayer zone of growth. Here and in Figs. 2 and 3: stained with hematoxylin and eosin.

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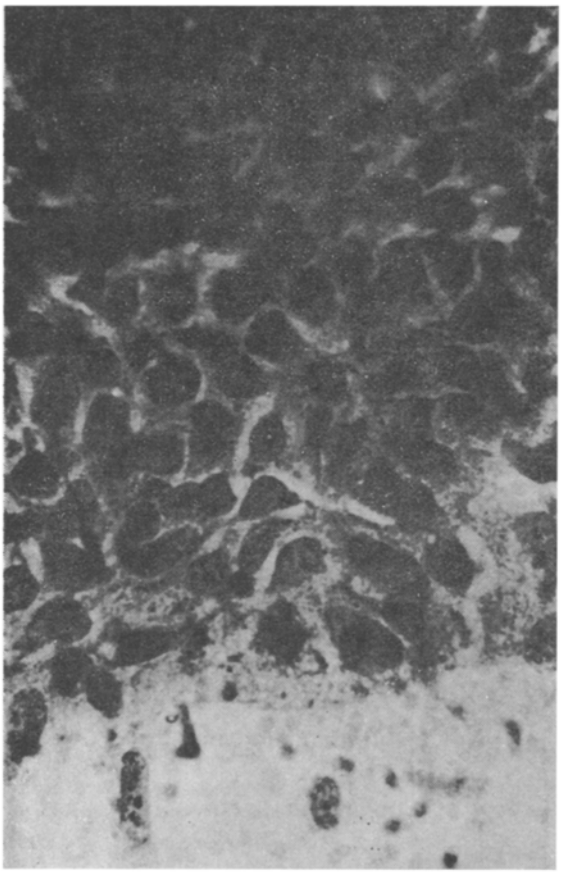

Fig. 2

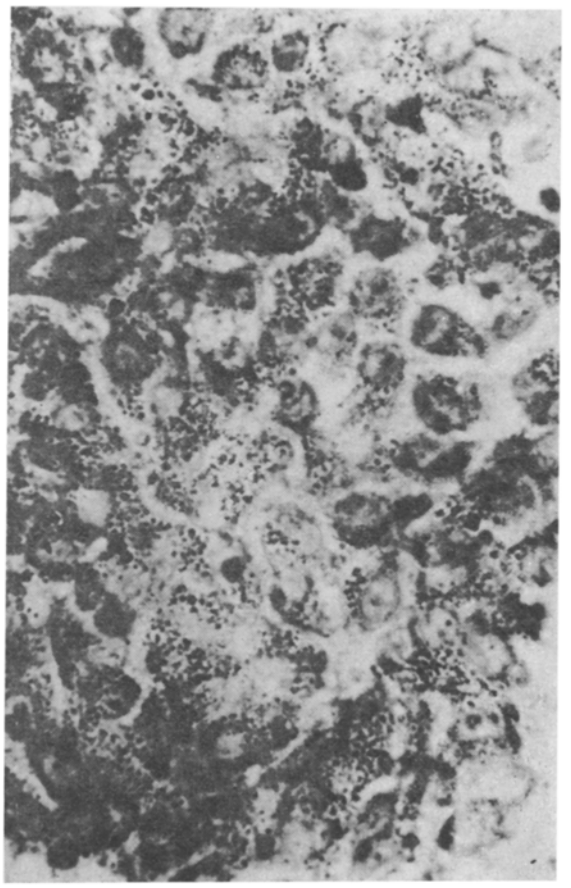

Fig. 3

Fig. 2. Transition from stratified zone of attachment to monolayer zone of growth (400 ×). Monolayer growth of hepatocytes in peripheral zone.

Fig. 3. Monolayer zone of growth (400 ×). Numerous pigment granules in cytoplasm of heptatocytes.

EXPERIMENTAL METHOD

The source of the cultures was the liver of 9-12-week human embryos. Material was obtained during operations to terminate pregnancy on medical grounds. The embryonic liver was carefully washed with Hank's solution with antibiotics (penicillin 500 U/ml, streptomycin 500 μg/ml or kanamycin 500 U/ml), cut into fragments measuring 2-3 mm, and carefully washed in several portions of Hanks's solution with antibiotics to remove blood. The washed fragments were placed on a watch glass and covered with 0.1% solution of collalytin (a product of the Meat Industry Research Institute, Moscow) in Hanks' solution. Contact between tissue and collalytin lasted 5-10 min at room temperature (20-22°C). Next, to remove the collalytin, the suspension of fragments was repeatedly washed with Hanks's solution and cut into small pieces with ophthalmologic scissors. The microfragments measuring 0.2-1.0 mm thus obtained were introduced into flasks of different capacity, and the material was uniformly distributed over the surface of the glass. After 10-15 min appropriate volumes of growth medium (medium 199 with 10% bovine serum) were poured into the flasks. Some of the microfragments were seeded on coverslips, placed in penicillin flasks with growth medium. After different periods of culture the coverslips with the cultures adherent to them were fixed with Bouin's mixture and stained with hematoxylin and eosin and by Mallory's method. The preparations were subjected to cytologic investigation (Figs. 1-3).

EXPERIMENTAL RESULTS

Multiple microfocal cultures consisting of a central stratified focus of attachment surrounded by a monolayer zone of growth, which gradually spread on account of outward movements of the hepatocytes from the explants and their division by mitosis, appeared on the

surface of the glass 24-36 h after seeding. The width of the zone of growth at the stage of complete culture formation was 0.3-1.0 mm. In the peripheral part of the monolayer zone of growth, the undulating membrane of the hepatocytes was clearly visible. With an increase in the period of culture the monolayer zone of growth freed itself from cells of the hematopoietic series and from mature forms of peripheral blood cells. By the 6th or 7th day of culture destruction and necrosis of the central stratified zone of the explant were observed in some cases: deposition of pigment took place in this area (as a result of destruction of erythrocytes and death of hepatocytes). Meanwhile, in the monolayer zone of growth the hepatocytes preserved their structure: they contained many granules of bile pigments and also vacuoles (evidently corresponding to drops of lipids that dissolved during histologic treatment of the material) in their cytoplasm. The monolayer regions of the cultures, consisting of hepatocytes, were preserved for 10-20 days, after which they underwent destruction.

These results are evidence that cultures of human embryonic liver can be obtained by a method used previously by the writers to obtain pancreatic islet cells. The zone of growth of these cultures, freeing itself during culture from contaminating elements (cells of the hematopoietic series), and consisting of a monolayer of typical hepatocytes, is particularly interesting.

The possibility of using hepatocyte cultures in experiments on animals and in clinical practice will be a topic for the writer's future research. Experimental transplantation of such cultures into different organs and tissues of xenogeneic recipients and, in particular, of animals with experimental hepatic failure, is a particularly interesting problem.

LITERATURE CITED

1. R. A. Babikova, V. N. Blyumkin, and B. I. Shal'nev, Byull. Éksp. Biol. Med., No. 3, 350 (1977).
2. V. N. Blyumkin, R. A. Babikova, and N. N. Kauricheva, Technical Recommendations [in Russian], Moscow (1985).
3. V. N. Blyumkin, N. N. Skaletskii, and N. I. Kauricheva, Byull. Éksp. Biol. Med., No. 6, 764 (1984).
4. É. I. Gal'perin and S. R. Karagelyan, Transplantation of the Liver [in Russian], Moscow (1984), pp. 5-70.
5. V. I. Shumakov, V. N. Blyumkin, and S. N. Ignatenko, Probl. Éndokrinol., No. 1, 18 (1985).
6. V. I. Shumakov, V. N. Blyumkin, and S. N. Ignatenko, Probl. Éndokrinol., No. 5, 65 (1985).
7. V. I. Shumakov, S. N. Ignatenko, and V. N. Blyumkin , Transplantation and Artificial Organs [in Russian], Moscow (1984), pp. 19-21.
8. A. Guillouzo, A. Guguen-Guillouzo, and M. Bourel, Triangle, 20, No. 4, 121 (1981).
9. M. Malik, J. L. Touraine, H. Perrot, and J. Traeger, Proceedings of the 16th Congress of the European Dialysis and Transplantation Association, Amsterdam (1979), pp. 413-418.