ORGAN CULTURES OF EMBRYONIC LIVER SYNTHESIZING SERUM PROTEINS

E. A. Luriya, R. D. Bakirov,
G. I. Abelev, and A. Ya. Fridenshtein

UDC 612.35:612.646/.015.348-085

Proliferation of liver and hematopoietic tissue took place and differentiation was maintained in organ cultures of liver fragments from 16-18 day-old mouse embryos on millipore filters for 24 days in vitro. Foci of intensive hematopoiesis were found in contact with liver tissue. These cultures synthesized serum albumin and α_f-globulin.

* * *

Organ cultures have not yet been adequately used as a means of cultivation of embryonic liver. However, in investigations when this principle of cultivation at the boundary between the liquid and gaseous phase of the nutrient medium was used [5-7], the most convincing morphological evidence that liver cells can be grown in vitro was obtained.

In the present investigation the method of organ cultivation on membrane filters was used to obtain long-surviving cultures of mouse embryonic liver in which proliferation took place and differentiation of liver and hematopoietic tissue was maintained. This could be concluded from the morphology of the cultures and also from the fact that they synthesized certain plasma proteins: albumin and embryonic serum α_f-globulin, synthesis of which takes place in parenchymatous cells of the liver [1-3].

EXPERIMENTAL METHOD

Pieces of liver (2-3 mm in diameter) from line CBA mouse embryos aged 16-18 days were explanted on AUFS millipore filters (pore size 0.6-0.9 μ) placed above nutrient medium in Conway dishes by the method described previously [4]. In some cases 20 mg each of L-glutamine and sodium β-glycerophosphate was added to 100 ml of culture medium. In one experiment radioactive glycine was added to the medium in a dish containing six 7-day cultures. The filters with the cultures were fixed after 3-24 days with alcohol-formol, and stained totally. After dehydration and clearance in xylol, total preparations were made. Some cultures were embedded in paraffin wax and series of sections 6-7 μ in thickness were cut and stained with alum-hematoxylin. Altogether 86 cultures were used. A micromethod of double diffusion in gel was used to determine α_f-globulin, with the aid of a test system for α_f-globulin [1, 2]. The test system consisted of rabbit antiserum against serum of newborn mice exhausted with plasma of adult mice, and of the serum of newborn mice taken in optimal dilution (1:128). The test preparations were compared with a standard test system.

Serum albumin was determined in a similar manner in the preparations. As test system in this case rabbit serum against serum of adult mice, and purified albumin taken in optimal dilution were used. By parallel dilution of the serum and antigen this test system was equalized in intensity with the test system for α_f-globulin.

The determinations were carried out in culture media before and after concentration. For concentration, 5 ml of medium was precipitated with 5 ml of 10% TCA and the precipitate was dissolved in 1 ml physiological saline with alkalification. Preliminary experiments showed that under these circumstances no inactivation of antigen takes place. Pure medium for cultivation and the culture media of nonhepatic cultures were used as controls.

Laboratory of Immunomorphology and Laboratory of Immunochemistry of Cancer, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Active Member of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 95-99, March, 1969. Original article submitted June 29, 1968.

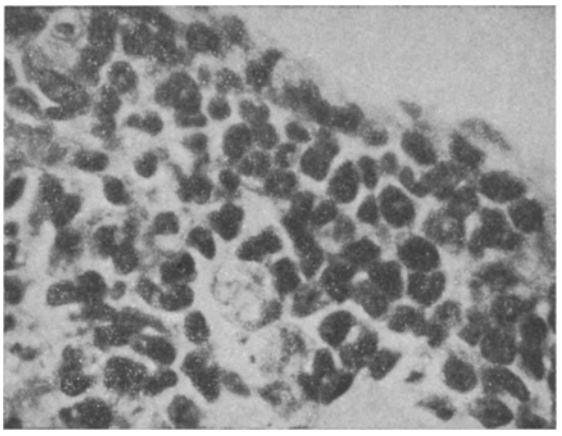

Fig. 1. Sections through 6-day explant of embryonic liver. Hematoxylin, 200×.

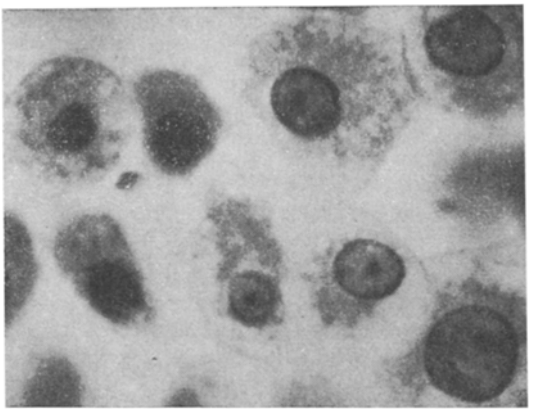

Fig. 2. Zone of growth of 12-day culture of embryonic liver. Hematoxylin, 800×.

TABLE 1. Serum Albumin (a) and Embryonic α_f-globulin in Organ Cultures of Mouse Embryonic Liver

Culture	Antigens	Time of cultivation (in days)								
		without glutamine						with glutamine		
		3	5	7	9	12	15	19	23	25
Liver of 18-day embryos	A	+++	++	±						
	α_f	+++	+++	+	+	+	+			
	A	+++	++							
	α_f	+++	+++							
	A	+++		+++	+++	+++	++			
	α_f	+++		+++	++	+				
	A							+++	++	+
	α_f							+++	++	+
Bone marrow of adults	A	—		—	—	—	—			
	α_f	—		—	—	—	—			
Embryonic bone	A	—		—	—	—	—			
	α_f	—		—	—	—	—			

Legend: +++ strongly positive reaction for α_f-globulin (albumin); antigen concentration in culture medium equal to its concentration in test system; ++ antigen concentration smaller than in test system; + weak positive reaction; — no antigen detected in medium.

To determine the uptake of labeled amino acids into α_f-globulin and albumin, the method of immunoautoradiography was used [3]. A solution of glycine-C^{14} was added to the nutrient medium to give a concentration of 2 μCi/ml. After incubation for 2 days with the label the culture medium was concentrated, and the precipitation reaction in agar was then carried out by the usual method. The agar plates were washed, dried, and exposed on film. The blackening of the autoradiograph produced by the labeled precipitate indicated incorporation of label into the antigen.

EXPERIMENTAL RESULTS

In the first 6 days, growth of epithelial membranes and extensive zones consisting of hematopoietic cells were observed on the filters around the explanted pieces of liver. The epithelial cells had a large vesicular nucleus with one or two nucleoli. They had sharply defined borders and were polygonal in shape. They were joined together by cell bridges and formed a membrane. On the under surface of the filter cells resembling branching histiocytes were growing, presumably stromal cells which had penetrated through the pores of the filter. In the center of the explants necrosis took place, while in the peripheral areas the layers of epithelial cells and myeloid cells remained intact (Fig. 1).

After 8-24 days the growing epithelial membranes on the upper surface of the filters became stratified. The epithelium in them assumed the characteristic morphology of liver cells and here and there it was arranged as bands resembling the columns found in the liver (Fig. 2). Numerous myeloid cells were arranged on the membrane, forming large hematopoietic foci containing hematopoietic cells at different stages of maturation. Proliferation of liver cells took place in the central parts of the fragments. However, hematopoietic tissue was found only in the peripheral part of the fragments, in close contact with liver cells; the central zone of the explants contained no hematopoietic tissue.

The results of determination of α_f-globulin and albumin in the course of growth of the cultures showed that the cultures synthesized these proteins during 24 days of growth (Table 1). The degree of synthesis correlated with the state of the culture. The occurrence of true synthesis of α_f-globulin was confirmed immunoautoradiographically—from incorporation of labeled amino acid into the investigated antigens. In this experiment, it must be emphasized, the culture was taken after no more than 6 growing fragments remained in the dish. Besides albumin and α_f-globulin, the culture media contained one further unidentified antigen.

Proliferation of both epithelial and hematopoietic tissue found in embryonic liver thus takes place in organ cultures,and their differentiation continues. Much of the initially explanted liver tissue constituting the center of the explants degenerates in the first days. At the periphery of the fragments the liver cells remain intact and form an extensive zone of growth in the form of characteristic epithelial membranes growing over the upper surface of the filters.

Both inside the fragment and in the membranes formed by them, the epithelial cells had the characteristic morphology of liver cells, and numerous mitoses were found in them. The character of differentiation of these cells could be judged from the specific synthetic activity which they retained. The presence of prolonged and intensive synthesis of serum proteins in the cultures was demonstrated conclusively.

Reaccumulation of α_f-globulin and albumin in high concentration took place in the cultures during frequent exchanges of culture fluid. Finally, incorporation of labeled amino acids into the investigated proteins is direct evidence of the occurrence of synthesis of albumin and α_f-globulin in the culture. Growth of typical connective-tissue elements migrating from the explant through the pores of the filter took place over a lower surface of the filters. This selective arrangement of the growing epithelial and connective tissues on different surfaces of the filter was explained by the size and mobility of the liver cells and Kupffer cells and by differences in their sensitivity to the oxygen concentration in the medium.

A characteristic feature of the cultures described above was the long persistence of intensive hematopoiesis where the hematopoietic cells were in contact with embryonic liver cells. The morphology of the hematopoietic tissue until the 24th day of cultivation indicated continuing histogenesis of hematopoietic cells. In fact, extensive foci consisting of young hematopoietic cells, whose life cycle do not exceed 3-4 days, were found in cultures at this period. Consequently, these were cells whose histogenesis had continued in vitro and not surviving elements. Numerous mitoses were observed among the hematopoietic cells. It remains unexplained whether the prolonged hematopoiesis in these cultures was connected with some special property of embryonic hematopoietic tissue, by virtue of which it can persist for a long time in organ culture, or with some influence of embryonic liver cells on hematopoietic tissue.

LITERATURE CITED

1. G. I. Abelev, S. D. Perova, et al., Transplantation, 1, 174 (1963).
2. G. I. Abelev, Progr. Exp. Tumor Res. (Basel), 7, 104 (1965).
3. G. I. Abelev and R. D. Bakirov, Vopr. Med. Khimii, No. 4, 378 (1967).
4. E. A. Luriya and I. E. P'yanchenko, Byull. Éksperim. Biol. i Med., No. 6, 81 (1967).
5. G. M. Chen, Exp. Cell Res., 7, 518 (1954).
6. W. Hillis and F. Bang, Exp. Cell Res., 26, 9 (1962).
7. H. Watenable, 42, 685 (1966).