ORGAN CULTURES OF THE LIVER OF ADULT MICE POISONED WITH CCl_4

R. D. Bakirov, T. A. Eliseeva,
A. Ya. Fridenshtein, and G. I. Abelev

UDC 615.917:547.412.133]-07:616.36-07

The intensity of growth was compared in organ cultures of liver fragments of adult mice 3, 24, and 72 h after exposure of the mice to CCl_4 vapor. Proliferation of the hepatic parenchyma was observed. The largest zone of growth was found in cultures explanted 72 h after CCl_4 poisoning. These cultures synthesized specific protein – serum albumin (detected by the gel-diffusion reaction). Liver explants of adult control mice did not grow and did not synthesize albumin under these conditions.

* * *

The parenchymatous tissue of the adult liver grows poorly in tissue cultures, but in submerged cultures regenerating liver tissue forms a mixed connective-tissue and epithelial zone of growth [4]. There is considerable difficulty in assessing the character of the epithelial cells in it.

In the present investigation the method of organ cultivation on membrane filters resulted in growth and differentiation in vitro of parenchymatous cells of the regenerating adult liver. Evidence of this was given by the morphology of the cultures and the fact that they synthesized serum albumin.

EXPERIMENTAL METHOD

Male mice of line C3HA, aged 2-3 months, were exposed to CCl_4 vapor for 15 min [1]. The liver of animals sacrificed 3, 24, and 72 h after poisoning, and also the liver of control mice, were used for cultivation. The liver was cut up into 2-3 mm fragments and washed in 3 changes of medium No. 199. The fragments were then placed on the surface of AUFS millipore filters and cultivated in a Conway dish by the method described previously [3]. The medium was changed after 48-72 h. Each Conway dish at the beginning of cultivation contained 13-14 cultures prepared from the liver of the same animal. The cultures were fixed on the 1st-15th day of cultivation. The filters were stained as total preparations with alum-hematoxylin, and sections cut from the fragments were stained with hematoxylineosin and by the PAS method and counterstained with hematoxylin. Altogether 74 cultures were studied.

Serum albumin in the culture medium was determined after each change of medium by Ouchterlony's double diffusion in gel method in the semimicromodification of Gusev and Tsvetkov [2]. A standard test system was used to determine albumin. This consisted of a rabbit antiserum against the serum of adult mice and the albumin preparation taken in optimal concentrations, obtained from mouse serum after zonal electrophoresis in gel. The culture media were concentrated by addition of an equal volume of trichloroacetic acid solution to the medium. A 10% solution of NaOH was added drop by drop to the precipitate for formed after centrifugation until it had completely dissolved. In this way the culture medium was concentrated 5-7 times. The results of the precipitation reaction, carried out at room temperature, were recorded after 24 h and estimated semiquantitatively from a standard scale [1].

EXPERIMENTAL RESULTS

Massive necrosis of the transplanted tissue without appreciable signs of regeneration took place in cultures of normal liver. No zone of growth was present around the pieces of the filters, or single fibroblast-like cells appeared.

Laboratory of Immunochemistry of Cancer and Laboratory of Immunomorphology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the AMN SSSR O. V. Baroyan). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 5, pp. 72-75, May, 1969. Original article submitted June 29, 1968.

TABLE 1. Serum Albumin in Culture Medium of Adult Mouse Liver

Day of cultivation	Experiments Series I			Day of cultivation	Experiments series II			
	normal liver	after CCl_4 poisoning			after CCl_4 poisoning			
		24 h	72 h		3 h	12 h	24 h	72 h
4	+++	+++	+++	1	++	++	++	+++
6	–	+	++	4	+	±	+	++
8	–	±	+	6	+	+	+	+
11	–	±	++	9	+		+	+
13	–	–	±	13	±		+	+

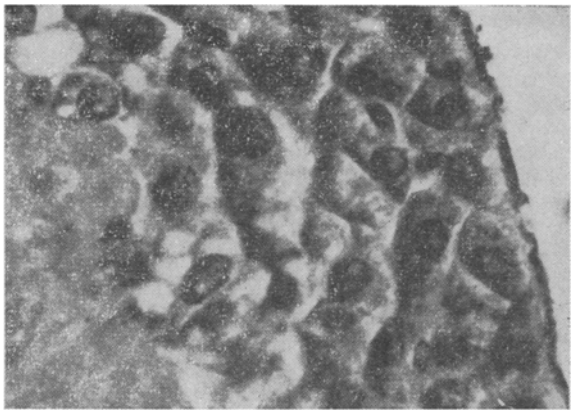

Fig. 1. 12-Day culture of liver explanted 72 h after CCl_4 poisoning. Hematoxylin, 200 ×.

During the first 4 days of cultivation, migration of single fibroblasts was observed on the filter in liver cultures explanted 3 h after exposure of the mice to CCl_4, while on the 6th and 9th days a small zone of growth appeared in half of the cases, consisting of liver cells with a small admixture of fibroblast-like cells. Inside the fragments necrosis of the hepatic parenchyma took place, with the preservation and proliferation of a small number of fibroblast-like cells.

In cultures explanted 24 h after CCl_4 poisoning, after cultivation for 24 h migration of single connective-tissue cells was observed on the filter, and after 4 days the cultures were surrounded by a connective-tissue zone of growth. After 6 days this zone of growth around the fragment consisted largely of typical liver cells forming characteristic columns, but no continuous membranes were present. The peripheral part of the zone of growth consisted chiefly of connective tissue. After 8-18 days the pieces were surrounded by a wide, stratified zone of growth, consisting of connective tissue and liver cells. Inside the fragments on the 4th-6th day most of the liver tissue was necrotic, but a layer of intact liver cells remained on the surface of the fragments. On the 8th-13th day mitoses were found in this region, while the zone of surviving liver cells had increased in size and consisted of between 4 and 6 cell layers.

In cultures explanted 72 h after CCl_4 poisoning, after cultivation for 24 h the fragments were surrounded by a large zone of growth consisting of hepatic and connective-tissue cells. At all later periods the zone of growth in these cultures was much larger than in cultures of liver tissue explanted 24 h after CCl_4 poisoning.

On the 6th-13th day the extensive, stratified zones of growth consisted of connective-tissue cells lying on the upper surface of the filters and covered with large epithelial membranes. These consisted of several layers of cells, mostly collected into small columns of 4-10 cells. The hepatic cells proliferated intensively, and the tendency to form columns was more marked in the places where they lay on the sheet of connective tissue and not directly on the filter surface.

Starting on the 4th day and continuing until the end of observation, inside the fragment central necrosis was accompanied by proliferation of liver cells so that after the 8th day large areas at the periphery of the explants consisted of viable hepatic tissue (Fig. 1).

The liver cells lying in the zone of growth and inside the fragments possessed the characteristic morphological properties and contained glycogen.

After 48 h, serum albumin was found in the culture fluid (1st change) of normal liver and of liver taken from the animals 3, 24, and 72 h after CCl_4 poisoning. In subsequent (until the 13th day) changes albumin was most clearly identified in medium in which liver explanted into the culture 72 h after CCl_4 poisoning was incubated. Albumin was detected in traces in the culture medium into which the liver was placed 24 h after CCl_4 poisoning. During cultivation of normal liver no albumin was found in the medium (Table 1).

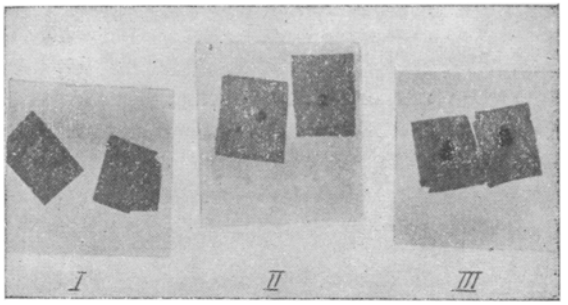

Fig. 2. Zones of growth of 11-day liver cultures explanted 24 h (II) and 72 h (III) after CCl_4 poisoning and without exposure to CCl_4 (I). Hematoxylin.

Adult liver tissue thus grows well under organ cultivation conditions in vitro only if the liver of mice poisoned with CCl_4, i.e., undergoing regeneration, is used. Under these circumstances, proliferation of hepatic cells, the formation of epithelial membranes, and the formation of structures resembling hepatic columns are observed both in the zone of growth and inside the fragment. On the 4th-12th day of explantation a clear quantitative difference was observed between the degree of proliferation of the liver cells (the size of the zone of growth) in liver cultures of mice poisoned with CCl_4 3, 24, and 72 h before explantation; the liver explanted after 72 h grew much more intensively at these times than that explanted after 3 h (Fig. 2). In the course of further cultivation this difference disappeared. Accumulation of albumin in the culture media of liver organ cultures during repeated changing of the medium is evidence of synthesis of this protein in culture. The possibility of elution of albumin from liver fragments during the investigation can be ruled out by the case of a culture obtained from liver of a normal mouse, where albumin was detected only in the first change of medium. The degree of accumulation of albumin in the medium corresponded basically to the morphology of the cultures. The content of this protein in cultures of liver explanted 72 h after CCl_4 poisoning was always greater than in cultures taken at different times after exposure to CCl_4.

Albumin synthesis is additional evidence of growth and functioning of cells of the hepatic parenchyma in the cultures. Other evidence is given by the morphology of the cultures. Consequently, after CCl_4 poisoning changes take place in the liver, increasing in degree from 3 h to 72, promoting growth of liver cells under explantation conditions.

A correlation exists between the degree of lymphocytic infiltration of the liver explanted in vitro at different times after CCl_4 poisoning and the degree of growth of the explant in vitro (Fig. 2). The possibility is thus not ruled out that lymphocytes may play an important role in the regeneration of the liver after CCl_4 poisoning.

LITERATURE CITED

1. R. D. Bakrov, Byull. Éksperim. Biol. i Med., No. 2, 45 (1968).
2. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
3. E. A. Luriya and I. E. P'yanchenko, Byull. Éksperim. Biol. i Med., No. 6, 81 (1967).
4. M. Abercrombie and N. Herkness, Proc. Roy. Soc., 138, 544 (1951).