correct distancing of the flexible light guide, thereby creating optimal temperature conditions for welding the vessel walls at all depths.

Because of the positive results of this experiment, laser welding of lymphatics and veins of small caliber, with the aid of a flexible light guide, can be recommended for clinical use.

LITERATURE CITED

1. O. K. Skobelkin, E. I. Brezhov, G. D. Litvin, et al., Khirurgiya, No. 3, 71 (1987).
2. O. M. Gomes, R. Marorus, R. Armelin, et al., Texas Heart Inst. J., **10**, 145 (1983).
3. K. K. Jain and W. Gorisch, Surgery, **85**, 684 (1979).
4. K. K. Jain, J. Microsurg., **1**, 436 (1980).
5. J. R. Morris and M. Carter, Laser Assisted Microvascular Anastomosis, Las Vegas (1980).
6. M. R. Quigley, J. E. Bailes, H. C. Kwaan, et al., Laser Assisted Microvascular Anastomosis, Las Vegas (1983), pp. 357-365.
7. R. A. White, G. Kopchok, and G. H. White, Proceedings of SPIE (Bellingham), No. 908, 364 (1988).

CORRECTION OF ACUTE HEPATIC FAILURE BY HEPATOCYTES CULTURED ON MICROCARRIERS

A. V. Bocharov, I. B. Fedina, V. G. Spirov, N. A. Bochkova, and A. A. Shnyra

UDC 616.36-008.64-036.11-092.9-08:[616.15-008.6-085.246.2:615.361.36.018.1

KEY WORDS: hepatocytes; microcarrier; acute hepatic failure.

Acute hepatic failure (AHF) is a serious clinical syndrome caused by extensive necrosis of the hepatic parenchyma. A disturbance of metabolism of the liver, connected with functional insufficiency of the hepatocytes, leads to the accumulation of toxins in the blood, disturbance of the balance between synthesis and degradation of plasma proteins, and changes in the hormonal status of the organism [1]. Existing methods of correction of AHF are aimed at removing accumulated toxins from the blood. Methods of exchange blood or plasma transfusion, hemoperfusion, and plasmapheresis are used for this purpose. However, despite these approaches to the treatment of severe forms of AHF, mortality in this group of patients remains high at about 70-80% [10]. The ineffectiveness of these methods of treatment, according to several authorities, is due to removal of biologically active substances and regeneration factors or their adsorption from the blood [8]. From this aspect, the use of biological adsorbents based on cultured hepatocytes for extracorporeal methods of treatment of AHF ought not only to lead to the effective removal of toxins, but also to accelerate the processes of regeneration.

This paper gives the results of a comparative study of the metabolic properties of hepatocytes cultured in Petri dishes and on microcarriers, and also the results of the use of a biological sorbent based on these cells as artificial liver support system (ALSS) for rats with AHF.

Department of Cellular Biology, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 590-592, June, 1990. Original article submitted October 10, 1989.

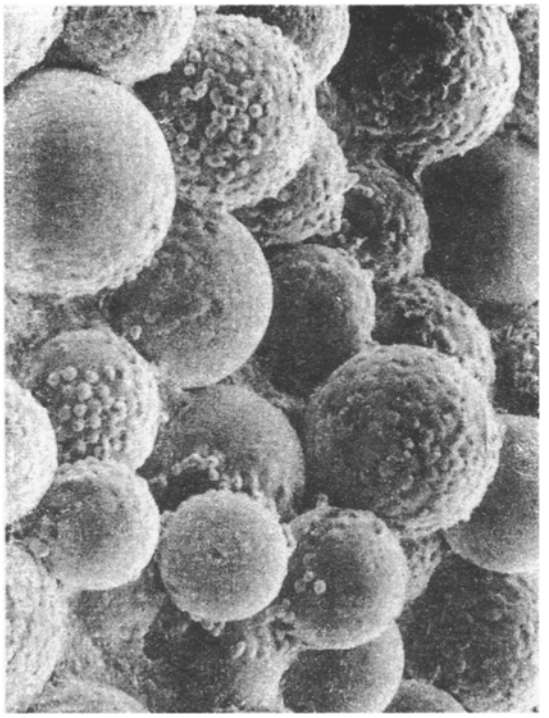

Fig. 1. Scanning electron microscopy of culture of rat hepatocytes on microcarriers 2 h after inoculation. 200×.

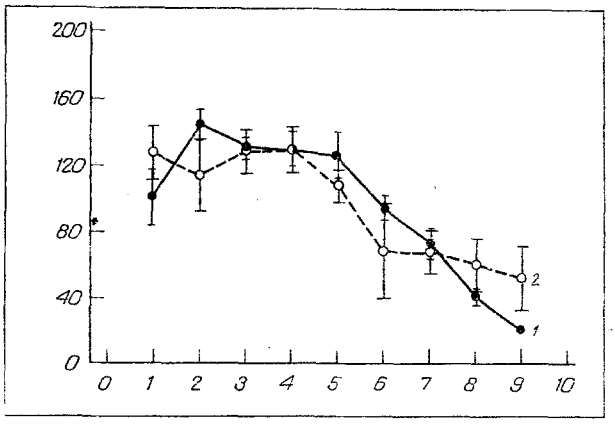

Fig. 2. Concentration of albumin secreted by hepatocytes in culture medium in Petri dishes (1) and on microcarriers (2). Abscissa, day of cell culture; ordinate, quantity of albumin secreted (in $\mu g/10^6$ hepatocytes in 24 h).

EXPERIMENTAL METHOD

Male Wistar rats weighing 300-350 g were used. The animals were kept on a standard laboratory diet in individual cages. The carotid artery and jugular vein were catheterized under superficial pentobarbital anesthesia (5 mg/kg body weight) [9]. AHF was induced in the rats by intraperitoneal injection of 2.5 g/kg of D-galactosamine ("Sigma," USA) or 3 ml/100 g of 8% CCl_4 solution (high purity grade, USSR) in castor oil. These doses caused death of 95-100% of the animals in the course of 40-48 h. Hepatocytes were isolated and cultured in Petri dishes by the method described previously [2]. Williams' E medium, containing 15 mM TES, 30 mM tricine, 20 mM HEPES, 10^{-5} M dexamethasone, 8 μg/ml insulin, and 100 μg/ml gentamicin ("Sigma").

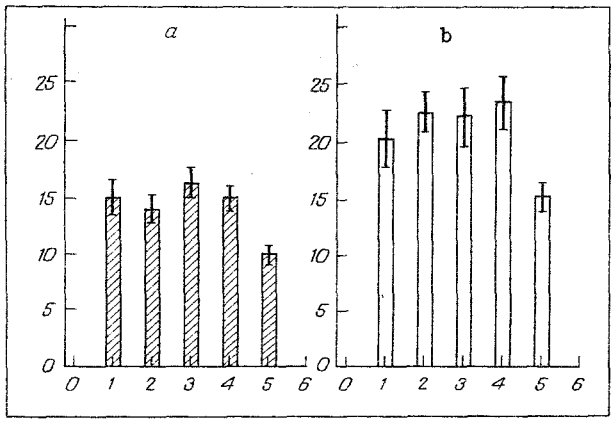

Fig. 3. Conjugation of bilirubin by hepatocytes cultured in Petri dishes (a) was on microcarriers (b). Abscissa, day of culture of hepatocytes; ordinate, rate of bilirubin conjugation (in $\mu g/10^6$ cells/h).

TABLE 1. Effect of Hemoperfusion Procedure through Biological Sorbents on Death of Experimental Animals with AHF

Exptl. conditions		Dying animals, %	
AHF	hemoperfusion	3rd day	7th day
CCl_4	Microcarrier	100(16/16)	100(16/16)
	Microcarrier with hepatocytes	18.2(2/11)	36.4(4/11/
D-galactosamine	Microcarrier	95(19/20)	95(19/20)
	Microcarrier with hepatocytes	33.4(4/12)	33.4(4/12/

Legend. Ratio of number of dying rats to total number of rats used in experiment given in parentheses.

A Biosilon microcarrier ("Nunc," Denmark), coated beforehand with human fibronectin at the rate of 5 $\mu g/cm^2$ of surface was used. To transplant the hepatocytes onto the microcarrier, 25 ml of previously oxygenated perfluorodecalin (PFD; from "Aldrich," USA), 1.5 × 10^8 hepatocytes, 5 ml of Biosilon, and 150 ml of culture medium were introduced into 250-ml culture vessels ("Techne," England). The cell culture vessel was placed on a magnetic mixer ("Techne") and the cells were incubated with continuous mixing at the rate of 5 rpm. After 2 h the PFD was removed and the medium replaced by 150 ml of fresh medium. The hepatocytes were then cultured during mixing at a speed of 25 rpm. Albumin secreted by the cells was determined by enzyme immunoassay [2]. To determine the rate of synthesis of bilirubin-glucuronide (BG) cells in culture were treated with bilirubin, stabilized in albumin solution [3]. The quantity of BG formed was determined as in [3]. The hemoperfusion procedure was carried out by the method described previously [9]. The minicolumn (2 ml) containing about 4 × 10^7 hepatocytes, cultured on the microcarrier, was used as the sorbent. Hemoperfusion through the column was carried out at the rate of 60 ml/h for 3 h.

EXPERIMENTAL RESULTS

Under the conditions of transplantation of the primary culture of hepatocytes on to the microcarrier developed as described above, the cells effectively adhered to the surface of the carrier. During the first 2 h of culture about 60% of the added cells interacted with the microcarrier and began to spread out into a monolayer (Fig. 1). The viability of the adherent cells was estimated by staining with 0.2% trypan blue, and it was not less than 80%. During the next 24 h the hepatocytes in culture completed their spread and acquired an epithelial morphology, which they maintained in culture for 7 days. The metabolic functions of the cells on the microcarrier were studied and compared with those of hepatocytes cultured under ordinary conditions in Petri dishes. The results of the investigation of synthesis and secretion of albumin are given in Fig. 2. Irrespective of the

conditions of culture, the hepatocytes maintained a stable level of albumin production for 5 days. The quantity of protein secreted in this period was about 120 $\mu g/10^6$ cells in the course of 24 h. On the following days of culture, protein secretion diminished, evidently reflecting processes of degradation and death of the primary cell culture. According to the results shown in Fig 3, the rate of synthesis of BG remained constant for 4-5 days of culture, and, expressed per 10^6 cells, amounted to 15 and 25 $\mu g/h$ for hepatocytes cultured in Petri dishes and on the microcarrier respectively. These data, together with the results of stable synthesis and secretion of albumin, are evidence that hepatocytes on the microcarrier maintain a high level of metabolism for 4-5 days and can be used as a sorbent for ALSS.

As a model of AHF in rats we used the hepatotoxins D-galactosamine and CCl_4 in doses causing death of 95-100% of the experimental animals in the course of 40-48 h. Table 1 gives the results of investigations in which the hemoperfusion procedure through a minicolumn with hepatocytes cultured on microcarriers was used in experimental AHF. The efficacy of the ALSS procedure was determined by the decrease in the acute and late mortality of the experimental animals (in %), estimated 48-72 h and 7 days respectively after injection of the hepatotoxins. According to the results, under these conditions only about 20% of rats receiving the lethal dose of CCl_4 died during the first 48-72 h, and about 40% died on the 7th day after injection of the toxin. For the model of AHF induced by Dpgalactosamine, the acute and late mortality did not exceed 40% in both cases. These results suggest that the proposed method of ALSS is sufficiently effective and does not depend on molecular mechanisms causing the development of the acute inflammatory process in the liver. Previous investigations showed that intraperitoneal injection of a suspension of liver cells [6], immobilized in alginate drops of hepatocytes [5], increased the survival rate of rats with AHF caused by injection of Dpgalactosamine. It must be pointed out, however, that by comparison with these methods, the procedure of ALSS now proposed has many advantages, based on the use of hepatocytes cultured on microcarriers. In the first place, hepatocytes on microcarriers are in direct contact with blood and can remove various metabolites and toxins which have accumulated during AHF from the bloodstream, whereas liver regeneration factors released during cell culture [5] can enter the bloodstream and thereby reach the affected organ. In the case of implanted liver cells, time is required for vascularization of the transplant [4]. Second, hepatocytes cultured on a microcarrier for 5 days preserve a high level of metabolic activity, and if used in clinical practice, this can solve the problem associated with preparation of the cell sorbent when needed.

LITERATURE CITED

1. A. F. Blyuger, Principles of Hepatology [in Russian], Riga (1975).
2. T. G. Vishnyakova and A. A. Shnyra, Biokhimiya, **54**, 694 (1989).
3. J. C. Annino and R. W. Giese, Clinical Chemistry: Principles and Procedures, Boston (1976).
4. Z. Cai and Z. Chi, Artif. Organs, **12**, 388 (1988).
5. A. A. Demetriou and A. Reisner, Hepatology, **8**, 1006 (1988).
6. L. Makowka and J. A. Falk, Science, **210**, 901 (1980).
7. L. Makowka and R. E. Folk, Surgery, **91**, 120 (1982).
8. Y. Nose, Artif. Organs, **12**, 291 (1988).
9. C. J. Ryan and J. M. Courtney, Hemoperfusion: Kidney and Liver Support and Detoxification, Washington (1980), pp. 27-36.
10. H. Sato and S. Teraoka, Artif. Organs, **7A**, 77 (1983).