regions depending on the degree of severity of the lesion suggest that proliferation and differentiation of CFU-GM in the subendothelium of the human aorta take place in early, mainly prefibrotic, stages of atherogenesis.

LITERATURE CITED

1. I. L. Chertkov and A. Ya. Fridenshtein, Cellular Bases of Hematopoiesis. Hematopoietic Precursor Cells [in Russian], Moscow (1977).
2. C. W. M. Adams and O. B. Bayliss, Br. J. Exp. Path., 57, 30 (1976).
3. S. R. Apparicio and P. Marsden, J. Microscop. (Oxford), 89, 139 (1969).
4. R. S. Ash, R. A. Detrick, and E. D. Lanjani, Blood, 58, 309 (1969).
5. D. W. Barnes and J. F. Loutit, Lancet, 2, 1138 (1967).
6. N. B. Everett and W. D. Perkins, Stem Cells of Renewing Cell Population, New York (1976).
7. J. C. Geer, H. C. McGill, and J. Strong, Am. J. Pathol., 38, 263 (1961).
8. J. C. Geer, Lab. Invest., 14, 1764 (1965).
9. D. A. Horwitz, A. C. Allison, P. Ward, and N. Kight, Clin. Exp. Immunol., 30, 289 (1977).
10. L. Jonasson, J. Holm, O. Skalli, et al., Arteriosclerosis, 6, 131 (1986).
11. D. Metcalf, Hemopoietic Colonies, Berlin (1977).
12. D. J. Moffat, C. Rosse, and J. M. Yoffey, Lancet, 2, 547 (1967).
13. S. E. Salmon and R. N. Buick, Cancer Res., 39, 1133 (1979).
14. E. L. Soboleva, A. I. Kolesnikova, A. G. Konoplyannikov, et al., Proceedings of Poster Communications, 7th International Symposium on Atherosclerosis, Melbourne (1985), pp. 126-134.
15. H. C. Stary, Atherosclerosis-8: Proceedings of the 6th International Symposium, Berlin (1983), pp. 462-466.

CORRECTION OF METABOLIC DISTURBANCES IN EXPERIMENTAL CIRRHOSIS OF THE LIVER BY CRYOSURGICAL DESTRUCTION AND PLASMA FLOW RESECTION

N. P. Mikaelyan, I. V. Stupin, and I. V. Sleta

UDC 616.36-004-092.9-02:615.832.9]-07

KEY WORDS: liver, cirrhosis; cryosurgical destruction; metabolic disturbances

In order to stimulate regeneration of the liver in cirrhosis, resection or cryosurgical destruction of part of the liver has been used successfully [5]. The possibility of using plasma resection has been demonstrated experimentally in liver surgery [4, 6, 7]. However, when large vascular trunks have to be divided, unless the liver is completely excluded from the circulation, bleeding is unavoidable. In addition, the incandescent gas flow of the plasma jet can cause embolism.

In the investigation described below, in order to eliminate complications during the operations we studied the possibility of liver resection in experimental animals by plasma flow after preliminary freezing of the resection line.

EXPERIMENTAL METHOD

Experiments were carried out on 36 Chinchilla rabbits weighing 3-3.5 kg. In six series of experiments the effects of cryosurgical destruction, plasma resection, and a combination of the two methods, used on the intact and cirrhotically changed liver were compared. Experimental cirrhosis of the liver was produced in rabbits by subcutaneous injection of 40% CCl_4 solution by the method in [8]. All operations on the liver were performed under

Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 604-607, May, 1989. Original article submitted May 10, 1988.

0007-4888/89/0005-0698$12.50

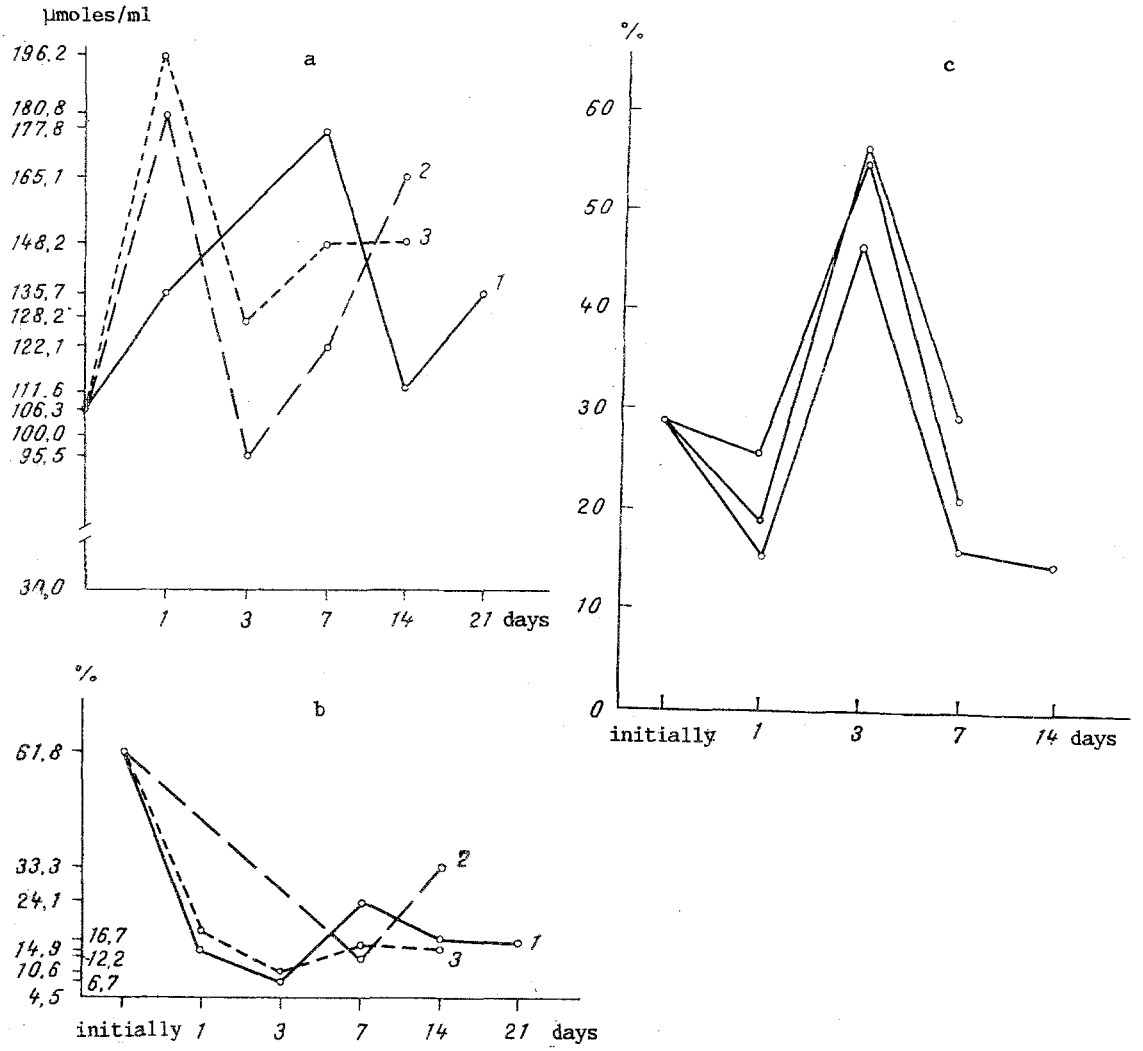

Fig. 1. Change in MDA concentration (a), serum AOA (b), and insulin-binding activity of lymphocytes (c) after different forms of intervention. 1) Plasma flow resection; 2) cryosurgery; 3) combination of cryosurgery and plasma flow.

hexobarbital anesthesia. Blood for biochemical testing was taken from the marginal vein of the rabbit's ear before the operation and on the 1st, 3rd, 7th, and 14th days after the operation. Concentrations of total protein and lipids, and glucose, and activity of alkaline phosphatase (ALP), creatine phosphokinase (CPK), and aspartate- and alanine transaminases (AST, ALT) were determined by kits from "Lachema," Czechoslovakia. Accumulation of malonic dialdehyde (MDA) and the antioxidative activity (AOA) of the blood serum, characterizing activity of lipid peroxidation (LPO) also were studied by the method in [3]. The effect of cryosurgery and plasma resection on reactivity of the cells was studied on models of lymphocytes relative to the number of insulin-binding sites on the plasma membranes by the method in [12], and expressed as a percentage.

EXPERIMENTAL RESULTS

Metabolic changes observed in the blood serum in the first week after the various operations on the intact liver may have been the result of compensatory or adaptive responses of the body to the various forms of injury, combining effects of different factors accompanying the operation. Moreover, the high blood enzyme levels may be based on the outflow of enzymes into the blood from various organs directly or indirectly involved in the process, and also from muscles traumatized during the operation. Evidence in support of these views is given by data in the literature, demonstrating increased activity of several enzymes (aldolase, transaminases, ALP) in the blood serum of animals during the first days after

ordinary resection of the liver, and restoration of their normal activity by the 10th-14th day [1, 9]. Metabolic disturbances (hypoglycemia, hypoproteinemia, an increase in the MDA concentration due to the effects of plasma, hyperglycemia, and hypoproteinemia associated with cryosurgical destruction), observed in the later stages of observation, after the use of the techniques of surgical treatment separately, canceled each other out when these factors were combined. The combined use of low temperatures and plasma resection was not accompanied by any further increase in severity of the inflammatory-destructive reaction of the liver tissue to trauma.

The experimental results indicate that after injection of the hepatotropic poison there was a sharp rise in the MDA concentration (from 35.13 ± 1.14 to 106.3 ± 5.66 μmoles/ml; $p < 0.001$), characterizing the state of LPO (Fig. 1a) and a decrease in AOA of the blood serum (from 61.8 ± 9.3 to 36.5 ± 7.1 μmoles/ml; $p < 0.05$; Fig. 1b). Transaminase activity also was increased (ALT by 2.5 times and AST by 1.3 times; $p < 0.05$) and hyperglycemia was observed: the glucose concentration rose from 3.8 ± 0.6 to 6.1 ± 0.9 μmoles/liter ($p < 0.05$).

It will be clear from Fig. 1a that with all forms of intervention on the cirrhotic liver, LPO was activated ($p < 0.05$) at all times of observation. After cryosurgical destruction of 10% of the weight of the organ in animals with experimental cirrhosis of the liver, transaminase activity returned to normal, whereas when plasma flow resection was used alone or in combination with cryosurgical destruction, transaminase activity rose considerably in the first week after the operation, after which it showed a tendency to return to normal. However, the MDA concentration in all cases remained high even 3 weeks after the operation; the serum AOA under these circumstances was reduced by more than half ($p < 0.002$).

Consequently, metabolic changes in intact animals in the late stages after intervention returned to normal, and a combination of plasma flow resection and low temperatures was not accompanied by any increase in the intensity of inflammatory and destructive reactions of the liver to trauma. Under the same conditions of intervention on the cirrhotic liver, free-radical lipid oxidation was intensified. Free-radical lipid oxidation is known to continue without interruption in all tissues of living organisms, and it is one type of normal metabolic process. Products of free radical oxidation exert their effect on enzyme activity, change the chemical composition, physical properties, permeability, and structure of biological membranes and, in turn, this is reflected in processes of cell metabolism and plays an important role in normal vital functions [2]. Meanwhile disturbance of regulation of free-radical oxidation is an important step in the pathogenesis of various diseases (including in cirrhosis of the liver). The combined use of cryosurgery and plasma resection, as will be clear from Fig. 1a, does not abolish free-radical lipid oxidation, and this must be taken into account in surgical practice.

The study of the state of the insulin receptors in the plasma membranes of lymphocytes showed that 24 h after cryosurgery and resection, in conjunction with the action of the plasma flow, the sensitivity of the cells to insulin was significantly reduced (specific insulin binding with lymphocytes amounted to 15.7% compared with 28.9% initially; $p < 0.005$; Fig. 1c). On the 3rd day there was a regular increase in the insulin-binding activity of the lymphocytes, which is a feature of the inflammatory process. By the end of the 7th day the reactivity of the cells to insulin was again reduced. Negative correlation was found between specific binding of insulin with lymphocytes and the plasma glucose level ($r = -0.3$). With plasma flow must therefore be combined with pharmacological correction and, in particular, with administration of antioxidants and of substances strengthening cell membranes.

No significant differences were found between changes in the remaining parameters studied.

LITERATURE CITED

1. A. S. Borovkov, A. F. Blyuger, and G. E. Vinogradov, Methods of Investigation in Surgical Diseases of the Liver [in Russian], Moscow (1973), pp. 169-179.
2. E. B. Burlakova, Bioantioxidants in the Regulation of Metabolism under Normal and Pathological Conditions [in Russian], Moscow (1982).
3. B. I. Gubskii, Biochemistry of Animals and Man [in Russian], No. 2, Kiev (1978), pp. 72-84.
4. V. S. Savel'ev and L. A. Serykh, Vest. Khir., No. 1, 7 (1986).
5. B. P. Sandomirskii, N. I. Kots'ko, and L. N. Zolochevskaya, Vest. Khir., No. 4, 24 (1979).
6. O. K. Skobelkin, E. I. Brekhov, G. D. Litvin, et al., Khirurgiya, No. 5, 75 (1987).

7. I. V. Stupin, V. S. Volkoedov, A. S. Bereskov, and I. M. Sapelkina, Acute and Chronic Lesions Due to Cold. Thromboembolism of the Pulmonary Artery [in Russian], Moscow (1982), pp. 171-172.
8. L. S. Rubitskoi and N. R. Korotkina, Byull. Éksp. Biol. Med., No. 7, 122 (1960).
9. Sh. Ya. Shelegiya and É. M. Tsutseashvili, Enzymes in Laboratory Diagnosis [in Russian], Moscow (1973), pp. 74-75.
10. W. G. Cahan, Fed. Proc., 24, 241 (1965).
11. G. S. Cooper, Fed. Proc., 24, 237 (1965).
12. P. Freychet, J. Roth, and D. M. Neville, Jr., J. Biol. Chem., 249, 2249 (1974).

EPIDERMIS AS A GRAFT

A. A. Ostrovskii

UDC 615.361.771.03:617-089.843].076.9

KEY WORDS: full-thickness skin defect; epidermis; transplantation

In the study of the morphogenetic properties of the transplanted epidermis, in some cases after excision of a skin graft by means of a dermatome it is placed in medium containing trypsin, where after a short time the cutaneous epithelium is separated mechanically from the dermis [2, 3]; in other cases, to obtain an epidermal graft, small pieces of skin are cultured in vitro until the formation of epithelial growths [7] or until the formation of a single sheet of epithelial cells [5, 6]. The third method is associated with division of pieces of skin into cells with the aid of trypsin, followed by culture either of the whole cell suspension [1] or of the subsequently isolated population of keratinocytes [10], until a single epithelial sheet is obtained. A relatively simple method of separation of the epidermis from the dermis, eliminating the need to culture cells of the cutaneous epithelium in vitro and (or) to treat them with trypsin, also is known [9]. This method is based on exposure of an area of skin to a partial vacuum. Under these circumstances all the layers of the epidermis become separated together with the cells of the stratum germinativum, whereas the basement membrane remains attached to the dermis [4]. The viability of the separated epidermis has been proved by the study of its morphological and histochemical properties [8].

The aim of this investigation was to study the possibility of using epidermis, separated from the dermis by exposure of an area of skin to a partial vacuum, as grafting material.

EXPERIMENTAL METHOD

Autografting of the epidermis was carried out on noninbred female laboratory rats weighing 200-240 g. As a first step, under ether anesthesia a bed was prepared to receive the graft, for which purpose the rat's hair was shaved in the region of the upper third of the spine and the skin was treated with alcohol. A circular incision 2 cm in diameter was made in the interscapular region down to the subcutaneous fatty areolar tissue. By means of two rows of U-shaped sutures, the base of a chamber shaped like a hollow truncated cylinder was sutured to the outer edge of the incision. The circular skin flap remaining inside the base was then removed. As a result, a fullthickness skin defect was formed on the surface. A gauze pad, soaked in nitrofurazone solution, was placed on it. A lid was fitted above the base of the chamber and fixed by means of a strong rubber band. After 24 h, the donor area was prepared under ether anesthesia. The hair was pulled out on the animal's right side and the fur accurately shaved. The skin was washed with soap and water and dried. The chamber was placed on the prepared area, with a hole 2 cm in diameter, covered with stretched Kapron gauze with a mesh of 0.5 × 0.5 cm, and was placed in contact with the skin. Air was withdrawn from the chamber for 45 min, gradually reducing the pressure during the first 15 min to -0.6 kg/cm^2, and thereafter maintaining it at that level for the remainder of the time. During exposure to the negative pressure, the skin of

Department of Biology, Grodno Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 5, pp. 607-609, May, 1989. Original article submitted April 5, 1988.

0007-4888/89/0005-0701$12.50