9. M. Colic and A. Dujic, Period. Biol., **85**, Suppl. 3, 47 (1983).
10. A. J. Dunn., J. Receptor Res., **8**, No. 1-4, 588 (1988).
11. J. Linder, Arch. Path. Lab. Med., **111**, No. 12, 1118 (1987).
12. D. D. F. Ma, A. N. Ho, and A. V. Hoffrand, Clin. Exp. Immunol., **55**, No. 2, 273 (1984).
13. P. L. Osheroff, Cell. Immunol., **60**, No. 2, 376 (1981).
14. W. Savino, M. Dardenne, and J. F. Bach, Clin. Exp. Immunol., **52**, No. 1, 7 (1983).
15. W. Savino, M. Dardenne, C. Marche, et al., Am. J. Path., **122**, No. 2, 302 (1986).

EXPERIMENTAL MORPHOLOGICAL EVALUATION OF WELDING OF LYMPHOVENOUS AND VENOVENOUS ANASTOMOSES BY CO_2 LASER WITH FLEXIBLE LIGHT GUIDE

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KEY WORDS: lymphatic vessel; anastomosis; CO_2 laser; welding.

Microvascular surgery is a rapidly developing field. Microvascular anastomoses are nowadays usually formed by means of vascular suture. When anastomoses of very small diameter are created, a frequent complication is stenosis followed by thrombosis of the anastomoses due to hyperplasia of the intima at the site of insertion of the foreign body (the suture material). One way of improving the technique of anastomosis is by using the laser beam to weld the edges of the vessels. An experimental and clinical investigation of various sources of laser radiation showed that they were effective for ensuring vascular union [1-7]. An important limitation of the use of the CO_2 laser has hitherto been the absence of any suitable system for transmitting and aiming the radiation and maintaining constant power density. A moveable mirror and ball light guide is not suitable because it does not guarantee the maintenance of a constant distance to the tissue, and correspondingly, a constant power density. Circular welding with aiming of the laser beam through a microscope is unsuitable because of the impossibility of completely rotating the vessels.

The aim of this investigation was to develop a method of creating lympho- and venovenous anastomoses with the aid of radiation from a CO_2-laser, conducted along a flexible light guide.

EXPERIMENTAL METHOD

Experiments were carried out on 13 male and female dogs weighing from 13 to 24 kg. To form lymphovenous and venovenous anastomoses, vessels of both the superficial and the deep lymphatic and venous systems were used. The diameter of the anastomosed vessels was from 2.5 to 3.5 mm for veins and from 0.6 to 1.2 mm for lymphatics. The operations were performed under sterile conditions in operating theaters, under general anesthesia. After an adequate depth of anesthesia had been achieved, 2 ml of methylene blue was injected into the soft tissues of the dog's hind limb, after which the skin covering the aponeurosis was divided through a longitudinal incision along the medial surface of the thigh, below Poupart's ligament, and the

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vessels were exposed. The lymphatics in this case were colored with dye. Lymphatics with a diameter of 0.6 mm or more were regarded as suitable for lymphovenous anastomosis. All lymphovenouses were of the end-to-side type, and only one venovenous anastomosis was of the end-to-end type. The operations were performed with the aid of microsurgical instruments and a magnifying glass (×4). After choice of the region for the anastomosis the vein was taken up on a holder and clamped with a hemostat distally to the site of the proposed anastomosis. The veins were then dissected over a distance of 2-3 mm and part of their adventitia was removed. An oval-shaped venotomy hole was made in the vein, its diameter about equal to the diameter of the lymphatic. During manipulations of the lymphatic, it was not stretched. In some cases, when technically possible, the aventitia of the lymphatic was removed. The edges of the venotomy hole were apposed to the edges of the afferent end of the divided lymphatic. The end of the light guide, 0.5 mm in diameter, was then applied to the edges of the vessels and welding was carried out. In our opinion, the optimal site for lymphovenous anastomosis is the region of the vein below a valve. An important factor in the formation of an anastomosisis manipulation of the lymphatic when filled with lymph. Particular attention must be paid to ensuring that the walls of the vessels are contained by the spot of the laser beam, depending on their thickness. If a lymphovenous anastomosis is formed, the spot of the laser beam must include the vessel walls, when apposed, as follows: one-third of the wall of the lymphatic and two-thirds of the wall of the vein, and in the case of a venovenous anastomosis, with walls of equal thickness, to spot in the case of a venovenous anastomosis, with walls of equal thickness, the spot of the laser beam must include the walls of both vessels at and equal distance. If there is a greater difference in thickness of the walls of the apposed vessels, only the surgeon's experience can predict the direction which the spot of the beam will move. Latency of the anastomoses was tested by pressing the vein and injecting methylene blue into the lymphatic distally to the anastomosis. On the basis of the results obtained, the power of welding of 0.15-0.25 W, a distance 1.5-2.0 mm from the light guide to the welded tissues, and an automatic continuous mode with a duration of action of the laser of 1 sec must be taken as optimal parameters. The duration of action of the laser for welding one lymphovenous anastomosis averaged 10-15 sec.

The dogs were killed 3, 6-8, and 13-16 days and 1 year after the operation. The anastomoses were fixed in 10% formalin solution and embedded in paraffin wax. Due to the small size of the anastomoses and, in some cases, to the development of sclerotic changes around them also, serial sections cut to a thickness of 7 μ were studied. The sections were stained with hematoxylin and eosin, by Van Gieson's method, and with "Fenaf" stain for elastic tissues.

EXPERIMENTAL RESULTS

Nonpatency and stenosis of the anastomoses and the development of aneurisms within their region and along the course of the lymphatic and venous vessels were not observed. The study of histological preparations at all times of the experiment showed that the vessel walls were directly apposed and the layers of the walls of one vessel appeared to be the continuation of walls of the other vessel (Fig. 1). The endothelial lining was continuous in character both along the course of the vessels and along the anastomosis line (Fig. 1b). Muscle cells along the anastomosis line preserved their normal structure. In some cases the region of the anastomosis differed from other areas of the walls by a rather higher concentration of collagen fibers. This was seen particularly clearly in the region of the acute angle of the anastomosis, and must be attributed to technical difficulty of welding in this zone. This same factor also lies at the basis of the hyperplasia of the intima, discovered in two cases, with the formation of a pillow-shaped prominence (Fig. 1c). Connective-tissue and elastic carcass of the vessels preserved their usual structure and direction. Necrosis of the walls of the lymphatics due to the action of laser radiation was not found. It was observed only in two cases in the walls of veins, where thrombosis of the vessels later developed. Incorrect apposition of the vessel walls with the formation of "pseudovalvular" structures was found in two cases of lymphovenous anastomoses on the 6th and 8th days of the experiment. Embolism of a main vein of the paravasal cellular tissue, due to penetration of the paravasal cellular tissue through the venotomy hole into the lumen of the vein, also was found in two cases on the 8th and 13th days. The cellular tissue remained viable because of the vessels supplying it. Thrombosis of the lumen of the lymphatic vessels, unconnected with the action of laser radiation on their wall, was observed in three cases on the 8th day. It developed because of mechanical injury, as shown by the absence of coagulation necrosis of the walls. Nonpatency of the anastomoses occurred in three lymphovenous and one venovenous anastomosis, which was of the end-to-end type, and was due in all cases to thrombosis of both anastomosed vessels. Sclerotic changes in the paravasal cellular tissue, visible as early as the 6th day, were associated mainly with inflammatory changes in the surrounding tissues. Sclerosis and hypertrophy of the walls of the anastomosed vessels were not found at any time of the investigation (Fig. 1d). The positive results observed in the late period of the experiment can be attributed to skill acquired in the technique of microsurgical welding of lymphatics and veins. Adequate welding, insuring accurate apposition of the edges of the vessel walls, not causing dystrophic or necrotic changes in them or scar formation along

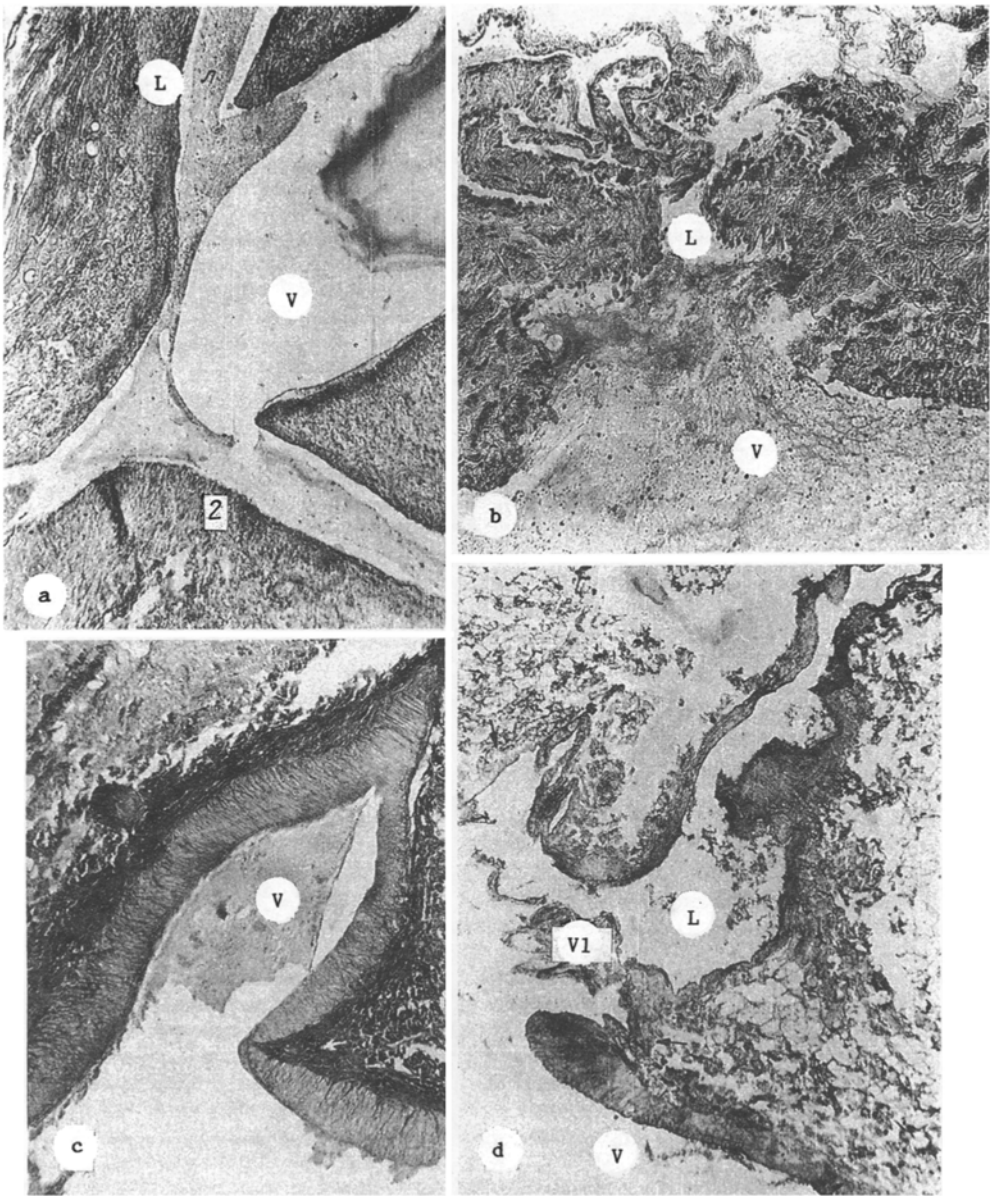

Fig. 1. Lympho- and venovenous anastomoses at different times of investigation. a) Two anastomoses on one vein, 3rd day. 1) Lymphovenous, 2) venovenous anastomosis. L) Lymphatic, V) vein (here and in Fig. 1b-d). Layers of walls of one vessel appear to be continuation of walls of the other vessel. Endothelium can be traced throughout its length. Hematoxylin and eosin (here and in Fig. 1b, d); 24× (here and in Fig. 1c, d); b) lymphovenous anastomosis, 13th day. Walls of lymphatic and venous vessels preserve their normal structure. Region of welding (arrows) without signs of proliferation of connective tissue. Walls of anastomosis on luminal aspect covered with juicy endothelium. 100×; c) Venovenous anastomosis, 6th day. Course of collagen fibers in adventitia of vessels is circular in character. In region of acute angle of anastomosis (arrow) a fibrous plaque can be seen on the intimal aspect, with penetration of collagen fibers from adventitia to intima, enabling region of welding of vessels to be clearly visualized. Van Gieson's stain; d) lymphovenous anastomosis, 1 year. Walls of lymphatic and venous vessels thin, with no evidence of sclerosis or hypertrophy. No scar tissue present at site of apposition of vessels (arrow). Surrounding cellular tissue shows no sign of sclerosis. Vl) Valve.

the line of the anastomosis, but guaranteeing the integrity of the endothelial lining in the vessels along their course and in the zone of the anastomoses at all times of the experiment, testifies to the correct choice of parameters of the operation and the

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

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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.

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