LONG-TERM TRANSPLANTATION OF THE NEONATAL UMBILICAL VEIN: AN EXPERIMENTAL MORPHOLOGICAL STUDY

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UDC 616.149.8-053.31-089.843-091+
615.361.149.8.07

KEY WORDS: bioprostheses; umbilical vein; electron microscopy.

The choice of material for artificial replacement of blood vessels is nowadays quite extensive. The best solution is to use an autograft or homograft with ability to preserve the structures of the vessel wall for a long time. The use of an autologous vein as graft in some cases may be difficult, first, because of the need to perform an additional operation in patients with various kinds of vascular diseases and, second, because pathological changes leading to rapid failure of the graft are often present in autologous veins. Consequently the attention of investigators has been drawn to the possibility of large-scale use of the vein of the neonatal umbilical cord [2]. The definite immunologic "areactogenicity" of this graft and its weaker tendency to undergo thrombosis compared with autologous veins [6, 8] have been noted by investigators. A morbid anatomical investigation of bioprostehses of umbilical veins in patients dying from intercurrent diseases 3-4 years after transplantation of the umbilical veins has demonstrated good adhesion of the bioprostheses with arteries [3].

However, the use of untreated native umbilical bioprostheses is accompanied, although to a lesser degree than when autologous veins are grafted, by changes in the umbilical vein characteristic of the rejection reaction: thrombosis of the lumen of the vein, necrosis of the graft wall, aneurysm development, and so on [5, 7]. The use of native umbilical bioprostheses for transplantation has thus not been adopted. Bioprostheses are used nowadays only if treated with substances which reduce their immunologic reactivity [4, 5, 9]. In particular, the "Biograft" umbilical prosthesis, produced by the firm of "Meadox," which is treated with 2% glutaraldehyde solution [5], is being used on quite a wide scale. However, this method does not guarantee integrity of the structures of the umbilical vein. The development of new methods of treating the bioprosthesis, with preservation of the structures of the vascular wall, is therefore an important problem.

This paper describes a comparative morphological investigation of bioprostheses of the umbilical vein and of bioprostheses obtained by the method of Kuzin et al. [1].

EXPERIMENTAL METHOD

A vascular graft of the human umbilical vein was used in experiments on 39 male dogs. Under ether and oxygen anesthesia part of the external iliac artery, 12 cm long, was removed from the animals and replaced by a bioprosthesis made from the umbilical vein, from which only the outer layer of Wharton's jelly was removed. The bioprosthesis was fixed externally with synthetic gauze. The bioprosthesis was excised 24 months after transplantation, and fixed in 10-15% neutral formalin buffered by Lillie's method. Pieces excised from it were embedded in celloidin and paraffin wax. Sections were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, with fuchselin by Weigert's method, for fibrin by Shueninov's method, and by the Gram–Weigert method. Part of the native material for electronmicroscopic investigation was embedded in a mixture of Epon and Araldite, after fixation in 2% glutaraldehyde solution and 1.5% OsO_4 solution. Ultrathin sections were stained with lead citrate and examined in the JEM-100B electron microscope. A segment of native umbili-

Department of Pathological Anatomy and Department of Vascular Surgery, and Tissue Conservation Group, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 485-489, October, 1985. Original article submitted January 18, 1985.

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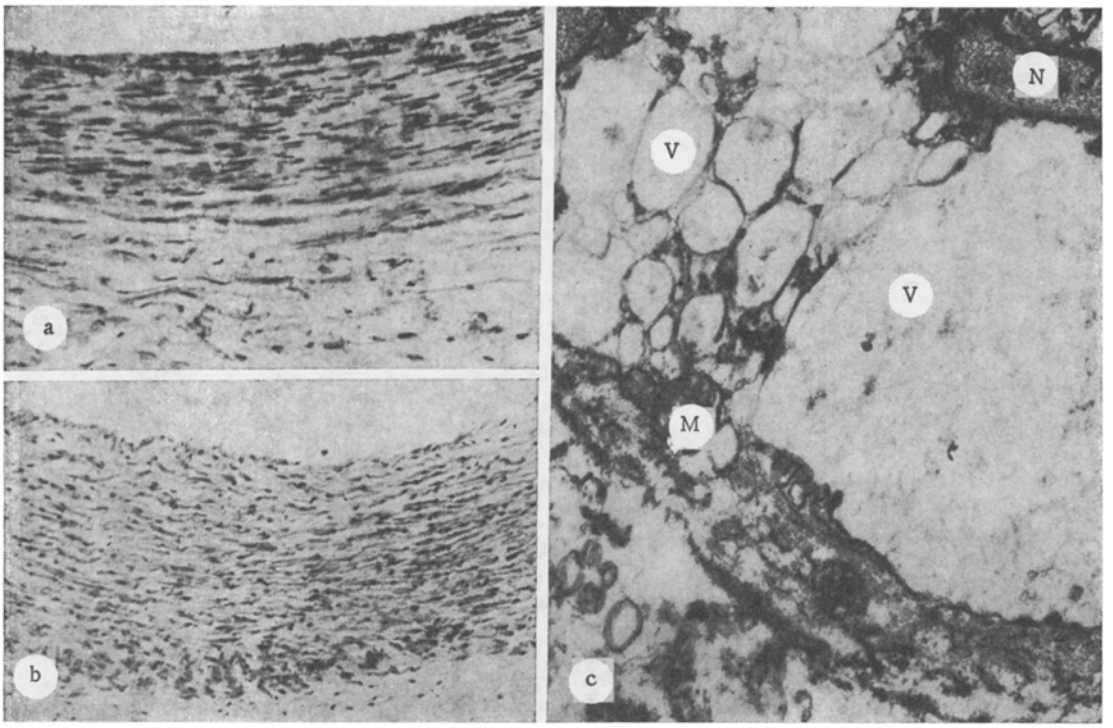

Fig. 1. Structure of umbilical vein and of "Biograft" bioprosthesis: a) preparation of native umbilical vein — muscular layer of vein is thin, adventia poorly developed, vein surrounded by a wide layer of Wharton's jelly. Hematoxylin and eosin, 900 ×; b) specimen of "Biograft" bioprosthesis. Vein wall friable. Hematoxylin and eosin, 90 ×; c) fragment of "Biograft" bioprosthesis — vacuolation of cytoplasm of myocyte with many small and large vacuoles (V), and with destruction and homogenization of myocyte mitochondria (M). N) Nucleus of myocyte. 24,000 ×.

icus and preparations of the "Biograft" bioprosthesis were used as controls for the histological and electron-microscopic investigation.

EXPERIMENTAL RESULTS

The control preparation of native umbilical vein had the ordinary structure of a thin-walled vein with thin intima, a moderately pronounced media, and poorly developed adventia, surrounded by a wide layer of amorphous Wharton's jelly. The electron-microscopic investigation showed that the media of the vein is formed by a large number of smooth-muscle cells, with elastic fibers arranged on their surface. Collagen fibers were mainly gathered into bundles and were distinguished by clear longitudinal and transverse striation (Fig. 1a). Histological investigation of control specimens of "Biograft" bioprostheses showed that the vein wall was somewhat edematous, friable, and surrounded by amorphous Wharton's jelly (Fig. 1b). However, electron-microscopic investigation revealed much more profound changes in the media of the vein. Destruction of smooth-muscle cells was observed in the media, with marked vacuolation of the cytoplasm of the mycoytes and homogenization of the mitochondria. This is evidence of considerable structural changes in the "Biograft" bioprosthesis as a result of conservation (Fig. 1c).

Histological investigation of preparations of the umbilicus treated by the method of Kuzin et al, 18-24 months after the beginning of the experiment, showed complete preservation of the structures of the intima and media of the vein. The adventia of the vein and the Wharton's jelly surrounding it were sclerosed. In the sclerosed layer of Wharton's jelly surrounding the vein inclusions of fatty areolar tissue and foci of lymphoid cells could be seen. In some places small foci of lymphoid cells were observed in the substance of the outer layers of the hypertrophied muscular coat of the vein. Cellular infiltration of this kind is evidently a manifestation of the reduced response of the experimental animals to the graft (Fig. 2a).

The formation of quite numerous arteries and veins of small and medium caliber, with hyperplasia of the intima and hypertrophy of the muscular layer in the outer layers of the

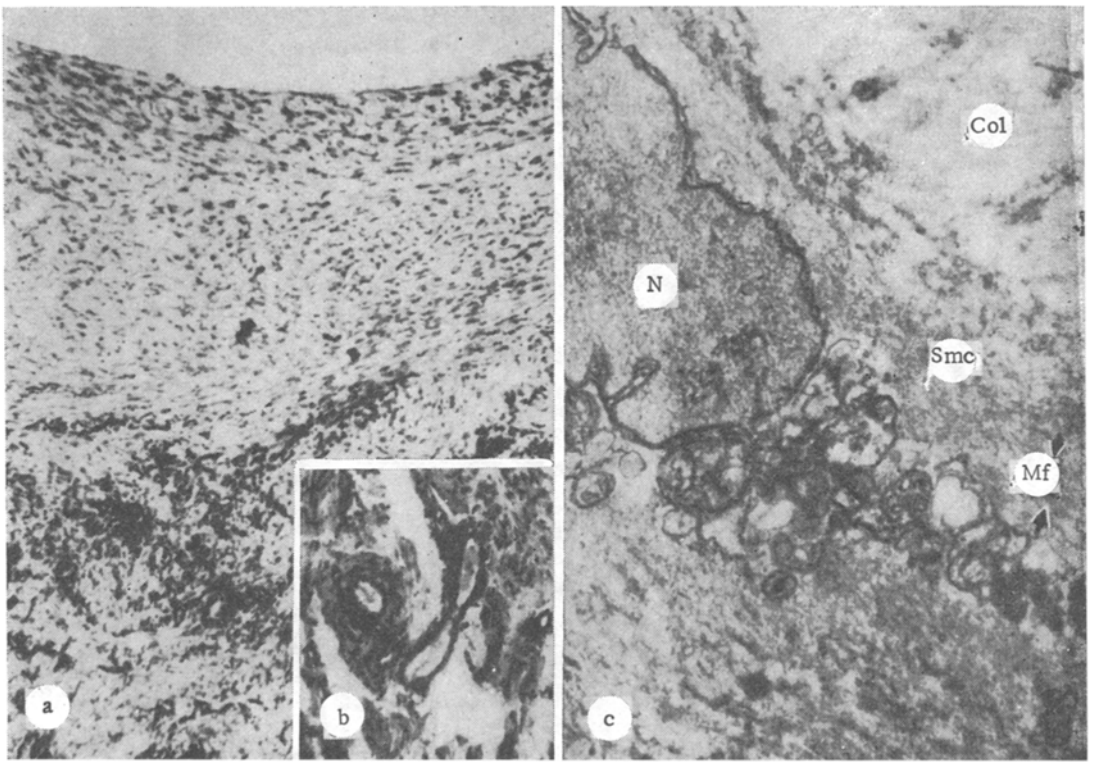

Fig. 2. Structure of graft of umbilical vein 24 h after beginning of experiment: a) vein wall thickened, pronounced muscular layer, adventia and periadvential tissue wall developed and contained foci of lymphoid cells. Hematoxylin and eosin, 90 ×; b) vessels of different calibers in periadvential tissue of umbilical vein. Hematoxylin and eosin, 160 ×; C) ultrastructure of muscular coat of vein graft: smooth-muscle cells (Smc) with unchanged nuclei (N), mitochondria (M), and myofibrils (Mf) arranged among bundles of collagen fibers (Col). 24,000 ×.

adventia and in the substance of the Wharton's jelly was highly characteristic. A system of powerful collaterals, due to adaptation of the transplanted venous bioprosthesis to a blood flow of arterial type evidently takes place in this situation (Fig. 2b).

Electron-microscopic investigation of the vein wall showed that the ultrastructure of the vein also was completely preserved. In the media of the vein, large smooth-muscle cells with unchanged, often hypertrophied and enlarged nuclei, with numerous mitochondria containing many mitochondrial cristae and myofibrils, were distributed among the numerous bundles of elastic fibers and infrequent bundles of collagen fibers. Many of the mitochondria were enlarged (Fig. 2c).

Morphological manifestations of transplantation immunity were observed in the bioprosthesis in 10 animals. In six cases large concentrations of lymphoid cells, focal edema of the intima and destruction of the endotheliocytes, and foci of necrosis of the intima were seen in the adventia of the vein and in the substance of the muscular layer. In four cases necrosis of the greater part of the intima of the vein and of part of the muscular coat next to the intima was observed. The boundaries of the necrotic part of the vessel wall were often indistinct and it was possible to draw a sharp line around the zone of necrosis only after staining by Shueninov's method — the necrotic tissue was permeated by fibrin. In other parts of the bioprosthesis, necrotic intima and part of the media were founded by a barrier formed from lymphocytes. Many clusters of lymphocytes were observed in the sclerosed adventia and around the numerous remnants of kapron gauze in the perivascular tissue (Fig. 3a). The preserved muscular coat or its outer part usually consisted of a layer of muscle fibers. Here and there on the necrotic intima fresh juxtamural thrombi consisting of accumulations of fibrin with no signs of organization were visible (Fig. 3b).

In none of the animals was an obturating thrombus of the vein biograft or a thrombus with signs of organization observed. This morphological picture is evidence that necrotic and thrombotic processes developed in the vessel wall shortly before the material was taken for histological investigation.

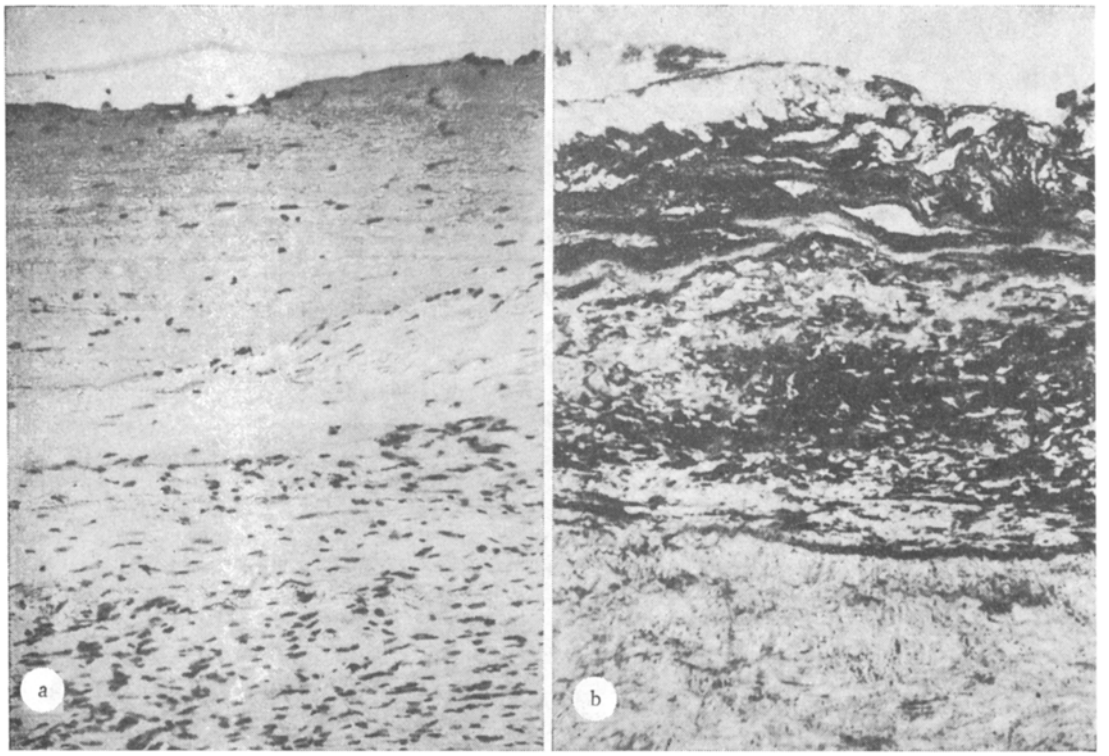

Fig. 3. Structure of modified vein 24 months after transplantation: a) necrosis of intima and of part of muscular coat of vein. Hematoxylin and eosin. 90 ×; b) juxtamural thrombus on area of intima of grafted vein that is necrotic and permeated by fibrin. Stained by Shueninov's method for fibrin. 90 ×.

The method of processing of the umbilical vein bioprosthesis used in these experiments, incidentally, was directly responsible for some of its morphological changes. Treatment of the bioprosthesis by the suggested method resulted in most cases in complete morphological preservation of the structures of the vein wall. This is confirmed by the results of histological investigation and of electron-microscopic study of the ultrastructure of the biograft.

The time course of the morphological changes at all stages of the experiment (until 24 months) was characteristic of adaptive processes observed in vein walls when the blood flow is modified and also during transplantation of veins. The important role of Whartons' jelly for preserving the structure of the venous graft and preventing thrombosis of the graft and necrosis of its wall is beyond question. In four cases when the most marked morphological changes were observed in the graft, the outer layer of Wharton's jelly had been removed most radically. Formation of new collateral vessels was not observed in the perivenous tissue at any of these times. This is a problem which requires further study, other considerations apart, because according to data in the literature [3], after transplantation of an umbilical vein biograft from which the Wharton's jelly had been removed, proliferation of collateral vessels was not observed in the preparation. It must also be noted that in cases when replacement of the intima by connective tissue was observed, thrombosis of the vein was not found in the preparation and its patency as a graft was preserved.

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