THE USE OF FREEZE-DRIED ARTERIES AS ARTERIAL VASCULAR PROSTHESES

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Despite much research into the development of vascular prostheses, the best solution to the problem as yet is to use biological living material, such as autologous veins or arteries [2, 5, 11]. The advantages of these vascular prostheses, ensuring good late results of their use, include rapid regeneration of the endothelium, maintenance of the mechanical strength of the vessel wall, combined with preserved elasticity. However, the use of autologous vessels as plastic material is not always possible (repeat operations, a loose type of structure of the vessel, involvement in a pathological process). In these cases synthetic prostheses or biological nonviable prostheses are usually used, to serve as a supporting structure into which the body's own cells can penetrate. They all possess several important disadvantages which make the replacement operation less effective, especially in the late period and in the case of replacement of a medium-sized or small artery [6, 14]. Modern methods of freeze-drying make it possible to obtain biological traits which have preserved their viability and biological properties, despite a long period of keeping [3, 8, 10, 12, 13]. The use of freeze-dried vessels as biological prostheses encourages the hope that the advantages of fresh biological prostheses can be maintained. Another advantage is that after freeze-drying the immunogenic properties of the object are evidently depressed [3, 4, 9], suggesting that allogeneic material can be used as a prosthesis.

The aim of this investigation was accordingly to determine the suitability of freeze-dried allogeneic arteries of small and medium caliber for use as a biological prosthesis during plastic repair of an artery. The arteries were chosen on physiological grounds, and also allowing for the development of degenerative changes in the wall of veins implanted into the arterial bed [7].

EXPERIMENTAL METHOD

There were two series of experiments. In series 1, part of the left renal artery 1 cm long was resected in 40 chinchilla rabbits weighing 3-4 kg, and in its place a freeze-dried allogeneic rabbit artery was fitted and sutured. In the 2nd series, in 20 mongrel dogs weighing 14-28 kg part of the femoral artery 4-5 cm long was resected and in its place a freeze-dried femoral or carotid artery obtained from an allogeneic dog was sutured. The artery was freeze-dried by the method described in [1] with certain modifications. Arteries removed and washed to remove blood were immersed in a cold (+8 to +10°C) cryoprotective solution, consisting of a solution of NIITiIO-2* for kidney

*Abbreviation for Research Institute of Transplantation and Artificial Organs.

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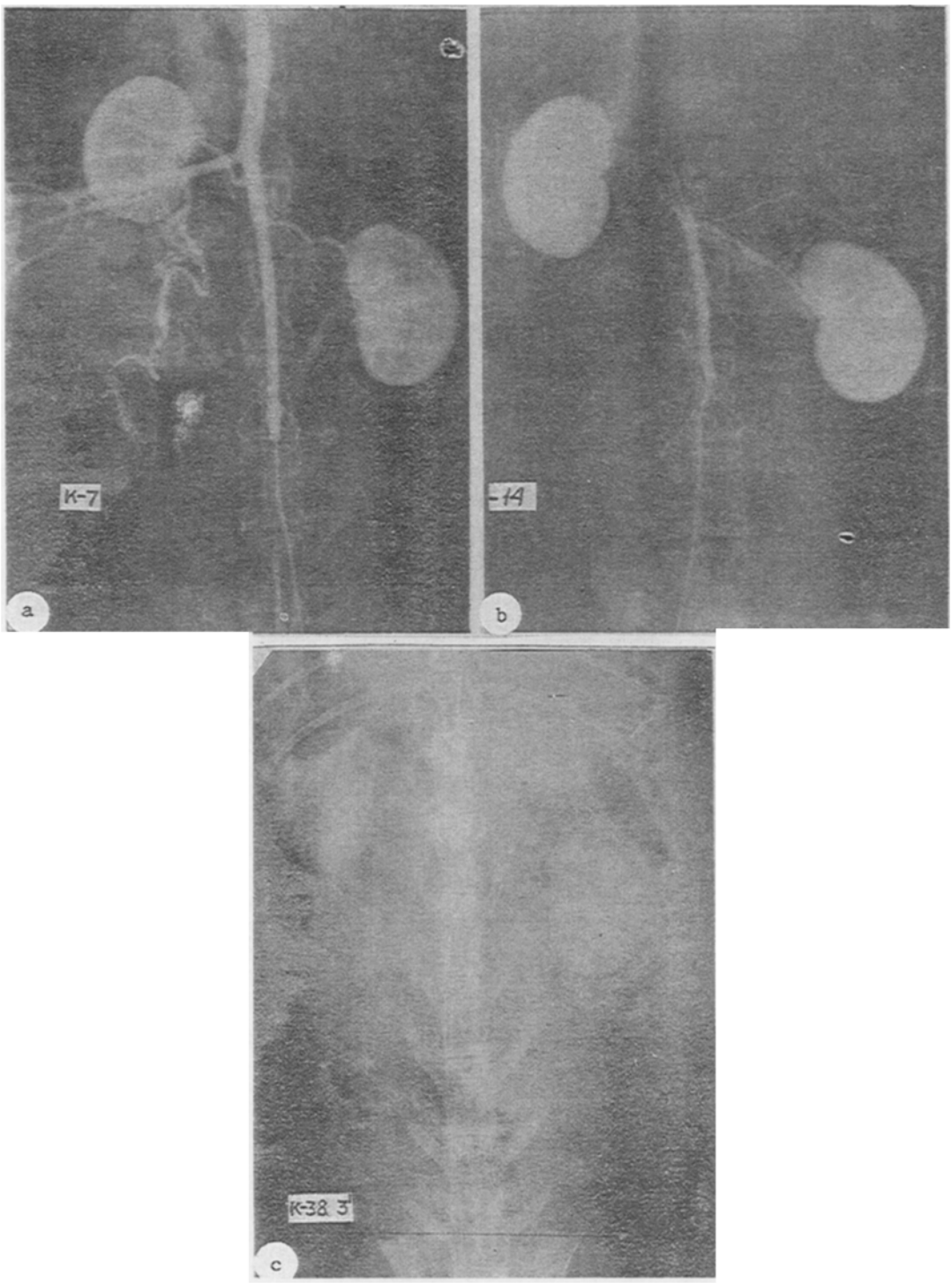

Fig. 1. Results of roentgenologic investigation of rabbits after replacement of left renal artery by a prosthesis: a) angiographic picture 2 months after fitting prosthesis. Prosthesis patent, corresponding in size to recipient's own artery; b) angiographic picture 3 months after fitting prosthesis. Moderate dilatation of prosthesis; c) excretory neurography 3 months after fitting prosthesis (the same rabbit as in Fig. 1b). Excretory function of left kidney preserved.

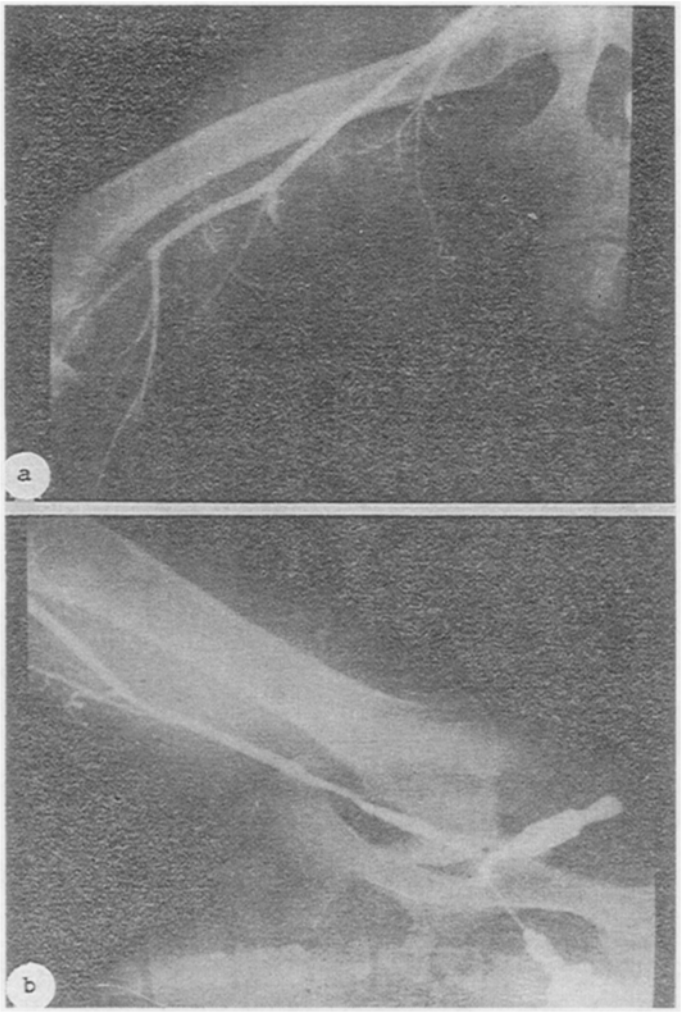

Fig. 2. Results of angiographic investigation of dogs after replacement of femoral artery by a freeze-dried graft: a) angiogram 2 months after implantation of prosthesis. Prosthesis patent, corresponding in diameter to recipient's own artery; b) angiogram 4 months after implantation of prosthesis. Moderate diffuse stenosis of graft.

conservation with the addition of human serum albumin 20 g/liter, sodium selenite 20 μg/liter, and dimethyl sulfoxide (DMSO), the concentration of which was raised in the course of 45 min to 15%. After another 15 min the arteries were removed, dried, and frozen at the rate of 1-3°C/min to −70°C, after which they were immersed in liquid nitrogen and kept for 7-30 days.

Before transplantation the containers were removed, heated in a water bath at +37°C, and the arteries were immersed in solution for rinsing out the cryoprotector (a solution of NIITiIO-2 with 88 g/liter of mannitol at 50 g/liter of DMSO). After incubation for 20 min with periodic washing of the lumen of the artery it was transferred into a similar solution but without DMSO, and containing 63 g/liter of mannitol, and another 20 min later into a solution containing 30 g/liter of mannitol. The artery was ready for transplantation after 20-30 min. In series 1

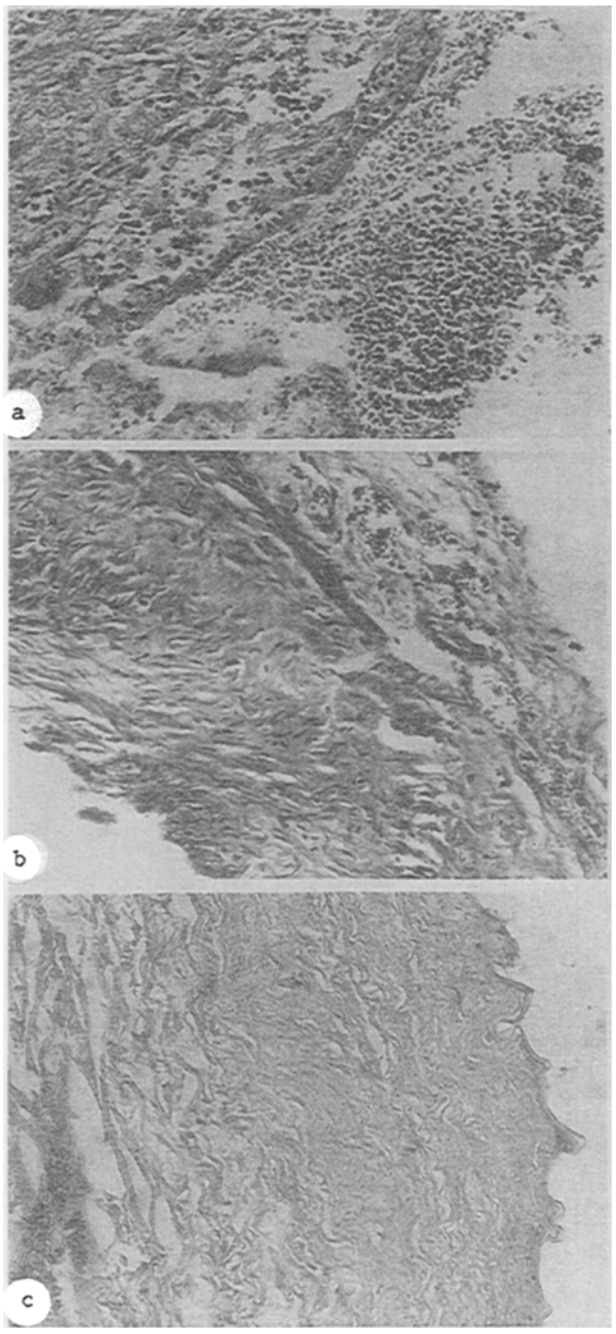

Fig. 3. Microscopic picture of wall of freeze-dried graft after different times after implantation: a) 3 days, b) 2 weeks, c) 6 months. Stained with hematoxylin and eosin. 200×.

anastomosis of the graft was carried out by a microsurgical technique and operative microscope, with atraumatic 8/0-9/0 thread. In series 2 anastomoses of the vessels was carried out with 6/0 atraumatic thread and with a continuous suture.

TABLE 1. Volume Velocity of Blood Flow along Femoral Artery at Different Times after Replacement by Freeze-Dried Allograft

Stage of investigation	Volume velocity of blood flow (ml/min)
Before implantation of prosthesis	83±9
Immediately after implantation	98±8
1 month later	95±14
3 months later	95±18
6 months later	78±6
12 months later	87±11

In the postoperative period the state of the grafts was evaluated between 1 week and 1 year later, on the basis of clinical and roentgenologic data, and also data of electromagnetic flowmetry and histologic investigation. No anticoagulants and no immunodepressants were used.

EXPERIMENTAL RESULTS

In the experiments of series 1 the patency of the implanted freeze-dried arteries was preserved for 1 week after transplantation in 80% of the rabbits undergoing operation. All eight cases of thrombosis developed during the first days, possibly as a result of technical errors during the operation. In the remaining animals, the bioprostheses remained latent throughout the period of observation. No late thromboses were observed. Angiography in the late stages revealed a virtually normal picture in 27 of the 32 long-functioning grafts (Fig. 1a). In five cases the freeze-dried bioprosthesis was dilated, and in four of these it was combined with moderate stenosis of the distal anastomosis (Fig. 1b). Excretory urography showed that the excretory function of the kidneys was preserved, even in the experiments in which stenosis of the anastomosis was found (Fig. 1c).

In series 2, 19 of the 20 freeze-dried allogeneic arteries preserved their patency when observed up to 1 year after the operation. Angiography, performed at different times after the operation, showed that the inner surface was even in all cases. The diameter of the implant until 1 month corresponded to that of the by-passed artery. After 3 months, moderate diffuse stenosis of the graft was observed in three experiments, but it did not subsequently progress (Fig. 2a, b). The blood flow in the shunted artery did not change significantly throughout the period of observation (Table 1). In cases when stenosis of the grait was found angiographically, no significant changes were observed in the blood flow.

Histologic investigation of grafts removed at various times after implantation revealed a similar time course in both series. Desquamation of the endothelium with exposure of the basement membrane over nearly all the surface, and with preservation of only separate endothelial islets, was found 3-7 days after implantation. The smooth-muscle cells of the tunica media had blurred outlines, their aggregation was disturbed, and discrete foci of necrosis could be seen. After the 3rd day foci of infiltration of the adventitia by lymphocytes and histiocytes was observed, and increased in intensity later (Fig. 3a). After 1 week the endothelial lining of the implanted artery was restored, and after 2 weeks the inner surface of the graft was completely endothelized; in some cases, moreover, the endothelium actively proliferated to form stratified structures. Replacement of the necrotic foci by connective tissue, with the development of subendothelial sclerosis of the transplanted artery took place after 2-4 weeks. Preserved bundles of smooth-muscle fibers were surrounded by layers of connective tissue. Infiltrating lymphocytes and histiocytes penetrated from the adventia into the tunica media (Fig. 3b). In the later period (3-12 months) the picture did not change significantly, with the exception of gradual disappearance of the infiltrating lymphocytes and histiocytes and an increase in degree of sclerosis of the arterial wall toward 6 months (Fig. 3c).

The results of these experiments thus showed that freeze-dried arteries, kept for up to 1 month at −196°C can be used as bioprostheses to by-pass arteries of small and medium caliber. Their use ensure good functional results in the late period, evidently because of early endothelization of the graft. The gradual sclerosis of the transplanted artery of medium caliber which developed in certain cases led to hemodynamically unimportant constriction of its lumen, which did not progress later than 3 months after transplantation. In arteries of small caliber this may be accompanied by the formation of a moderately large aneurysm (in 15% of cases).

Despite potential depression of the immunogenicity of the allogeneic artery as a result of freeze-drying, rejection reactions were observed to develop after the early stages, but their intensity increased slowly and they caused no unfavorable effects on function of the prosthesis. Signs of rejection disappeared in the late period.

Stabilization of the morphologic picture 6 months after implantation of a freeze-dried artery encourages the hope of stable function of the graft for a long period of time.

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