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The Use of frozen and freeze-dried homologous arterial grafts

Clyde E. Penner
University of Nebraska Medical Center

THE USE OF FROZEN AND FREEZE-DRIED HOMOLOGOUS ARTERIAL GRAFTS

Clyde Eugene Penner

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College of Medicine, University of Nebraska

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INTRODUCTION

The purpose of this paper is to review the literature regarding the use of frozen and freeze-dried arterial homografts with particular reference to the techniques of freezing and of preservation of these grafts.

HISTORY

Transplantation of an arterial segment was first performed in animals in 1896 by Jaboulay. In 1903, Hopfner described the successful transplantation of vascular segments using Payr's magnesium rings and a non-suture technique. That same year, Exner reported his experiments in vessel transplantation and in 1907, Stich and co-workers described similar experiments. (1)

The many studies reported by Carrel (2,3) between 1905 and 1912 provided the first definite evidence that homologous arterial and venous grafts could be used to replace excised vascular segments. Carrel's fundamental work established several facts--1) that successful restoration of the continuity of blood vessels could readily be accomplished by the proper suture methods, 2) that arterial and venous segments could be transplanted from one animal to another of the same species and that these transplants would function for long periods of time without thrombosis, and 3) that venous

grafts could be used in replacing arterial segments.

Villard, Watts and others (4) confirmed these observations, but during the following thirty years, little practical application of the knowledge was made. This was apparently due to several factors, but primarily it was because surgeons were reluctant to use autogenous vein grafts as substitutes for arterial segments, and it was difficult to obtain fresh homologous arterial segments when they were needed.

After a lapse of several decades, these experimental studies have been revived and techniques evolved for clinical application of vessel grafts. To Peirce, working in Gross's laboratory should go the credit for reintroducing the arterial graft. Since 1948, however, many surgical laboratories across the country have been studying this problem vigorously.

One of the most serious problems incident to the use of vascular grafts has been the lack of a satisfactory method of storage for the grafts from the time of donation to the time of use. Several methods have been used with varying success. These have included formalin and alcohol fixation; storage in gases, petrolatum, and nutrient media at near-freezing temperatures; quick-freezing with storage well below 0°C., and freeze-drying with storage at room temperature.

CRITERIA FOR SELECTION OF HOMOGRAFTS

Pate and Sawyer et al. (5) in 1952 stated that a vascular graft should fulfill the following requirements: the graft should transmit an adequate amount of blood for a prolonged period of time after implantation, without thrombosis, rupture or other detrimental physiologic effects; the technical details of processing should be minimal; storage should be independent of outside factors such as climatic conditions, power failures, contamination of solutions, etc., and should require minimal care; and the grafts should be capable of prolonged storage, allowing stock-piling and reducing waste.

H. Swan (6) stated in 1952 that homografts must be obtained sterile from young individuals, from two to 35 years of age, previously in good health, who die a sudden or relatively acute death. The vessels must be obtained within six hours post-mortem. Due to the problems of obtaining such grafts, the procurement of an adequate supply of segments for the "artery bank" is often difficult, even in large communities. The panel of elgible donors may be increased in communities where high-voltage cathode ray irradiation can be employed to sterilize segments from autopsy material. These machines however, are not widespread.

P. DeCamp (7) in 1953 agreed with Swan in details regarding the selection of homografts. He states that in removing the grafts from the donor, the entire aorta is dissected free, the adventitia removed and branch vessels ligated flush with the surface of the parent vessel. The grafts are then cut to the desired lengths and placed in the containers in which they are to be stored.

METHODS OF GRAFT PRESERVATION

E. C. Peirce, II and associates (8) in 1949 stored blood vessel segments using six different methods, ie. 1) in 10% homologous serum and balanced salt solution at 6 to 11'C., 2) in helium under pressure at 0'C., 3) in various salt solutions at 6 to 11'C., 4) in 100% serum at 1 to 4'C., 5) in serum ultrafiltrate at 1 to 11'C., and 6) in the frozen state at -76'C. Of these various types of grafts, the grafts stored in nutrient solutions contained the highest percentage of viability on tissue culture and gave the best results after transplantation. Only two of 34 grafts stored at -76'C. showed positive growth on tissue culture. Twelve of 14 such grafts failed because of thrombosis or breakdown of the graft.

Gross and co-workers (9) in 1949 used 25 arterial grafts, which had been stored in a balanced salt solution containing 10% homologous serum at 2 to 4'C., to bridge

experimental defects in dogs. In none of these dogs was there a death due to disruption of the graft. Three dogs had grafts which completely thrombosed. In the remaining 22 dogs, the grafts were known to be carrying blood at intervals of four days to ten months after operation.

A blood vessel bank was established in New York in 1951 by Keefer and associates (10). They found that vessels stored by Gross's method were not satisfactory for grafting if stored for longer than four to six weeks. They found no rupture of grafts stored for longer periods, but these vessels did show a high incidence of thrombosis. These authors feel that if the graft can be used within a relatively short period after storage begins, the graft stored in nutrient media is of greater value than other types of grafts because the former usually retain some viable cells.

On the other hand, MacPherson, Nabatoff, Deterling and Blakemore (11) in 1951 could not correlate the function or fate of venous homografts with the results of tissue culture at the time of implantation. These authors used the solution recommended by Gross for storage of their vein segments.

Coleman, Deterling and Parshley (1) in 1951 were unable to duplicate the results of Peirce et al., in

that they were unable to demonstrate viability of grafts preserved in nutrient solutions for periods longer than 38 days. They were also unable to demonstrate viability of any of their frozen grafts at the time of implantation despite the fact that the cultures were often studied microscopically for as long as one month.

The work of Peirce et al. and Gross et al. was repeated by Graham (12) in 1952 with similar results. Graham used Hank's modification of Tyrode's solution as his preservative. He concludes that this solution is only suitable for short-term storage of vessel segments and is wasteful for longer preservation, and that more satisfactory methods of storage were needed.

Peirce (13) in 1952, asserted again that viably preserved vessels should be used for homologous arterial grafts. He states that generally speaking, undoubted graft failures (thrombosis, and suture line disruption) occur within a few days, or at most, weeks after implantation. During this period a non-viable graft appears peculiarly subject to infection and attendant disruption. Possibly also, intimal changes predispose to thrombosis. A suitably preserved viable graft, on the other hand, behaves for a time much as an autograft and can actually contribute to the healing during this critical period.

In addition, it maintains a viable and relatively physiologic lining. Consequently, disruption is infrequent and thrombosis unusual. For these reasons, Peirce believes arterial homografts employing viable tissue to be less hazardous than those utilizing devitalized vessel segments.

Peirce lists the following criteria for viable grafts: arteries removed from one to four hours after death and used fresh; arteries preserved by refrigeration above freezing for periods up to about two weeks in simple unbuffered salt solutions and up to fifty days if physiologically buffered and balanced media are used. He states that arteries frozen; arteries fixed in formalin, zephiran, or alcohol; arteries stored late; or arteries stored in any uncontrolled fashion should be assumed to be non-viable until evidence indicates otherwise.

Studies, including those with slow and rapid freezing methods, have demonstrated occassional fibroblastic growth on tissue culture, but it is considered wise to assume that frozen tissue is non-viable for all practical purposes. There are some indications that newer methods of freezing tissue, or carrying it past the eutectic point more rapidly may make it necessary to alter this conclusion.

However, due to the waste of graft material and the high cost of maintaining blood vessel banks using the nutrient media for storage, Deterling, Parshley and Blunt (14) in 1953 carried out further experiments aimed at determining the true importance of viability in regard to functional success of homografts, and at evaluating other methods of preservation if viability were not all important. In addition to grafts stored in nutrient media at 4°C., these men also used grafts which were frozen, irradiated or lyophilized. In none of these grafts was viability demonstrated on tissue culture, but all types of grafts showed a high degree of functional success.

Many investigators have conducted experiments with frozen and freeze-dried homografts in an effort to make vessel segments more readily available for use when they are needed. These methods are attractive because they are relatively simple, they are economic, and they provide for long-term storage and hence, stock-piling of vessel grafts. The concept that frozen tissues might be kept for long periods of time in a condition suitable for surgical use has been the subject of such investigators as Keith, who in 1934 observed new bone formation after transplanting frozen bone segments; Mider and Morton, who found proliferation in the sites

of transplants of frozen rat skin; Webster in 1944 and Strumia and Hodge in 1945, who successfully transplanted skin grafts which had been freeze-dried and slowly frozen respectively; and Garber and Bush, who in 1950 successfully transplanted frozen homologous bone segments.

The object of rapid freezing of tissues is to maintain the protoplasm and tissue fluids in a vitreous state without crystal formation with resultant intracellular expansion and disintegration of cell structure. The critical temperature range is usually given as between 0' and 30'C. Since the eutectic point of a calcium chloride solution is -54.9'C. and the eutectic point of a combination of salts such as found in tissue fluids would be even lower, most investigators believe that the storage of tissues must be done at temperatures below -60'C.

The concept of preserving tissue by quick freezing has been based on the assumption that at low temperatures cellular metabolism is suspended. Under certain conditions, if the cells have not been disrupted by the freezing process, cellular activity can be resumed when the tissue again reaches normal environmental temperatures. The extensive work of Luyet (15) and others has suggested that in mammalian tissue there is least disturbance of the cell structure when freezing is very

rapid; and that this is probably due to the small size of the ice crystals formed in quick freezing. Crystals forming during slow freezing are larger and chiefly extracellular, so that a concentration of the intracellular fluid occurs. This concentration of intracellular fluids may allow super-cooling to occur without freezing and thus explain the rare survival of tissue occurring after slow freezing.

The cellular protoplasm is a colloid suspension. It is well known that such gels can exist in several physical states depending upon the amount of water in the gel, the temperature, and the rate of temperature changes. Thus, at various temperatures, such colloids exist as a semi-solid gel, a macrocrystalline solid, a microcrystalline solid, or as an amorphous solid. If freezing is accomplished slowly, and orderly lattice formation has time to take shape, macrocrystals result. If the thermal gradient is sufficiently rapid, the colloid can pass from the gel state to an amorphous or vitrified state without crystallization. This process is somewhat analogous to sublimation in which a solid passes into a gaseous phase without liquefaction.

It is therefore desirable in a freezing method to lower the temperature of the tissue as rapidly as possible particularly through the temperature range in

which crystallization is apt to occur. To obtain this objective, a steep thermal gradient is required, and the thickness of the mass cannot be large. Observers have found that tissue smaller than one mm. in diameter survived poorly after freezing and cubes of one cm. also had areas of extensive destruction of cells, particularly in the inner portion which was the last to freeze. It was the hope of many investigators that blood vessels, because of their hollow lumen and relatively thin walls, would possess an ideal tissue mass for freezing.

Blakemore and Lord (16) were the first of modern investigators to preserve blood vessels by freezing. They studied the use of homologous and heterologous vein grafts in bridging arterial defects. In their experiment in 1945, vein segments were frozen in helium in hermetically sealed tubes at -72'C. (dry ice and alcohol). These vessels were successfully transplanted after being stored for three months. The temperature of storage in this work is not given, and no tissue culture studies were reported.

Using essentially the same technique, Gross and coworkers (17) in 1948 attempted to increase the period of viability of vessel segments over that produced by simple cold storage in nubrient media. They stored the grafts at -72'C. for periods ranging from two to 35 days

before implanting them into recipient dogs. These men found that only t wo of the twelve vessels so treated were viable on tissue culture. Nine of the twelve recipient dogs died within five to 30 days from hemorrhage at the suture lines. Two grafts became partially thrombosed. One animal was kept for six months, and at autopsy the graft showed moderate intimal sclerosis. These results led the authors to the firm conviction that storage of vessels by freezing would not be a satisfactory method for graft preservation.

Meanwhile, other investigators were more successful in the transplantation of frozen arterial segments. By 1951, Hufnagel and Eastcott (4) had developed and evaluated two methods for the preservation of arteries by freezing in an effort to determine the optimal rate of cooling. The first, reported in 1947, used a mixture of solid carbon dioxide and ether as the freezing medium. This produced a temperature of below -70°C. in the freezing bath. Direct immersion of the arteries in this mixture was not possible because residual ether present on the vessel when it is warmed would be toxic. The grafts obtained under aseptic conditions were sealed in sterile, thin-walled Pyrex tubes. Since air is a poor conductor of heat, the air in the tube was replaced by helium under a pressure of 40 cm. Hg. The

sealed tubes were cooled in an ice bath to 6'C. They were then placed in the carbon dioxide and ether bath and allowed to equilibrate. The arteries were then removed from the freezing bath and stored in a carbon dioxide refrigerator at -70'C.

In an effort to secure more rapid freezing and thence the theoretical ideal a second method was evolved in 1951 by these two investigators. This method utilized the very low temperature (-195'C.) obtained with liquid nitrogen as the cooling agent. Liquid nitrogen is a generally available liquefied gas which is not toxic to tissue and is non-explosive. Direct immersion of the vessel segments in this medium is permissable. Small arteries can be frozen directly by placing them in liquid nitrogen and allowing them to come to equilibrium which occurs in about 15 seconds. When large vessels were used, equilibrium could be reached in 25 to 30 seconds. Just before this occurred, cracking sounds were usually heard, and upon examination after thawing, thin longitudinal cracks could be seen in the intima. This complication has been avoided by the use of a two-phase freezing technique which uses liquid nitrogen to lower the temperature very rapidly but does not allow the final temperature to go below -70'C.

Arterial segments have been preserved by both methods

for intervals of one to 180 days before transplantation. The functional results of such implantation have been excellent in all arteries with a lumen larger than 5 mm. In the last 55 consecutive grafts performed by Hufnagel and Eastcott, no thrombosis has occurred, probably due to the increase in technical skill. Homologous grafts have been followed for periods up to three years, and the majority for at least three months. The overall incidence of complications for this group of grafts was approximately 3% and did not vary greatly in the two rapid freezing methods. Grafts which were frozen by placing them in a standard deep freeze and keeping them at temperatures of -17 to -20'C. showed a very high incidence of thrombosis. The rapidly frozen homografts, even when stored for long periods of time had no greater incidence of thrombosis than fresh homografts. Grafts which had been stored for periods up to six months did not differ from those kept for shorter periods. The maximum effective storage period had not been completely determined, but they thought it was longer than six months and appeared to be indefinite. Grafts stored above 0'C. in salt solutions gave similar good results for the first four weeks of storage, but after that time, the fate of these grafts was uncertain.

Deterling, Coleman and Parshley (18) in 1951 froze

arterial segments at -72'C. using dry ice and alcohol. The grafts were then stored for one day to eight months using two methods--1) one set of vessels was transferred to a commercial deep freeze and stored at -27'C. and 2) the other set of vessels was stored in dry ice at a temperature of -60 to -70'C. The authors implanted 43 grafts stored by these methods into two groups of dogs. Of the 43 dogs, 17 were allowed to survive for long-term studies and had been observed for periods up to ten months at the time of publication. The grafts in these 17 dogs appeared to be functioning well in all instances. Grafts of the remaining 26 dogs were studied immediately post mortem by 1) tissue culture, 2) photographs of the gross specimen, and 3) histologic preparation. In 22 dogs with grafts which had been stored at -27'C. and which received no anticoagulants, a satisfactory function was observed in 16 or 73%. The other 27% had complete occlussions. In 13 dogs with grafts stored at the same temperature but which received anticoagulants, full function was found in 12 or 93%, only one of the grafts becoming occluded, and this in a dog which had received inadequate amounts of the drugs. In eight dggs which received grafts stored at -60 to -70'C., there was found no instance of complete occlussion, whether or not anticoagulants had been used. Deterling and his

co-workers state that their results with the worst set of grafts, that is, those stored at -27' and used without anticoagulants, were much better than those observed with frozen grafts by Gross and his associates, and only slightly worse than the results of their series employing grafts stored in a nutrient medium at 4'C. The former authors' results with grafts stored at -60' or below coupled with the use of anticoagulants are fully as satisfactory as the results with grafts in the animals studied by Gross and co-workers.

In 1952, Swan, Feehan et al. (19) stated that the objection to freezing tissue by direct immersion in liquid nitrogen or hydrogen was the formation of gas bubbles on the surface of the tissue which produce an uncontrolled, irregular, insulating layer. To prevent this, isopentane, a low boiling (30'C.) paraffin compound which freezes at -195'C. was used by these authors as a cold transmission medium without bubble formation. Isopentane, however, is mildly toxic to tissue. They reported that Snell found tissue immersed in this substance showed evidence of toxicity after one hour, and after 24 hours of immersion, isopentane is lethal to tissue. Swan and associates froze vessels using different media at both -196 and -78'C. both rapidly and slowly. They found the greatest per cent of tissue culture

viability using nutrient media such as 1) serum, Ringer's solution plus 10% serum or 2) Ringer's plus 10% serum plus 10% glucose, with the exception of those vessels frozen in isopentane, but because of the known toxicity of this substance, this technique is not practical. They found no significant difference in the survival rates of vessels frozen at -196'and 78'C. as long as the rapid method was used at the latter temperature. They thought the most practical method of freezing was the use of dry ice and alcohol. These authors stored grafts at -78' and at -30'C. but found no viability after storage at -30'. At -78' they found that the tissue would remain viable for at least 25 days.

Proper thawing of frozen arterial segments is essential to produce routine functional success of homografts. It is generally agreed that the thawing of tissue should be as rapidly accomplished as possible to prevent the formation of ice crystals with resultant cell destruction during the transition to the fluid state. Swan, Feehan et al. (19) in their work in 1952 thawed their frozen homografts using two procedures--some were thawed slowly by leaving them at -10'C. for 15 minutes, at 4' for 15 minutes, and then at room temperature. Others were thawed rapidly by agitating in Ringer's solution heated to 37'C. Microscopic sections of vessels thawed slowly revealed small clefts

between the elastic fibers of the media presumed to be the result of ice crystal formation. These defects were not demonstrable in the vessels thawed rapidly. Hufnagel and Eastcott (4) had used large volumes of Ringer's at 37'C. to thaw the frozen graft segments in their work in 1951 and were not able to demonstrate defects in the graft walls.

Attempts were made by several investigators to simplify the storage of vessel grafts by freeze-drying them. In their effort in 1951, Marrangoni and Cecchini (20) placed the arteries to be preserved in sterile jars in a deep freeze for 24 hours. The vessels were then transferred to a lyophilizer, dried at a temperature of -15 to -25'C. and kept under a vacuum (0.001 mm.) for 72 to 96 hours, removed and placed in sealed jars and then kept at room temperature until transplanted. Tissue culture studies at this time showed these grafts to be essentially non-viable segments. They had the appearance of rigid tubes when placed in the sealed jars. After hydration with physiologic saline, they became elastic, taking on the appearance of normal vessels except they were more pale. Vascular channels of satisfactory size resulted in most instances when these vessels were transplanted. These grafts resembled other homografts histologically as described by Swan and others.

Other experiments with freeze-dried arterial homografts were carried out by Pate and Sawyer in 1952 and 1953 (5,21), and by Jordan and associates (22) in 1953.

Pate and Sawyer stated that although Carrel and many subsequent investigators have indicated that tissue must be alive for satisfactory transplantation, it has been repeatedly observed, in many later experiments, that homografts slough or die and are replaced after grafting even though they may survive and proliferate for short periods of time. In fact, with the exception of a few cases in identical twins, there have been no valid reports of permanent survival of mature homologous tissue after implantation in its normal site. Therefore, one cannot assume that it is necessary to have viable transplants if they serve only a simple mechanical function. Furthermore, from a theoretic standpoint, the presence of injured living cells may be deleterious. These cells give off metabolites and toxic products of degeneration which may be actually harmful. These products may add to the incidence of autolysis, thrombosis, and surrounding tissue reaction, with resultant weakness of the suture lines and increased amounts of scarring. Freeze-drying may allow longer periods of storage at room temperature without special care, routine shipment from the point of processing to the point of subsequent

use, and stock-piling.

In their experiment, Pate and Sawyer placed homografts in ampuls partially submerged in liquid nitrogen at -196'C. While still at this low temperature, the grafts were placed under a vacuum of 50 to 20 μ Hg. pressure in a modification of a commercial freeze-drying machine. The water was sublimated from the ice to the vapor stage, thereby reducing the possibility of crystal formation. The tissues were then allowed to warm to room temperature while being maintained under a vacuum of at least 50 μ Hg. pressure. Removing heat of sublimation by evaporation kept the tissues frozen during the early stages of dehydration. The vacuum was maintained for 72 hours, during which time the temperature slowly rose to 20'C. The tissues were then removed and sealed in glass ampuls under a pressure of about 100 to 200 μ Hg. The vacuum sealing was used in a effort to exclude oxygen and water since the authors believed exposure to these agents causes deterioration of the graft during long periods of storage. The ampuls with their grafts were stored at room temperature. At the time of implantation, the grafts were placed in normal saline for 20 to 30 minutes for reconstitution. At this time the microscopic sections and physical properties were essentially the same as those of fresh aortic tissue.

After implantation of the grafts, the animals were examined frequently for skin color, temperature, femoral pulsations, and gross neuro-muscular function of the hind legs. Aortograms were done each month on the long term animals.

Pate and Sawyer found that freeze-dried grafts, even when preserved for as long as six months, show a significant reduction in mortality compared to fresh grafts (2 vs 14%); decrease in incidence and degree of thrombosis (2 vs 23%); and decrease in unsatisfactory results (4 vs 23%). Overall, they found freeze-dried grafts to be superior to fresh homologous grafts with 96% of the former classed as excellent, while only 55% of the latter were classed as excellent.

Studies of the effects of various storage times on the results of implantation revealed that there is no significant difference in the results obtained with grafts preserved for one, six, twelve, or even 25 weeks.

Jordan and associates used the same technique , with few minor exceptions, that was used by Pate and Sawyer. Except for severalfailures due to technical errors, they obtained good results upon transplantation of freeze-dried grafts. They state that lyophilization could solve the disadvantage of maturing which is the greatest single deterrent in other methods of vessel preservation.

STERILIZATION OF GRAFT SEGMENTS

One of the major problems in graft preservation has been that of obtaining sufficient quantities of sterile blood vessel segments. Many methods of sterilization of vessel segments have been tried experimentally. Attempts to decontaminate grafts with chemical antiseptics and antibiotic combinations have been consistently unsuccessful. In 1948, the Dept. of Food Technology at the Mass. Institute of Technology reported the marked bactericidal action of high voltage cathode-ray irradiation in the sterilization of food. With the cooperation of John Trump at M. I. T., Meeker and Gross (23) in 1951 carried out irradiation of intentionally contaminated blood vessel segments using a compact 3-mev. electrostatic generator, which produced high-voltage cathode rays that were able to penetrate organic material to a depth of 1.5 cm. Other investigation of the use of cathode-ray irradiation to sterilize blood vessel segments has been carried out by Hui, Keefer and associates (24) in 1951, and by Brunnen (25) in 1953.

These investigators have used irradiation dosages varying from 15,000 to six million roentgen equivalent physical units (R. E. P.). Irradiation dosages have been applied to grafts at various temperatures, ranging from room temperature to temperatures well below freezing.

Meeker and Gross, using graft segments intentionally contaminated with 24-hour pure cultures of various organisms, found that sterilization of these segments could be produced by irradiation with dosages of from 1.5 to 2.0 million R.E. P., and this dosage of irradiation did not cause tissue damage as long as it was applied to segments which were kept at temperatures below -50°C. The above dosages applied at room temperature, and dosages in the range of 3.0 to 6.0 million R.E.P. applied at any temperature, uniformly produced tissue damage.

Hui, Keefer and associates found that as little as 15,000 R.E.P. were needed to sterilize grafts subject to chance contamination. For 100% sterilization of vegetative bacteria in concentrations of 10^5 to 10^{10} organisms per milliliter, as well as spores and viruses, a dosage of 1.6 million was found to be necessary. As much as 3.2 million R.E.P. were needed to sterilize grafts heavily contaminated with a pure culture of a hemolytic streptococcus. These authors found that dosages of 3.2 million R.E.P. or below cause little recognizable change in aortic grafts although the cells are killed. Minor changes seemed proportional to the duration of storage rather than differences in dosage. Irradiated vessels retained better gross and microscopic

appearance than non-irradiated vessels stored at various temperatures, with best results in both being noted at -70°C.

Brunnen, in an attempt to comfirm the work of Gross and Meeker, and Hui et al, obtained effective sterilization with dosages of 1.5 million R.E.P., but found a high percentage of thrombosis in the grafts after they had been transplanted.

TECHNIQUES OF GRAFT IMPLANTATION

Johnson and Kirby (26) in 1950 used different suture techniques in end-to-end anastomoses of abdominal aortas of pigs to observe the effect upon the growing aorta. Before being sacrificed, the pigs and their aortas had increased in size about 800%. These authors found that when catgut or interrupted silk sutures were used, the results appeared to be perfect. The site of the anastomosis was difficult to detect and obviously had grown as the aorta increased in size. When a continuous silk everting mattress suture was used, there was constriction at the site of anastomosis and dilatation beyond it. The loop of the silk suture had straightened out as much as possible, but had cut through the vessel wall and was covered only by a thin layer of intima. It obviously constricted the lumen and prevented growth of the vessel at the site of anastomosis.

Swan (6), in 1952, stated suture anastomosis is best suited for insertion of blood vessel grafts with all the niceties of technique of vascular suture--strict asepsis, avoidance of tissue injury by trauma or desiccation, careful stripping of the adventitia, careful placement of small non-traumatic sutures, careful wound closure without dead space or necrosis--to be strictly observed. In addition, the employment of a graft of proper size is of paramount importance. The lumen of the graft when distended by blood pressure, must match as closely as possible the lumen of the recipient vessel, thus forming a vessel in continuity of uniform or tapering caliber. If either anastomosis forms a constriction, or if the graft distends, thus creating a sudden change in the size of the vessel, turbulence and eddy currents are created which strongly favor intraluminal thrombosis and failure. All arteries with an internal diameter of three mm. or larger may be grafted successfully. The maximum safe length is not certain, but relatively long ones (six to ten inches) have been used experimentally in the aortas of dogs. Swan has successfully transplanted clinically an aortic graft of five inches in length, and a femoral graft of three inches in length.

FATE OF HOMOGRAFTS

Swan et al. (27) in 1950 after storing grafts in nutrient media at 3-8'C concluded the duration of storage (up to six months) was not a significant factor governing the immediate functional success of the transplant, however, microscopic evidence strongly suggested that degenerative changes appeared earlier and were more extensive in grafts stored longer in time than 40 days. Serial microscopic studies indicated that the adventitia and the intima of these grafts were totally replaced by similar layers derived from the host. At least part of the media apparently survived. The elastic tissue remained for periods up to ten months. They found that some smooth muscle tissue survived in grafts stored less than forty days.

In frozen grafts stored at temperatures well below freezing, Deterling and co-workers (18), in 1951 found that within hours after implantation the grafts were invested in inflammatory-like adhesions. This reaction increased and after a few weeks the graft was heavily supported by a thick layer of new fibrous tissue. The same reaction was noted when autografts were used. When the surrounding adhesions were dissected away, the graft appeared white, tough, and thinner than the adjacent normal aorta, although not appreciably thinner at the

time of implantation. Minor dilatation was observed in a few grafts but there was no aneurysmal formation. It appeared that up to two weeks was necessary for the process of re-endothelization of the grafts. The authors at this time, were unable to determine any significant difference, at least functionally and from early microscopic studies, resulting from variations in treatment of the graft before quick-freezing or in the process of thawing. It appeared, however, that the degenerative changes observed in grafts stored at -27' were more severe and progressive than in grafts preserved at a much lower temperature. These writers found similar changes in grafts stored in nutrient media. The results of the work of these men indicate that viability of the aortic homograft per se is not a requisite for success in vascular grafts. Apparently, all homografts, even when used in the fresh state, merely act as a framework or prosthesis which in time is entirely infiltrated by cellular elements from the host.

Hufnagel and Eastcott (4) in their work in 1951, also found that the described histological changes occurred in homologous grafts whether or not the grafts were fresh, and were not dependent upon the type of preservation used. They noted that growth of new blood vessels into the graft from the surrounding tissue was evident soon after implantation. They felt that the

presence of an intact elastic lamina was an important factor in the prevention of overdistension of the graft wall.

According to Swan (6), after his work in 1952, the elastic tissue in the media of homografts persists for a period of at least two years. The host elaborates a new inner layer which grows in from each end of the graft. At first, primarily fibrocellular, this layer undergoes remarkable differentiation, the inner cells assuming endothelial forms. Swan also states that the graft serves merely as a scaffold upon which the host builds a new blood vessel using the elastic lamina for the foundation.

In 1952, Peirce (13) described the typical appearance of homografts several months after transplantation. He states that at this time the various grafts tend to resemble one another, that is 1) the media is almost acellular, 2) there is little muscle or collagen remaining in the media, 3) there is marked condensation of the elastic tissue, the fibers of which have lost support, but retain staining characteristics, 4) a heavy new adventitia, rich in collagen is seen, 5) a new intima is formed which is heavier toward suture lines, and 6) occassional amorhpous calcium deposits and, rarely, bone are seen in the media.

McCune et al. (28), in 1952, stated that except for foriegn tissue reaction and suture technique, the most important single factor in the success or failure of any homologous tissue graft is the development of an adequate blood supply from the host. Beside the blood supply entering the graft from vascular tissue surrounding it, most investigators feel that the graft also receives some nourishment from the blood passing through its lumen. McCune and associates found that as early as one week after grafting, an exhuberant growth of collateral vessels could be demonstrated in the graft walls derived chiefly from surrounding structures, but to some extent, from the ends of the recipient's aorta. They found no evidence of vessels originating from the lumen of the graft to supply the inner layers, but seemed to think that nutrition in some form filters through the new layers of the graft to supply these inner cells.

THE CLINICAL USE OF ARTERIAL HOMOGRAFTS

Hufnagel and Eastcott (4) list the following uses for arterial grafts. They may be use to treat:

I. Coarctation of the aorta--long constriction.

II. Tetralogy of Fallot
   a. To increase the length of the subclavian or innominate artery.
   b. Pulmonary widening--direct.

III. Vascular rings
   a. When both branches are large.

IV. Aneurysm
   a. Aortic.
      1. Thoracic.
      2. Abdominal.
   b. Peripheral-arterial.

V. Arterio-venous fistula
   a. Restoration of the artery.

VI. Obliteration of thrombotic arterial disease
   a. Thrombosis of the aortic bifurcation.
   b. Localized arterial occlussion in any major vessel.

VII. Trauma
   a. Restoration of a major vessel to an extremity or organ damaged by injury.

VIII. Tumor
   a. Resection of a major vessel involved by tumor and restoration with a graft.

To date, there have been only a few cases of actual clinical application of arterial grafts. Gross (29), in 1950 reported the surgical treatment of 100 cases of coarctation of the aorta. Homologous aortic grafts were used in six of these cases. These grafts varied in length from four to five cm. At the time of his writing, the patients had been followed for three months to 1½ years with complete relief of hypertension seen in all. In 1951, Gross (30) reported the treatment of thirteen subsequent cases of coarctation using homologous grafts. Sixteen of these grafts had been stored in Tyrode's solution at 2 to 4'C. while the other three grafts had been frozen and stored in a carbon dioxide refrigerator at -50'C. for from two to 72 days.

These patients had been followed for periods ranging from several months to three years. Two of the 19 patients in their total series died on the fourth postoperative day due to renal shutdown. The remaining 17 patients were alive at the time the article was published. The results were termed excellent in that the arm pressures dropped to normal and the blood pressures in the legs were increased above those in the arms.

In 1950, Swan et al.(31) used a homograft which had been stored for 52 days in Ringer's solution to treat a coarctation in a 16 year old boy. Two years later the graft was still functioning well. The next year, Swan and Morfit (32) used homografts in the treatment of three patients having radical excision of sarcoma in the femoral area requiring removal of significant portions of the femoral artery. They obtained good functional results in two patients; the graft in the third patient failed because of inadequate wound closure, and the patient's leg had to be amputated. In 1952, Swan (33) also reported the use of homografts to repair a bullet wound of the femoral artery, a false axillary aneurysm, and a false femoral aneurysm. All patients obtained good functional results.

Beattie (34) in 1951, and Martin and Lynn (35) in 1951, also report their use of homografts in the treatment of coarctations in clinical cases.

SUMMARY

Since the work of Carrel in the first part of this century, there has been considerable interest in the possibility of using homologous arterial segments to replace diseased or damaged arteries. Only in the last decade, however, has the idea become widespread, and many experiments have recently been carried out in an effort to determine a method whereby vessel segments may be stored in a manner so that they will be suitable for transplantation.

Many investigators, notably Carrel and more recently, Gross and Peirce, were of the opinion that in order to produce a successful transplant, the vessel segments must remain in a viable state throughout storage. As a result, these men stored grafts in nutrient media (Tyrode's or Ringer's solutions plus homologous serum) at above-freezing temperatures (4to 6'C.) They found that certain cells of the vessels would remain viable if stored in such a manner, but only for a period of approximately forty days.

Due to the difficulty in obtaining sufficient quantities of sterile vessels and the short time in which they would remain viable during storage, it was found that suitable vessel segments could not always be obtained when needed.

For this reason, other investigators such as Hufnagel and Eastcott, Coleman, Deterling and Parshley, and Swan became interested in preserving arteries at temperatures well below freezing after having been quick-frozen initially. By rapidly passing through the critical temperature range for tissues (0 to -30'C.), it was hoped that homografts could be preserved for long periods of time without cell destruction. Grafts handled in this manner were usually frozen rapidly in a dry ice and alcohol mixture at a temperature of -70'C. and then stored in a dry ice refrigerator at the same temperature. These grafts were found to be non-viable after storage, but results after implantation into host animals were almost uniformly satisfactory.

Further investigation convinced many of these authors that viability of the homograft was not the all-important factor in obtaining good functional results with transplanted vessel segments. By studying grafts at autopsy after varying periods of implantation, it was found that even if there were viable cells in the tissue at the time of grafting, these cells soon died and were removed and replaced with tissue growing in from the host. The elastic portion of the media remained intact for the longest period (about two years) and it is considered essential to have this layer intact in order for the

transplantation to be successful. The recipient animal uses the elastic layer as a foundation upon which new intimal and adventitial layers are laid down.

To further simplify the problem of blood vessel storage, Pate and Sawyer and others have freeze-dried arterial homografts. In this procedure, the segments are dehydrated at below-freezing temperatures, allowed to warm and are then stored in sealed containers at room temperature. The vessels are reconstituted in saline before implantation and at such time they appear very similar to fresh homografts. Good results have been obtained with these grafts in experimental animals, and investigators using this method have found less undesirable reactions using grafts of this type than with fresh grafts.

Due to the fact that arterial homografts serve only as a prosthesis upon which the host animal builds new blood vessel layers using the elastic tissue of the graft as a foundation, this writer believes that the freeze-drying method will eventually come to produce most of the arterial segments used for grafting. This method is simple, it is economical, it allows for stock-piling of homografts, and it seems to produce results which compare favorably with those of other types of grafts. While it does not appear that blood vessel grafting

will become a widespread surgical procedure, if used properly this procedure can save many limbs previously amputated and prolong lives of patients suffering from coarctation of the aorta.

CONCLUSIONS

1. Several methods of preservation of homografts are discussed with emphasis on quick-frozen and freeze-dried blood vessel segments.

2. The most practical method of quick-freezing arterial segments is the dry ice and alcohol bath. Storage should be at -70°C. (dry ice refrigerator).

3. Freeze-drying of arterial homografts appears to be the answer to the problem of storage of sufficient grafts to provide an adequate and constant supply.

4. The number of donors might be increased by the use of cathode ray irradiation in dosages of 15,000 to 1.5 million R.E.P. applied to unsterile segments.

5. Arterial homografts may be used to treat coarctation of the aorta, aneurysms(both aortic and peripheral), injured vessels, vessels involved by tumor growth, congenital lesions, etc.

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C. E. Penner

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