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HOMOLOGOUS TRANSPLANTATION OF ARTERIAL SEGMENTS PRESERVED IN A BLOOD VESSEL BANK

Preliminary Report on an Experimental Investigation in Dogs

By

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Forty years ago *Carrel* (1912) proved that preserved arterial segments could be successfully used as homologous vascular grafts to bridge experimental gaps in the major arteries. Vascular segments were removed under sterile conditions from recently killed dogs and preserved for several days before transplantation at a temperature slightly above freezing in different media, including defibrinated blood and isotonic saline. As a rule, these grafts healed satisfactorily and were able to carry out their vascular function in the recipient, despite certain histopathologic changes which gradually developed in their walls.

The practical clinical use of the homologous arterial graft was not evolved until recently. The need of a method of bridging long gaps in the major arteries became acute during the Second World War. But the advance of the diagnosis and surgery of the heart and central blood vessels has perhaps been an even more important factor in reviving interest in homologous arterial transplantation. The first cases of aortic coarctation to undergo successful radical resection and direct end-to-end suture were operated on by *Crafoord*. For cases of long coarctations in which direct end-to-end suture is impossible, *Blalock* (1951) has evolved a method of uniting the subclavian artery with the caudal aortic segment. Another method, particularly in long coarctation of the aorta, has been used by *Gross*. In his latest paper, *Gross* (1951) reported on not less than 19 cases in which the coarctation was removed radically and the aortic pathway was restored. In most of the cases the segments had been preserved for four to six weeks at two to four degrees Centigrade in a balanced electrolyte

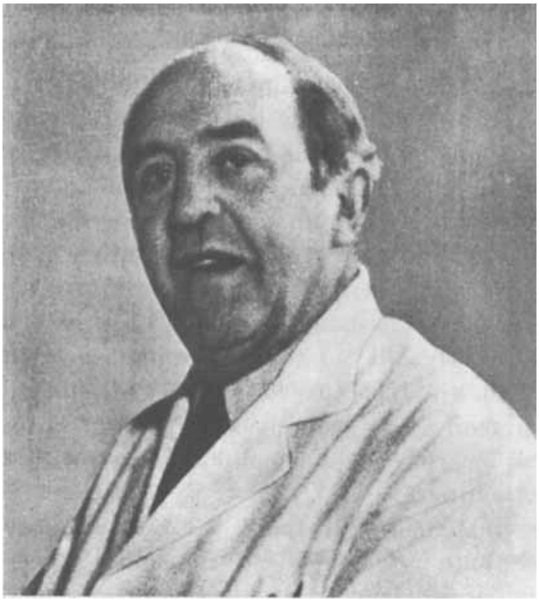
Waldenström largely succeeded in redressing this balance; he indicated to Swedish orthopedics the way towards a more medical–surgical outlook and gave it a scientific backbone. Much of what is written today in Swedish orthopedics is a product of his initiative.

His contributions to the sphere of medical teaching were perhaps not very comprehensive but his importance to his colleagues at the clinic in stimulating the scientific impulse was very considerable. To work in association with Henning Waldenström imposed upon one the need for a constant critical appraisal and re-valuation of theories and methods. This critical attitude never bore a trace of negativism, however. New viewpoints were always being taken up, tested and transformed. And it is a fitting confession and salute to him, I think, that we who have had the privilege of being his colleagues often continue to ask this question before composing our own speeches of articles: "Would Henning approve of this?"

Waldenström's path has always led through the sunlit spaces of life and he has always had the happy knack of enjoying such an advantage in a balanced way. Thus he possesses the power, so rare in Swedes, of being able to say no to unnecessary honours and unnecessary social relationships, which is so conductive to the peace of mind. His publications are relatively few, his style, apparently so simple, is crystal clear and on a high literary level; its "bouquet" is unfortunately lost in translation.

It is the custom to wish an older generation an "otium cum dignitate". The happiness of enjoying it is vouchsafed to comparatively few; Waldenström is one of these. He celebrates his 75th birthday with undiminished strength of body and mind. We so readily believe that this is the result of a way of life, distinguished by maturity of spirit, which many strive for but few are able to achieve.

Stockholm, July 1952. *Sten Friberg.*



AAGE BERNTSEN

In memoriam

Aage Berntsen died on the 16th of April, at the age of 66 years. He was born on the 16th of May, 1885, and grew up in a home in the midst of intellectual life, on Fyen; his father, Klaus Berntsen–the President of the Council and the "Politician"–impressed his personality deeply on their home, with his wide and intens interest in popular education in the Grundtvig spirit.

He was a poet and the collections of poems which he published all bore the stamp of his deep humanity, his penetrating powers of observation and feeling for the shadows and stranger aspects of every-day life. He knew how to express himself in verse in a rare and charming fashion, using the language of daily life. In later years he also devoted himself to painting.

Even though Berntsen was a heavy man and though his pace was never hurried, he excelled at sport, being a good tennis player and Denmark's champion at rapier fencing for several years.

After his medical examinations he took up general surgical training and was assistant surgeant at the Rigshospital, but general surgery with its many acute and dramatic situations, its surprises and disappointments oppressed Berntsen's sensitive mind and this was probably the reason why he felt more strongly the attraction of the more constructive surgery to be found in orthopedics where, in planning the schedules of treatment, he was able to make use of his artistic gifts and imagination. His bon sens was a great help to him, when he had to judge a patient's future outlook and often his view was that it would be preferable for him to carry out a minor intervention which improved the patient's condition rather than to undertake a major operation which involved a considerable risk.

After the period as assistant surgeon at Schaldemose Berntsen became "assistant surgeant" at the Samfundet og Hjemmet for Vanføre at Toldbodvej, (in 1935 surgeon at the Orthopedic Hospital) and in 1947 head surgeon at the orthopædisk Hospital in Copenhagen.

Berntsen was awarded his medical doctorate for his thesis "Varices in the lower extremities".

In 1934 he published a textbook on Orthopaedics and in 1948 "Operative Technique in Orthopedics"–schematic survey of orthopedic operations seen daily–a book which is highly esteemed by surgeons. Berntsen published a number of articles on orthopedic subjects, particularly the treatment of hip arthrosis and stabilising operations of the foot; in these articles there often appeared accounts of technical subtleties of which he wished to inform others.

From 1947 Berntsen was lecturer in Orthopedics at the University of Copenhagen.

Even if we shall never again see his characteristic figure nor listen to his many bon mots we shall preserve the memory of the loyal, true colleague, the brilliant surgeon with the lucid sensitive mind, and we shall remember the melancholy but charming undertones revealed in glimpses through which his cultivated and noble spirit expressed itself.

Let us honour his memory.

Copenhagen, June 1952. *A. Monberg.*

HENNING WALDENSTRÖM

75 years

Henning Waldenström completes his 75th year on the 14th of August. He comes of an old medical family. Both his father and grandfather were doctors and his father was Sweden's first professor of surgery. Like his brother, Henning devoted himself to surgery and after he finished his training became the first chief surgeon in the department for bone and joint tuberculosis at Sankt Görans Hospital, Stockholm. He was not appointed professor of orthopedic surgery until 1936, at the age of 58, and he was active as professor until 1942 when he retired on his pension, still almost a young man; he was and still is a truly living argument for those who consider that Swedes are compelled to leave their positions too early.

Although Waldenström's longest period of service lay within the field of bone and joint tuberculosis, the climax of his career must be said to occur during the years of his professorship, the six short years before the retirement age. Swedish orthopedics had previously been tied fast to social welfare in a way not very favourable to the development of the speciality, and had rather served as the complement of social welfare instead of vice versa as should have been the case.

solution containing nutrient elements and antibiotics, according to the principles described by *Gross, Bill & Peirce* in 1949. Other reports on the clinical use of blood-vessel bank materials have been published by *Swan, Maaske, Johnson & Grover* (1950), *Beattie, Cooke, Paul & Orbison* (1951), *Lam & Aram* (1951), *Oudot* (1951), *Hiertonn, Johansson & Unander-Scharin* (1952).

The need for a sure method of bridging gaps in the major arteries in various injuries and diseases has long been felt. In certain regions of the body the collateral circulation becomes inadequate if the flow in the main artery is cut off. This is frequently true of the extremities. Part of the leg is almost invariably lost as a result of ligature of the femoral and popliteal arteries due to war injuries. *Rose, Hess & Welch* (1946) stated that the amputation rate was 70 to 80 per cent in a series of vascular injuries, in which ligature had been done during the Second World War. Even if the collateral circulation is developed after ligature so that the vitality of the extremity is not lost, many cases have grave symptoms of insufficiency on exertion, which may even become incapacitating: *Shumacker* (1947).

Acute arterial injuries are relatively uncommon in peacetime. However, I have had occasion to handle a few of these cases. The situation is dramatic. For the surgeon the choice often is a difficult one between a crippling amputation and an attempt at reconstruction.

The availability of arterial segments of different calibers and lengths preserved in a blood-vessel bank would appear to provide the surgeon with new and improved possibilities of treating not only congenital deformities of the heart and the major arteries but also acute injuries to the central and peripheral arteries. It is also possible that a certain group of patients with incapacitating pain in the extremities due to limited arterial obliteration may be submitted to radical removal of the constricted area and bridging of the resultant gap with a vascular graft.

In other words, homologous arterial transplantation with material from a blood-vessel bank would seem to be a method which can be applied in several different fields of surgery. Before establishing a blood-vessel bank for relatively general use in Sweden, it has been considered advisable to learn more about the functional and morphological fate of the grafts by means of exploratory experimental investigations.

Therefore, in the autumn of 1949, Dr. *T. Borg* and the writer began an investigation on dogs in which experimental defects in the abdominal aorta were bridged with homologous grafts. The preliminary results of our common experiments were reported in *Nordisk Medicin*

1950: 44: 1387. Since then I have added to the series of aortic grafts and have also transplanted 24 peripheral arterial grafts. In the following I shall summarize briefly the results of the functional and morphological investigations of these grafts, as well as certain observations concerning the viability of arterial segments preserved in a blood-vessel bank. The strength and elasticity of the grafts have also been studied.

Arterial segments removed under sterile conditions from recently killed dogs were preserved at 1 to 4°C. in sealed Ehrlenmeyer flasks containing 10 per cent homologous serum and phosphate-buffered Tyrode's solution, as well as penicillin. This method of storage is known as a *blood-vessel bank* in the present study.

Arterial segments which had been preserved in this way for ten days were examined in a Warburg apparatus, at which time almost 80 per cent exhibited significant tissue respiration. The technique used to remove the arterial segments and for their preservation was therefore considered to be sufficiently gentle to maintain viability. When the storage period was extended to three or four weeks, more than half of the segments lost their respiration. These figures correspond well with the results of other viability tests. *Gross, Bill & Peirce* (1949) examined tissue cultures from arterial segments in their bank and found positive cellular growth after storage for 30 to 40 days. *Deterling, Coleman & Parshley* (1951), who made their cultures from the intima and media only, could observe no cellular growth in segments preserved in the same manner for more than 23 days.

With the blood-vessel bank used in the present study as a model, it should be possible to count on well-preserved and even "living" grafts for two to three weeks. For practical surgical purposes, storage of about one week is required to allow the bacteriological tests, etc.

Experimental gaps in the abdominal aorta and the femoral artery in dogs were bridged with homologous aortic and arterial grafts of various kinds. Table 1 and 2 summarize the functional results of homografts. The transplantation of small arteries was frequently complicated by a thrombus, regardless of the length of the storage period. The chances of arriving at a functioning graft were much better in the case of arteries of greater caliber. Heparin was not used. From the technical of view, it was found to be of importance to have a sufficiently large and varied collection of segments stored in the bank so that the right size of graft could be found to fit each case. It is probable that this is of fundamental importance to function and also to the reconstructive processes in the area of the operation.

It was usually possible to determine whether the grafts were

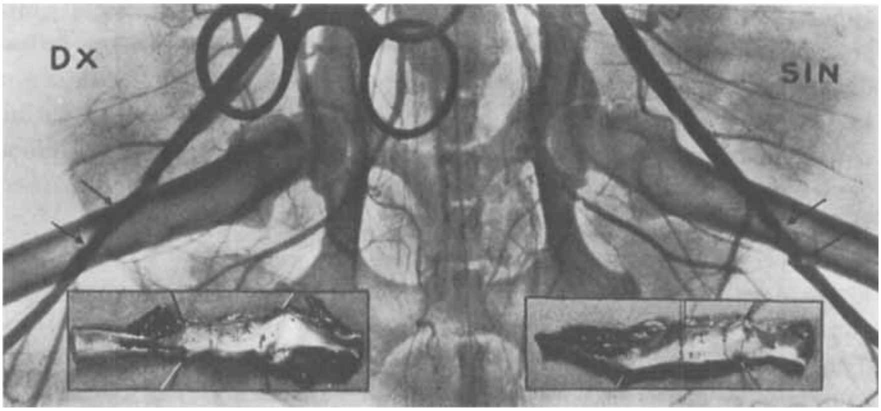


Fig. 1.
Arteriograms of peripheral homografts in the right and left femoral artery of a dog, made by umbradil-injection into abdominal aorta in vivo. The grafts, previously stored 2 days in the experimental blood vessel bank, are the segments between the arrows. On both sides there is a good patency 14 months after implantation. Photographs of the specimens show a smooth and glistening intimal surface.

functioning by observing the dogs' general activity and by palpation of the femoral pulse. Arteriography was then used to see whether a graft which permitted satisfactory flow of blood from the outset retained this quality throughout the period of observation. No secondary dilatation of the graft was seen. Figure 1 is an arteriogram of two femoral grafts, in place for 14 months. Before transplantation they had been preserved for 2 days in the bank. In some of the cases *arteriography* was carried out *at two different blood pressures,* i.e. Exposure I was made at 200 to 300 mm. Hg. and Exposure II at 30 to 70 mm. Hg. This revealed that the relationship between the diameter of the host vessel and that of the graft did not remain constant under different pressure conditions. The diameter of the host vessel increased and decreased with the blood pressure, while the graft appeared to be rather rigid. The grafts were fully capable of performing the function of a pipeline, but apparently did not have the capacity for functional dynamic adaptation.

After the period of observation had come to an end and the dogs had been killed, a number of tests of tensile strength were made on strips of the aortic wall in and beyond the area of the operation. It was found that the actual anastomoses were at least as strong as the intact host aorta and that the grafts possessed considerable tensile strength. The risk of aneurysmal dilatation or rupture would therefore seem to be small. Elasticity curves provided an explanation for

TABLE 1

Functional results of aortic homografts.

Type of graft	Total number	Early hemorrhage from anastomosis	Thrombosis	Functioning grafts
I. *Removed under sterile precautions within 3 hours after death of the donor dog:*				
a. Stored 2–18 days in nutrient medium at +1 to +4°C	9	2	1	6 (Time graft in dog 1–15 months)
b. Stored 30–38 days in nutrient medium at +1 to +4°C	2	0	1	1 (Time graft in dog 2 months)
II. *Removed under sterile conditions 24 hours after death of donor dog*	2	0	0	2 (Time graft in dog 1–2 months)
III. *Devitalized in absolute alcohol*	1	0	small mural thrombi	1 (Time graft in dog 14 months)

TABLE 2

Functional results of homologous arterial transplants in small arteries.

Fresh	2	1	0	1 (Time graft in dog 14 months)
Stored 1–7 days in nutrient medium at +1 to +4°C	15	0	8	7 (Time graft in dog 4 days–14 months)
Stored 20–61 days at +1 to +4°C	4	0	2	2 (Time graft in dog 14 months)
Devitalized in KCN or C_2H_5OH	3	0	3	0 —

the apparent rigidity shown in the arteriograms at different blood pressures. They revealed that the grafts had very little pliability compared with the intact host aorta.

Histopathologic study of homologous grafts of aortic and peripheral arterial segments revealed certain typical reconstructive processes common to both the viable and the non-viable segments. The intima disappeared during storage or at any rate shortly after implantation. In the media the muscle cells gradually succumbed, but the elastic structure appeared to be very resistant. In the borderline area between the graft itself and the surrounding tissue of the host, the collagenous connective tissue was generally so vital and normal that it seemed in some cases as if connective tissue cells from the original adventitia of the graft had survived. On the other hand, specimens which had unquestionably been devitalized at the time of the operation showed the same picture with an uninterrupted transition into the connective tissue of the granulation tissue. In these cases the collagenous bundles of vital connective tissue cells, visible between the elastic lamellae of the graft, must have been made up of structures growing in from the host. This corresponds to the process which *Nageotte & Sencert* (1919) called "connective tissue revivescence".

It was exceedingly interesting to study the attempts made by the host animal to build up a new vessel wall inside the graft. This apparently takes place by the precipitation of fibrin on the site of the dead intima. Fibroblasts then start to grow in, beginning at the sites of the anastomoses, and collagenous and elastic fibers are eventually formed. The process is a slow one, and several months generally pass before a real membrane has had time to develop. After a year, however, the new intima has become a thick membrane, often of the same thickness as the media, which is somewhat compressed at this stage. Of particular interest is the presence of smooth muscle cells in this layer. This was noted by *Carrel,* among others, during the first decade of the century, but was later forgotten. *Schloss & Shumacker* (1950) studied the same problem as applied to venous grafts.

In this material of homologous arterial grafts, some smooth muscle cells were seen in specimens after only a few months of observation, but in no great number until a longer period had elapsed. Their genesis aroused considerable interest, as well as the suspicion that they really consisted of fibroblasts transformed through metaplasia (*Schloss & Shumacker,* 1950). Another explanation is that they consist of smooth muscle cells that have grown in from the media. I have observed certain evidence that this is the case and am therefore inclined to share the opinion that they are not transformed fibroblasts but migrant muscle cells. In general there was no great difference in the histology of implanted viable and non-viable homografts. It should be pointed out, however, that the latter showed occasional thrombi, areas of myxomatous degeneration, calcium deposits and also breaks in the lamina elastica interna. I have therefore come to believe that devitalized and dead vascular segments are somewhat less satisfactory both functionally and morphologically, even if they are, at their best, quite capable of carrying out their function.

Much more extensive experimental material and a long period of observation are required before it will be possible definitely to answer the question whether the viability and method of storage before transplantation are of any importance to its histologic fate. It would also be helpful to investigate the relationship of the blood stream to the viable and the non-viable vascular wall. It is probable that factors in the graft which prevent thrombi and to some extent favor the immigration of cells from host vessels and surrounding tissue are of great importance to the success of the transplantation. What proportion of the graft receives its nutrition from the blood stream in the lumen and what proportion is nourished by the surrounding tissue is a question that deserves a special investigation. Nor has it been possible in the present study to take up the problem of the innervation conditions.

In other words, a great many questions and a host of problems still remain unsolved.

As far as can be judged at this point, the blood-vessel bank method, which has been tested experimentally herein, can also be used for practical clinical purposes. It is impossible to state precisely how vascular segments can safely be preserved before transplantation. It seems, however, that viability (tissue respiration or cellular growth) is not a *conditio sine qua non.* If the procurement of material for a clinical blood-vessel bank large enough for a city of the size of Stockholm proves to be as difficult in the future as it has been hitherto, it will be necessary to experiment with longer storage periods, as well as other methods of preservation.

Such investigations have recently been published by *Hufnagel & Eastcott* (1951) and *Deterling, Coleman & Parshley* (1951).

SUMMARY

1. Segments of aorta and small arteries preserved at a temperature of +1° to —4° Centigrade in a buffered Tyrode solution containing 10 per cent serum, have been used as homologous transplants for bridging experimental defects of aorta and femoral artery in dogs.

2. In arteries preserved in this manner for 2–3 weeks the oxygen consumption of the arterial tissue still was significant.

3. In regard to the healing and function of these grafts the technique and the size of the recipient and donor vessel were of paramount importance—the viability of the inserted segments of lesser importance.

4. The grafts usually were capable of performing the function of a pipeline, but apparently did not have the capacity for functional dynamic adaption.

5. In spite of marked histopathologic alterations of the walls of the graft, gradual degeneration and substitution of tissue from the host, there were no signs of aneurysmatic dilitation or any rupture. The tensile strength of the aortic grafts proved to be considerable.

Such investigations have recently been published by *Hufnagel & Eastcott* (1951) and *Deterling, Coleman & Parshley* (1951).

RESUME

1. Des segments d'aorte et de petites artères conservées à une température de + 1° à + 4° centigrade dans une solution tyrode tamponnée contenant 10 % de sérum, ont été utilisés comme transplantations homologues pour remplacer expérimentalement des parties défectueuses d'aorte ou d'artères fémorales chez les chiens.

2. Dans les artères conservées de cette manière pendant 2 à 3 semaines, la consommation d'oxygène dans les tissus artériels existait toujours.

3. En ce qui concerne la guérison et la fonction de ces greffes, la technique et la taille du vaisseau receveur et donneur présentaient une importance primordiale, la viabilité des segments insérés étant de beaucoup moins d'importance.

4. En règle générale, les greffes pouvaient assumer la fonction de canalisation, mais ne semblaient pas avoir la faculté d'une adaptation fonctionnelle dynamique.

5. Malgré des altérations histopathologiques marquées des parois des greffes, d'une dégénération graduelle et d'une substitution des tissus chez le receveur, il n'y avait aucun signe de dilatation aneurysmatique ou de rupture. La force de tension des greffes d'aorte s'est montrée considérable.

ZUSAMMENFASSUNG

1. Segmente von Aorta und kleinen Arterien, aufbewahrt in einer, 10 % Serum enthaltenden, Tyrode-Pufferlosung bei einer Temperatur von +1° bis +4° Celsius, wurden als homologe Transplantate zur Überbrückung von experimentellen Defekten der Aorta und a. femoralis an Hunden verwendet.

2. Bei Arterien, die in dieser Weise 2–3 Wochen hindurch konserviert wurden, war der Oxygenverbrauch des arteriellen Gewebes noch deutlich vorhanden.

3. Hinsichtlich der Einheilung und Funktion des Transplantates war die Operationstechnik und die Grösse des Empfänger- und Spendergefässes von ausserordentlicher Wichtigkeit – die Lebensfähigkeit des eingepflanzten Segmentes hingegen von geringerer Bedeutung.

4. Die Transplantate waren im allgemeinen fähig die Funktion einer Röhrenleitung zu übernehmen, hatten aber anscheinend nicht die Fähigkeit der funktionell-dynamischen Anpassung.

5. Trotz ausgeprägter histo-pathologischer Veränderungen der Wand des Transplantates, schrittweiser Degeneration und Gewebsersatz vom Wirtsgewebe aus, wurden keinerlei Zeichen von Aneurismabildung oder Ruptur gefunden. Die Festigkeit des Aortentransplantates gegenüber Dehnungsbelastung war bedeutend.

Derartige Untersuchungen sind neuerdings von *Hufnagel & Eastcott* (1951) und *Deterling, Coleman & Parshley* (1951) veröffentlicht worden.

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