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From the Orthopaedic Department of the Caroline Institute Stockholm.
Chief: Professor Sten Friberg

ARTERIAL HOMOGRAFTS

AN EXPERIMENTAL STUDY IN DOGS

By

TOR HIERTONN

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CATHERINE DJURKLOU

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TO THE MEMORY OF MY FATHER
WHO SAW THE BEGINNING OF THIS WORK

TO MY MOTHER

TO MY WIFE

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PREFACE

The idea for the present study sprang directly from the work on preservation and transplantation of arterial grafts published by *Gross* and coworkers in Boston in the autumn of 1949. My colleague, *Tage Borg,* and I then became fascinated by the possibilities for reconstructive surgery of peripheral arteries. Professor *Sten Friberg,* our chief, immediately approved and encouraged our plan to begin by learning what we could of the technique of vascular surgery and vascular grafting by means of animal experiments. It was thanks to Professor *Friberg*'s stimulating cooperation that we were able in 1949–1950 to organize an experimental blood vessel bank, as described by *Gross,* and to carry out a series of homologous aortic transplantations in dogs. The results, which were encouraging, were published in *Nordisk Medicin* by the writer in collaboration with *Tage Borg.*

Before organizing a blood vessel bank for clinical use, it was considered wise to continue experimentation with arterial homografts of various kinds. It fell to my lot to pursue the investigation and at the same time to make a special study of the functional and morphologic fate of the grafted segments after a longer period of observation. Studies of the tissue respiration of fresh arterial segments and those stored in the blood vessel bank were carried out in an attempt to answer the question whether grafts are more apt to be successful if alive (respiring) when transplanted.

At this time, when I present the results of my investigation, I would first of all express my heartfelt thanks to Professor *Sten Friberg,* who, throughout the course of my work, exterted a valued and stimulating influence both by his sound advice and his constructive criticism. Never can I forget the great confidence shown by *Sten Friberg* by his introduction of a blood vessel bank for clinical use at the Orthopaedic Department of the Caroline Institute. Nor can

I adequately express my appreciation for the great help he gave me in planning my studies abroad.

At the same time, I wish to tender my grateful thanks to *Tage Borg,* my friend and colleague, for his help at the outset of our common effort and for his generosity in letting me use the material of that period.

I would also pay tribute to the memory of Professor *Hjalmar Holmgren,* who so generously and enthusiastically put the resources of his institution at my disposition and who enriched the planning of the present work with inspiring suggestions.

The surgical part of the investigation was performed at the Surgical Clinic of the Royal Veterinary Institute. My sincere thanks are due to all the members of the Institute staff who facilitated my work there in various ways. In this respect I would make a special mention of Professor Emeritus *Gerhard Forsell* and of Professor *Nils Obel,* the present chief, whose wise guidance helped me through the various problems and complications that so often confront an experimental surgeon. Furthermore, I would like to express my personal gratitude to my friend, Dr. *Sten-Erik Olsson,* chief of the roentgen section of the same institute, for all his assistance with the arteriographic examinations. Some of the latter were performed under different conditions of blood pressure and in this connection I am indebted to Professor *Carl Georg Schmiterlöw,* chief of the pharmacologic section.

To Professor *John Runnström,* chief of the Wenner-Gren Institute, Department of Experimental Biology of the University of Stockholm, I would acknowledge my debt of gratitude for his kindness in providing quarters for my investigations on the oxygen consumption of arteriel tissue. During this part of my work I had the privilege and pleasure of receiving advice and counsel from Docent *Olof Lindberg.*

The major part of the histologic study was carried out at the Department of Histology of the Caroline Institute, the resources of which were kindly put at my disposition by Professor *Gösta Häggqvist.* Docent *Bror Rexed,* at the same institution, helped me with the histologic study. I am deeply indebted to him for his wise guidance and counsel.

Professor *Nils Ringertz,* then chief of the Department of Pathology of the S:t. Göran Hospital, showed a stimulating interest in my work.

He sacrificed much of his time in advising me on histopathologic questions and also in facilitating the practical organization of a clinical blood vessel bank. It gives me pleasure to express my great appreciation and gratitude to Professor *Ringertz.*

Mr. *Erik Steneroth,* Civil Engineer on the staff of the Royal Institute of Technology, was unsparing of his time and effort in assisting me in the studies on tensile strength, for which I am exceedingly grateful.

Docent *Lennart Goldberg* of the Caroline Institute was most helpful with the statistical analysis of the material, and I am deeply indebted to him for his support.

The positive interest that Professors *Clarence Crafoord* and *Philip Sandblom* unfailingly showed in my work was most stimulating and inspiring.

I am also indebted to Docent *Åke Lindbom* for advice concerning technique of reproducing arteriograms.

Dr. *Charles G. Johnston,* Professor of Surgery at Wayne University, College of Medicine, my respected chief and host in Detroit, facilitated in many ways the completion of the present investigation. Furthermore, his confidence in me made it possible for me to increase my experience in clinical vascular surgery. In the heartfelt gratitude I feel for Dr. *Johnston,* I would include my colleagues and friends on his staff. One of the latter, Dr. *Herbert Pedersen,* was good enough to go through parts of the manuscript.

I would also direct my sincere thanks to Miss *Karin Lundin,* photographer, Miss *Anna-Greta Lind,* laboratory technician, Miss *Puck Runefeldt,* roentgen technician, and the many others who have helped me in various institutions.

The expenses of the present study have been defrayed in part by grants from the King Gustaf V Jubilee Foundation, The Reserve Fund of the Caroline Institute, and the Orthopaedic Department of the Caroline Institute. I am deeply indebted to all these institutions. The financial burden of the work was also lightened by the doctorate fellowship awarded me by the Caroline Institute. The contrast medium "Umbradil" was generously furnished by the *Astra Pharmaceutical Corporation.*

Furthermore, grants from the State Medical Research Council, the

Norrbacka Institute, and the Department of Surgery of Wayne University, Detroit, enabled me to make an exceedingly rewarding visit to the vascular surgery centers of the United States for a period of seven months in 1952. I shall always be grateful for this opportunity to complete the present work and at the same time to enrich my experience of experimental and clinical vascular surgery with new and stimulating perspectives.

Detroit, June 1952. TOR HIERTONN

CHAPTER 1

INTRODUCTION. AN HISTORICAL SURVEY OF VASCULAR SURGERY

In times past surgeons used to check hemorrhage by cauterization with hot irons or boiling pitch. The ligature was in use in ancient times, then forgotten, and finally established by *Ambroise Paré* in the middle of the sixteenth century. For a long time the ligature remained the only definitive radical measure in the treatment of arterial damage and hemorrhage. It was not until after the advent of antisepsis and asepsis that the repair of damaged blood vessel began to gain ground. Even before that, however, surgeons had tried to realize the dream of repairing blood vessels in an effort to maintain their function. In the middle of the eighteenth century, for example, *Lambert* in England proposed a method of arterial suture, which his colleague, *Hallowell,* put into practice in 1759, when he successfully closed a gaping wound in the axillary artery by uniting the wound edges with a peg, around which he twined a thread. That operation fell into disrepute following the unsuccessful experiments of *Assman* (1773). In an historical survey of vein suture, *Clermont* (1901) mentioned a few early cases, but it was not until 1892, when *Schede* was able to report a number of vein sutures of his own, that the repair of injuries to veins came to be more generally employed. It is noteworthy that the first anastomosis between the portal vein and the vena cava (Eck's fistula) in man was performed by *Vidal* in 1903.

In modern times the first reports of effectively sutured arterial wounds, i.e., lateral arterial suture, were made by *Durante* (1892), *Heidenhain* (1895), *v. Zoege-Manteuffel* (1895) and *Israel* (1895). In 1899 *Dörfler* published two cases of lateral arterial suture and at the same time gave a survey of the other cases hitherto reported in the literature. It was quite clear that arterial suture was still regarded with some skepticism, due to the danger of insufficiency, hemorrhage from the suture holes, stricture, thrombosis and endarteritis and–in the successful cases–of the subsequent formation of an aneurysm.

The fear of such complications, however, was removed by the animal experiments of *Jassinowsky* (1889), *Murphy* (1897), *Dörfler* (1899), *Silberberg* (1899), and *Jacobsthal* (1900).

In order to facilitate uniting of completely divided vessels, a number of different methods were tested, among which those of *Murphy* (1897), *Gluck* (1898), and *Payr* (1900, 1904) were of most interest. See Figs. 1–3. Intravascular prosthesis of glass was also tried without success by *Abbe* (1894). The relatively simple suture technique, which later came to have its greatest use in anastomosis, was applied at an early stage by *Jaboulay & Briau* (1896). This method entailed eversion of the vessel ends with U-shaped sutures, or the interrupted horizontal mattress sutures. In reality the principles on which the technique of vascular surgery was later based and which often bear *Carrel*'s name were already to some extent in use at the turn of the century. *Carrel* (1902), however, improved the technique by using three stay sutures, to triangulate the opposed ends of the vessel. See Fig. 4. This greatly facilitated the anastomosis. In their first experiments with end-to-end anastomosis between the carotid and the jugular vein in dogs, *Carrel & Morel* (1902) tried to avoid including the intima in the suture. It is noteworthy that these animal experiments were stimulated by *Jaboulay*, who hoped in this way to improve poor circulation in the brain, for example, in encephalomalacia. In the course of the next few years, *Carrel* and his coworkers carried out a great many animal experiments thereby standardizing many of the techniques of vascular surgery: *Carrel* (1907, 1908, 1910, 1912); *Carrel & Guthrie* (1905, 1906); *Guthrie* (1912). The following prerequisites for satisfactory results were defined: gentle temporary hemostasis, peeling off the adventitia, avoidance of dehydration, triangulation with stay sutures, fine suture material, approximation of wide intimal surfaces by everting sutures through all layers of vessel. In the Scandinavian countries, important contributions to the technique of vascular suture at that time were made by *Jensen* (1903) and *Reinsholm* (1903).

Clinical circular arterial suture was done successfully for the first time in 1896, when *Murphy* (1897) operated on an aneurysm in a 27-year-old man, whose femoral artery had been penetrated by a bullet. After excision of the damaged area, the circulation was restored by a circular invagination suture (Fig. 1). Other examples of similar circular suture were reported by *Kümmel* (1900) and *Brougham* (1906). During that period *Matas* (1888, 1903) contributed his arteriorraphy to the treatment of aneurysm.

One of the experimental contributions from the beginning of this century was the demonstration that it was possible to bridge defects

in vessels with some type of graft. There were many successful cases of autogenous transplants, i.e., transplants from the same animal, and of homologous transplants, i.e., transplants from different animals of the same species. Transplantation of a vascular segment from an animal of another species, or heterogenous transplantation, was rarely successful.

Goyanes (1906) appears to be the first to have made clinical use of a vein *in situ* to restore continuity in an arterial defect. *Lexer* (1907) is generally accorded the honor of being the first to use successfully a free vein graft in an operation for aneurysm. He bridged an 8 centimeter long defect in the axillary artery with an autotransplant of a segment of the vena saphena magna. Circulation was restored, but the patient died five days postoperatively. The most quoted case, however, was that operated on in 1913, in which he used the saphenous vein to bridge a 16-centimeter arterial defect resulting from removal of an aneurysm in the iliac artery. Follow-up five years later showed a satisfactory flow of blood (1917). Thus, methods for the reconstruction of arterial damage had already been evolved at the time of the First World War. At that time excellent results in the treatment of aneurysms were reported by *Subbotitch* (1914), *Bier* (1915), *von Haberer* (1916, 1917). *von Bonin*'s survey included eleven cases of venous grafting, and *Warthmüller* (1917) discussed fifty-two cases from the literature. In 1925 *Lexer* published a survey of vascular reconstruction, in which he reported on fifty-eight cases of grafting, thirteen of them performed by him personally. Homologous transplantation was mentioned by *Lexer,* but was regarded at that time as a very uncertain procedure. The same year *Weglowski* (1925) reported fifty-one other cases of venous transplantation in connection with the treatment of traumatic aneurysm.

Although those good results were obtained in late reconstructive surgery, the high hopes that had been entertained for primary vascular repair in war injuries came to naught, because the delay between the injury and definitive treatment resulted in such complications as thrombosis and infection. This helped to create skepticism regarding vascular surgery, and since that type of injury was rarely encountered in peacetime, interest died out in many countries.

An important exception was the surgical treatment of *acute arterial embolism,* which has been pursued with enthusiasm and success in Sweden in particular. *Einar Key*'s basic work in 1922 revealed that occasional attempts at embolectomy had been made as early as the 1890's, and that the first successful operation of the kind was performed by *Labey* in 1911. *Key* reported thirteen successful peripheral

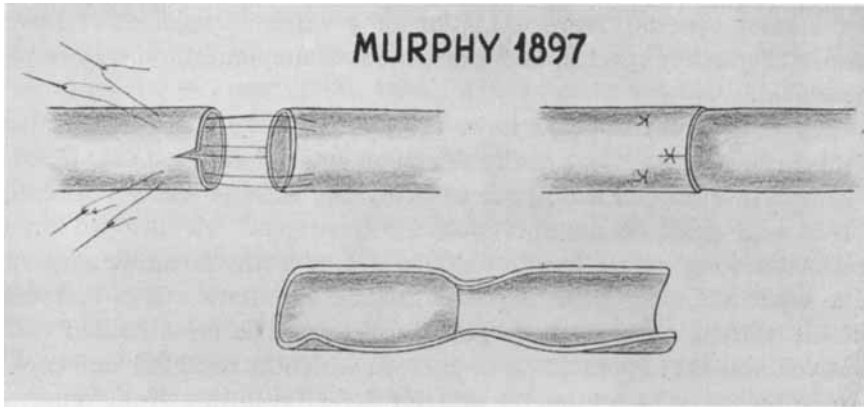


Fig. 1.

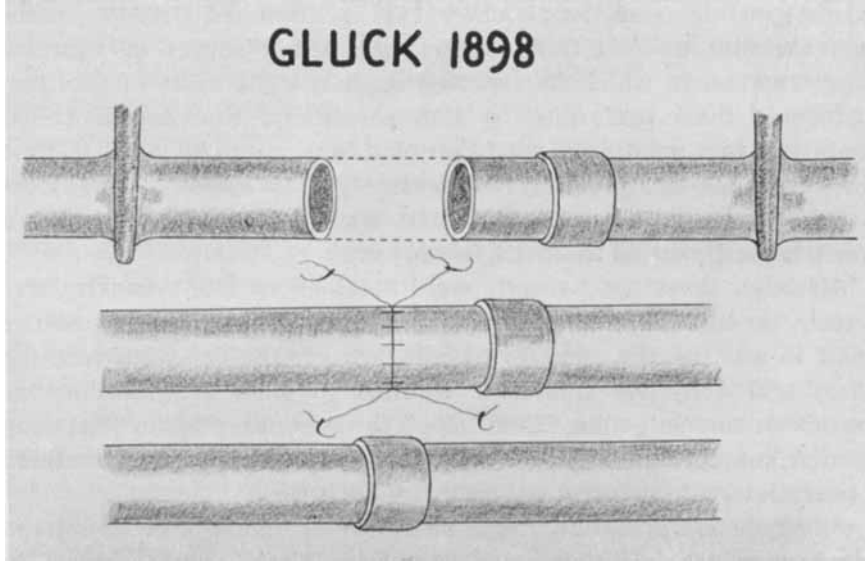
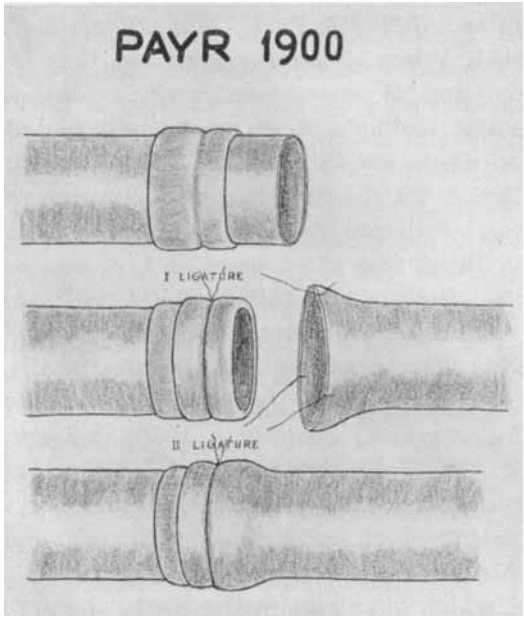


Fig. 2.



Figs. 1–3. Techniques of vascular end-to-end anastomosis.

embolectomies, six of them his own. The first of this series was done as far back as 1912. In the twenties practically every issue of *Acta Chirurgica Scandinavica* contained papers on successful operations for arterial embolism: *Michaelsson* (1924), *Olivecrona* (1924), *Aleman* (1926), *Söderlund* (1926), *Odelberg* (1926), *Nyström* (1926), *Perman* (1926). In 1929, *Key* published a general survey of embolectomies performed up to that time, and *Strömbeck* (1936) reported on the end-results of all the embolectomies carried out in Sweden up until 1932.

The dramatic removal of an embolus from the pulmonary artery, proposed by *Trendelenburg* (1907, 1908), was performed successfully for the first time by *Kirschner* in 1924. A few years later, *Crafoord* (1929) was able to save two patients and *Nyström* (1929) one patient by means of this operation.

An intensive period of development in vascular surgery began at the end of the thirties. In 1938 *Gross* performed the first successful operation for patent ductus arteriosus. The method was immediately adopted in several countries, and in Sweden its principal proponents were *Crafoord, Sandblom, Wulff* and *G. Pettersson.* The clinical aspects of the congenital cardiovascular malformations were studied in great detail, and experimental methods of correcting similar conditions in

animals were tested: *Blalock & Park* (1944), *Gross & Hufnagel* (1945), *G. Murray* (1948). When it became apparent that the surgical correction of cardiac and vascular anomalies was possible, research and study of diagnostic methods were greatly stimulated. Catherization of the heart and angiocardiography are two of the newer important diagnostic methods, which, together with improved techniques of anesthesiology and artificial respiration, made it safer for surgeons to operate upon heart and great vessels: *Crafoord & Nylin* (1945), *Blalock & Taussig* (1945), *Gross* (1945), *Potts, Smith & Gibson* (1946), *Brock* (1948), *Harken, Ellis, Ware & Norman* (1948), *Bailey* (1949), *Shumacker* (1951). The disorders and diseases of the heart and the central vessels, which today are treated surgically, were the subject of an illuminating survey by *Sandblom* (1950) in *Svensk Läkartidning* and by *Blalock* (1951) in the Journal of Thoracic Surgery. Most recently, the medical, roentgenologic and surgical aspects of the problem were discussed at the *Medicinsk Riksstämma* in Stockholm in 1951: *Mannheimer; Jönsson & Karnell; Werkö; Crafoord* (1952).

The interest which gave rise to the broad developments in heart surgery was also reflected in the attempts at the repair of *peripheral* vascular injuries during the Second World War. In an interesting analysis of 2,471 arterial injuries from World War II, *De Bakey & Simeone* (1946) drew attention to certain factors which made it impossible to practise reconstructive vascular surgery to any great extent: The long interval between the injury and adequate treatment, the lack of specially trained surgeons in the advanced medical stations, the combination of injuries with extensive destruction and grave risk of infection, and the large number of casualties and the urgency of purely life-saving operations. Although conditions for vascular repair were poor, there was obviously a great need for improvement; thus, a detailed study of 3,000 amputations revealed that the indication for operation was arterial damage in 20 per cent of the cases. To meet such a need, *De Bakey & Simeone* (1946) pointed out that general surgeons in World War II experimented with vascular prostheses of glass and plastic in order to restore the continuity in damaged arteries. The main purpose of the prostheses was to maintain circulation temporarily until the collateral circulation had time to develop. The tissue tolerance was relatively good, but the risk of thrombosis was considerable. Glass as well as silver prostheses had already been tested during the First World War by *Tuffier* (1915), among others. Another procedure, which caused considerable discussion, was the "non-suture-method" described by *Blakemore, Lord & Stefko* (1942). In principle, this method had long ago been used by several surgeons, notably *Nitze*

(1897), *Payr* (1900, 1904), *Höpfner* (1903), and *Lexer* (1907), for the purpose of reuniting completely severed blood vessels without sutures. The method is illustrated in Fig. 3, reproduced from *Payr*'s works. The central stump of the vessel was drawn through the metal tube, over which the end of the vessel was forced so as to turn the intima outward. The vessel stump so everted was attached by ligature No. I to the tube, after which it was invaginated into the peripheral vascular stump. The latter was attached over the invagination with ligature No. II.

The same principle has been used in bridging long defects in the arteries with free venous grafts. The method was tested in about forty cases during the war according to *De Bakey & Simeone* (1946). This method may be useful under certain circumstances, but most vascular surgeons now prefer direct suture procedures.

The surgeons of the Second World War had in many respects much better opportunities for reconstructive vascular surgery than their colleagues of World War I. Despite this, primary reconstructive operations appear to have been rare: *Killian* (1942), *Khenkin* (1944). In the definitive treatment of aneurysms, on the other hand, reconstructive surgery has been widely used. Here, the free venous autograft represents a major advance, which even in very difficult cases permits radical removal of the aneurysm and restoration of circulation. Several German workers, *Rhen* (1942, 1943), *Schneider & Bätzner* (1947), *Bätzner* (1947) have advocated *Carrel*'s and *Lexer*'s old principle of attempting to restore the normal flow of the blood by direct suture or venous grafting.

Parallelling the development of vascular surgery in the above fields was the approach to chronic obliterative arterial disease. As early as 1908 *Carrel & Leriche* planned the excision of a diseased arterial segment and replacement with a vein graft. Because of the extent of the disease noticed at the time of surgery, the operation was not performed (*Bérard,* 1908). In spite of the early conception of such an operation, the treatment of peripheral arterial disease consisted of various non-surgical measures, lumbar and cervical sympathetic block or resection, and amputation.

Because of the inadequacy and uncertainty of such treatment, other measures have recently been tried. "Thromboendarterectomy" followed by heparin treatment, has been successfully used to treat localized arterial obstruction in great arteries, *J. Cid dos Santos* (1947), *Leriche & Kunlin* (1947), *Bazy, Huguier, Reboul & Laubry* (1949), *Bazy* (1949), *Reboul & Laubry* (1950), *Fontaine & Hubinont* (1950), *Wylie, Kerr & Davies* (1951), *Crafoord & Hiertonn* (1952).

Early attempts to revascularize an ischemic extremity by arteriovenous anastomosis were made by many: *San Martin y Satrustegui* (1902), *Jaboulay* (1902), *Gallois & Pinatelle* (1903), *Wieting* (1908), *Roussiel* (1916), *Bernheim* (1931). There then followed a period of inactivity until at the Surgical Forum of the American College of Surgeons in Chicago in 1949, *Johnston & Jordan* again brought up the possibility of directing arterial blood in a distal direction through superficial or deep veins by means of an arteriovenous anastomosis. They stressed the importance of placing a ligature about the proximal portion of the vein after the fistula has become well established.

Finally, in the last few years, measures again have been proposed to replace the diseased portions of the vessels and restore the circulation. In the most recent literature, *venous autotransplants* for the bridging of defects in the peripheral arteries have been recommended by *Fontaine & Hubinont* (1951), *Kunlin* (1951), *Bouchard* (1950), *Fontaine, Buck, Riveaux & Hubinont* (1951), *Julian & Dye* (1952). The veins generally available for this purpose are the superficial femoral and the saphenous veins. In consideration of the valves, the transplanted vein should be reversed so that the distal part with its smaller diameter is sutured to the larger proximal arterial end. However, this frequently entails technical difficulties, which at times jeopardize the results. Faced with the ever-growing need for a method to replace defects in the aorta and the great vessels, American surgeons, in particular, have reverted to the old concept of fresh or preserved arterial homografts.

This method fell into disrepute after *Pirovano*'s unsuccessful attempt in 1910 to replace a defect in the iliac artery with a homologous arterial transplant. Recently, blood vessel banks have been established in several institutions, to be certain of an adequate supply of segments of aorta and peripheral arteries of suitable caliber and length. The New York Society for Cardio-Vascular Surgery is running an arterial bank with a fund donated by the New York Heart Association as described by *Keefer & al.* (1951). A blood vessel bank under military conditions was described by *Hurwitt* (1950). Arterial segments, removed under sterile conditions from fresh cadaver, are kept alive by preservation in a suitable medium for days or even weeks. *Gross* and coworkers (1949) were the first to publish a human series of homologous arterial grafts. In nine cases arterial grafts were used as shunts between the aorta and the pulmonary artery and in six cases to bridge the defect following resection of aortic coarctations. The preliminary results, which were very promising, caused considerable stir in the professional literature. They served as stimulus to vigorous research on the subject in various countries. In addition to extensive

experimental investigations on animals, *Gross* has already supplemented his clinical experience of homologous arterial transplantation: In a recent paper (1951), he reported on nineteen cases of aortic coarctation treated by resection plus free aortic grafting. Two of the patients died of uremia, and seventeen showed progressive improvement. Other surgeons, too, have reported on the clinical use of blood vessel bank material: *Swan, Maaske, Johnson & Grover* (1950), *Beattie, Cooke, Paul & Orbison* (1951), *Lam & Aram* (1951), *Oudot* (1951), *Dubost, Allary & Oeconomos* (1952).

As already pointed out, there has long been an acute need of a reliable procedure for bridging defects in the great arteries. In certain parts of the body the collateral circulation becomes inadequate if the flow in the main artery is blocked. This is true especially in the extremities. Ligature of the femoral and popliteal arteries due to a war injury is often followed by loss of a part of the limb. *Rose, Hess & Welch* (1946), for example, stated that amputation was done in 70 to 80 per cent of a series of vascular injuries submitted to ligature during the Second World War. Even if the collateral circulation develops after ligature, thereby saving the vitality of the extremity, the patient is often incapacitated by symptoms of insufficiency on exertion.

Acute arterial injuries are less common in peace time. When the situation is encountered it is difficult to decide which of the several possible operative procedures should be selected. In the past, those measures which have failed to restore the continuity of the injured vessel have given poor results. Since it has been demonstrated that arterial transplants are successful and since it is now possible to have available homologous arterial segments of different diameter and length, preserved in blood vessel banks, it is hoped that blood vessel continuity can be restored in acute injuries, and that better results will follow.

It is also possible that a certain group of patients with incapacitating pain in the extremities due to local chronic arterial obliteration can be advantageously treated by radical excision of the obstructed area and bridging of the defect with a vascular transplant. Likewise the field of tumor surgery may be advanced if major vessels can be sacrificed and replaced.

In other words, homologous arterial transplantation with material from a vessel bank appears to constitute a method which can be used in several areas of surgery. Before establishing such a bank for relatively general use in Sweden, it was considered advisable to institute experimental studies in order to learn about the functional and morphological fate of the transplants.

CHAPTER 2

THE "VIABILITY" OF PRESERVED ARTERIAL SEGMENTS

The words "viable" and "viability" are often used to indicate that a transplant contains living cells presumably capable of surviving in their new environment after transplantation. As will be shown later we still have not enough knowledge about the fate of transplanted tissue. Several biological methods have been used to demonstrate that a tissue or a cell is alive, but no histologic procedure for distinguishing between "live" and "dead" tissue has yet been evolved. Meantime, it must be borne in mind that the various layers and structures of an artery may vary with regard to both cellular activity and resistance after transplantation. The expression "viable graft" is, therefore, ambiguous, the more so since it constitutes a statement on the prognosis of the cells. It is used in the present study when discussing the findings of other workers to indicate the presence of living cells which may survive transplantation–in contradistinction to transplanted dead or devitalized tissue.

At the beginning of the century, *Carrel* found that arteries isolated from the organism could be preserved by refrigeration for days and even weeks and then used for grafting. Among the preserving media he tested were isotonic saline solution, Locke's solution, serum, defibrinated blood, and vaseline. *Carrel* (1910, 1912) was of the opinion that vascular segments stored in this way for 1 to 14 days remained in a state of "latent life". He based his conclusion on the fact that after transplantation the function was maintained and healing occurred by continuity without important gross changes. Degenerative changes were found, it is true, but they were less pronounced than with the transplantation of devitalized vessels. Although *Carrel* believed that the grafts were viable, his published reports included no good objective evidence in support of that conviction. *Bode & Fabian* (1910) removed arterial segments from dogs within one hour after the animals had been sacrificed. The vessels

were stored in Ringer's solution in the refrigerator for 1 to 60 days. After storage gross examination of the segments revealed them to be somewhat paler and tougher than fresh vessels. There was no microscopic evidence of degeneration. The authors came to no conclusion regarding viability, but felt that their studies indicated that, for successful grafting, vessels could be stored for periods of no longer than 35–50 days.

The foundation for tissue culture was laid by *Harrison* in 1907, when he proved that a tissue could be kept in a state of "active life" outside the organism as long as it was supplied with sufficient nourishment. The method was adopted by *Carrel* and rapidly developed into a biologic procedure of great value: *Carrel & Burrows* (1911), *Carrel* (1912, 1913). In a recently published handbook, *Parker* (1950) gave a survey of the development of this method and of its modern techniques. It has been found that a fragment of tissue removed from a living or recently sacrificed animal can be stored for a period at a low temperature in a suitable medium and still demonstrate active growth of cells. *Hanks & Wallace* (1949), for example, found cell growth in pieces of skin that had been stored for 8 days in Tyrode solution with a 10 per cent admixture of serum. It was considered that the blood cells increased the acidity and decreased the buffer action. Therefore, a noncellular fluid was preferred. It further appeared that at a temperature of $\pm 0°C$ the oxygen dissolved in the medium sufficed to maintain the very low rate of metabolism. Storage at an appreciably higher temperature required the addition of oxygen.

In 1949, *Peirce, Gross, Bill & Merrill* published their extensive investigations on various methods of storing vessel segments outside the organism. They found that the vessel segments retained their normal structure for a long period if they were stored at a temperature between $+1°$ and $+4°C$ in 10 per cent serum in a balanced salt solution. Parts of the vessel segments preserved in this way were studied in tissue cultures. With the help of a meticulous technique of removing the specimens and of regular checks and adjustment of the pH, etc., definite cellular growth could be demonstrated in cultures of arterial segments which had been preserved for as long a time as six to seven weeks. If isotonic saline solution without buffer action was used as a medium, however, cellular growth could not be demonstrated after such a long period of storage. The flasks in which the vessel segments were stored were at first closed with cotton. Later rubber stoppers were used instead, for it was found that the rate of metabolism in vessel segments stored at $+1°$ to $+4°C$ was not high enough to require a free exchange of oxygen and carbon dioxide.

Gross, Bill & Peirce (1949) stated that the viability of the arterial segments, determined by tissue culture, was of fundamental importance to healing and function. *Bätzner & Grupp* (1951) were of a similar opinion and stressed that only *viable* homografts could be considered for clinical use. According to their experimental results, a retained contractility was found in strips of arteries, preserved for as long as eleven days in a Tyrode's solution with 10 per cent serum at +1° to +4°C and that this quality of contractility invariably disappeared after a long period of storage, they recommended that bank grafts should be tested for contractility before being used. They found also that the vessel segments lost their contractility if stored in Ringer's solution for only a few days. The medium that permitted the longest storage with retained contractility in these experiments was found to be preserved blood, particularly when it was changed every fourth day.

Tissue culture following freezing methods for preservation of arterial segments showed no cellular growth according to *Deterling, Coleman & Parshley* (1951). Studying arterial segments in a blood vessel bank of the same type used by *Gross* and associates *Coleman, Deterling & Parshley* (1951) were unable to demonstrate cellular growth of grafts, stored longer than 38 days. Tissue cultures of vessels, stripped of adventitia, and so stored for more than 23 days revealed no growth of cells from the media and intima. Since those vessels, too, could be successfully transplanted and most parts of the grafts seemed to be replaced by the host it was felt that the demonstration of fibroblast viability at the time of operation was not of any great importance. The same opinion was held by *Swan, Robertson & Johnson* (1950). The transplantation of aortic segments stored in a nutrient medium for shorter periods and thus viable appears, however, to be somewhat superior to transplantation of similar segments stored for longer periods. Although several factors indicate that the viability of the aortic homograft *per se* is not a requisite for success it is evident that opinions differ about the importance of using a method of storage which is capable of keeping cells alive in preserved arterial segments.

The consumption of oxygen by an organ or a tissue is the result of a complex series of reactions. Compared with parenchymatous organs, such as the liver and kidneys, vascular tissue has a very low metabolism and consequently, a very low oxygen consumption. *Briggs, Chernick & Chaikoff* (1949) estimated the oxygen consumption of aortic tissue, for example, at one-tenth that of the liver and one-twentieth that of the kidneys. The major proportion of the measurable

metabolism of vessel wall specimens would appear to take place in the superficial layers, i.e., the intima and adventitia. They found no appreciable difference between the oxygen consumption in the thoracic and abdominal parts of aorta of rats.

No studies have hitherto been published on the tissue respiration of arterial segments stored in Tyrode's solution with 10 per cent serum at a temperature of $+1°$ to $+4°$C. It was therefore decided that tissue respiration studies should be carried out on bank grafts in an attempt to answer the question whether preserved grafts are more apt to be successful if alive (respiring) when transplanted.

CHAPTER 3

ARTERIAL HOMOGRAFTS—FUNCTIONAL AND MORPHOLOGIC ASPECTS

TRANSPLANTATION WITH FRESH ARTERIAL SEGMENTS

Höpfner (1903) was the first to make a successful experimental transplantation of a free arterial graft. The basis of the experiment was actually to ascertain whether extensive removal of the adventitia of arteries, by cutting the vasa vasorum, really led to nutritional damage in the wall and to secondary thrombosis–an opinion that was held at that time. *Höpfner* exposed a 4.5-centimeter section of the carotid in a dog. Vessel clamps were then applied proximally and distally, and the entire section was excised and turned, after which the anastomoses were accomplished by means of Payr tubes (Fig. 3). This represented the first successful autologous arterial transplantation. The transplant functioned as it should and was removed for study four and a half weeks later. At that time the wall was thickened, but otherwise the structure was normal. *Höpfner* also transplanted a segment of the carotid to the femoral artery and a segment of the femoral artery to the carotid in the same dog. This type of fresh autotransplant usually heals into place as a living structure without other appreciable changes than possibly some degree of intimal hyperplasia, as described by *Borst & Enderlen* (1909), *Stich & Zoeppritz* (1909), *Yamanoüchi* (1911) among others and, more recently, by *Miller, Callow, Welch & Mac Mahon* (1951).

After his success with autologous transplantation, *Höpfner* proceeded to implant a segment of the femoral artery of one dog into the carotid artery of another. After 45 days he inspected the graft, which had functioned admirably. There was good healing at the sites of anastamosis, and microscopic examination revealed no appreciable change of the graft. Because of such success, *Höpfner* expected great things of homologous transplantation of arteries in clinical practice–

particularly since all his experiments with autotransplantation of veins had failed.

A survey of the literature on vessel transplantation soon reveals that opinions have differed widely, particularly with regard to the arterial homografts. This is due to the fact that some workers based their conclusions mainly on the ability of the transplants to heal in place or to maintain function, while others stressed the histologic fate of the grafts after varying periods of time.

Capelle (1908) and *Stich & Zoeppritz* (1909) agreed with *Höpfner* that fresh arterial homotransplants healed with unchanged structure in the same way as fresh autotransplants. Arterial segments even from recently sacrificed animals were tested at an early stage. *Stich, Makkas & Dowman* (1907), for example, reported three functionally successful arterial homografts in which the segments were removed under sterile conditions from recently sacrificed dogs. They concluded optimistically that healing took place in the same way as with fresh autografts. The period of observation, however, amounted to only 4 to 11 days.

Other experimental surgeons of the same period, including *Ward* (1908), *Borst & Enderlen* (1909), *Villard, Tavernier & Perrin* (1911), *Yamanoüchi* (1911), *Ingebrigtsen* (1912), *Castiglioni* (1913), and *Goodman* (1917) studied fresh homografts after a somewhat longer period of observation and found definite degeneration in the smooth muscle of the media, a generally well preserved elastic structure, and new formation of an inner and outer layer of the wall. More recently *Williamson & Mann* (1947) had poor functional results with fresh carotid homografts on dogs. They did not feel that vascular homografts could be used in clinical practice. *Miller, Callow, Welch & MacMahon* (1951) found that 90 per cent fresh femoral homografts and 87 per cent of the preserved type functioned well under experimental conditions of a very critical nature. They felt that both types would be useful for replacement in cases of human arterial injury.

TRANSPLANTATION WITH ARTERIAL SEGMENTS, PRESERVED IN A LIVING STATE

If homologous arterial transplants were to become surgically practicable, it was necessary to evolve methods which permitted prolonged storage. *Carrel* (1910, 1912) was especially aware of this. He tested different media and then studied the functional and histologic fate of the grafts. As mentioned on page 22, *Carrel* considered that

arterial segments removed under sterile conditions at operation or within an hour or so of death could be kept in a state of "latent life" for some days, if stored in a cold place in a medium such as defibrinated blood or isotonic saline solution. He achieved good functional results with transplants so stored.

An examination of *Carrel*'s records reveals that the media of the transplants was generally well preserved in cases in which the period of observation was short, while it tended to degenerate after a period of observation of only two or three months. In the new layer of connective tissue which grew out over the inner aspect of the transplant, *Carrel* sometimes found, after a longer period of observation, cells resembling smooth muscle cells and also newly formed elastic fibers. These interesting observations have not always been confirmed by subsequent investigators.

Carrel (1910) stated that a criterion of a kind of viability in the vascular segments stored at low temperature in a suitable medium was their retention of a normal appearance for a long period after transplantation, as compared with the very rapid degeneration of the non-viable transplant. His opinion in this respect has been the subject of considerable discussion by other workers, and was, for instance, criticized by *Klotz, Permar & Guthrie* (1923), and recently by *Schloss & Shumacker* (1950).

Transplants, removed from sacrificed dogs one hour postmortem and stored at a low temperature in Ringer's solution for one to sixty days, were tested by *Bode & Fabian* (1910) in nine peripheral arterial transplantations in dogs. Most of the grafts developed thrombi and became obliterated. One transplant, stored for 24 hours, showed completely intact structure after nine days. Another graft, also stored for 24 hours before the operation, still retained its elastic structure after 38 days, although staining of the nuclei in the media could no longer be observed. One of *Yamanoüchi*'s (1911) two experiments with arterial segments from sacrificed dogs led to thrombosis. The other had a satisfactory lumen 36 days postoperatively. The adventitia was the site of active cell proliferation. The media was completely degenerated and the intima was thickened. In another series of eleven dogs, *Yamanoüchi* (1911) transplanted homologous arterial segments, which had been stored for one to ten days in various electrolyte solutions at low temperature. The period of observation varied from 2 to 143 days. The functional results in this series were very good, with thrombi developing in only one case. All the cases, however, revealed extensive histologic changes of approximately the same kind as with fresh homologous transplants, i.e., degeneration of the smooth muscle and

successive replacement with connective tissue. *Yamanoüchi* did not consider that homologous transplantation with material from surgical cases or cadavers would come to have any practical importance. In this connection, it made no difference whether the grafting was done with fresh or stored arterial segments, for the risk of thrombosis and necrosis was large in either case. *Lexer* (1919) was of the same opinion.

As mentioned before *Gross, Bill & Peirce* (1949) showed that if removed under sterile conditions within six hours of death segments of dog aorta stored at +1° to +4°C in a Tyrode's solution to which serum and antibiotics had been added, preserved vascular structure and viability for 35 to 40 days. They succeeded in transplanting such segments to the abdominal aorta of other dogs. The results were better than with transplants stored at —70°C. The histologic picture was not described.

Swan, Robertson & Johnson (1950) made 35 experiments using ordinary Ringer's solution with 10 per cent serum as a medium and a temperature of +3° to +8°C. Detailed histologic reports and photomicrographs revealed that, after homologous transplantation, the intima and adventitia were gradually completely substituted by connective tissue from the host vessel. The major part of the media degenerated. These processes took place more rapidly in grafts which had been stored for more than 40 days than in those stored shorter periods. The authors considered it probable that, in some cases, parts of the elastic structure and of the smooth muscle of the media were able to survive.

Dalem (1950) replaced the aortic bifurcation in dogs by homografts which prior to the implantation had been stored in 10 per cent serum in Ringer's solution. In 13 of the 20 animals the transplantation was successful. Time of observation varied from 39 to 232 days. Two of the functioning grafts (before implantation stored 36 resp. 39 days) were calcified. The histologic examination revealed degeneration of the media and formation of a new intima from the host vessel. There was a marked thickening of the adventitial layer of the graft. Thus, it was demonstrated that successful substitution of the terminal aorta and bifurcation by preserved homograft is possible in dogs.

Miller, Callow, Welch & MacMahon (1951) described necrosis of all layers in 20 functioning homografts from the femoral artery. Those transplants had been refrigerated for 1 to 22 days in Tyrode's solution containing serum. The period of observation was at most one year. All cellular elements in the media disappeared at an early stage and were replaced by an amorphous mass. Nearest the lumen, the trans-

plant was covered by a layer of new fibrocellular tissue, while externally it was surrounded by fibrous connective tissue.

The studies discussed above concerned short transplants. *McCune & Blades* (1951) used 18 to 22-centimeter long aorta segments. Before use, the segments were stored for 1 to 15 days at +1° to +4°C in a balanced salt solution with 10 per cent serum. The observation period was two months. The incidence of thrombosis was not related to the length of the graft. The histologic findings did not vary from those of shorter homotransplants. *McCune &* co-workers (1952) also made a study of the nutrition of blood vessel grafts. The new intima was presumed to receive its nourishment from the circulating blood, while most of the remainder of the graft wall was probably nourished by surrounding tissue. Consequently, the surgeon should always make sure that a free vessel graft is covered by muscular or other highly vascular tissue belonging to the host.

TRANSPLANTATION WITH DEVITALIZED HOMOLOGOUS ARTERIAL SEGMENTS

The necessity of using viable material to bridge a vessel defect has been questioned for many years. In *Carrel*'s (1908, 1912) experience, frozen and calcium chloride dehydrated transplants were far less successful than transplants which had been stored in a liquid medium as described on page 28.

The technique of preservation of vessels by means of freezing was studied by *Blakemore & Lord* (1945), *Hufnagel* (1947), *Hufnagel & Eastcott* (1951), *Deterling, Coleman & Parshley* (1951), *Meeker & Gross* (1951), and more recently freeze-drying (lyophilization) has been reported as a method for storage of vascular segments by *Marrangoni & Cecchini* (1951) and *Natellis & Visalli* (1951). It was assumed that frozen segments were non-viable. Using large vessels, however, the functional results with frozen grafts appear to have been comparable with those secured with the transplantation of viable segments. Judging by the various reports, the histologic changes in the transplants were of the same nature as those in other homologous arterial transplants. The advantage of frozen segments would appear to be that the method permits an almost unlimited period of storage. *Hufnagel & Eastcott* (1951) found, for example, that there was no functional or histopathologic difference between grafts that previously had been stored for six months in deep-freeze and those that had been frozen for only a few days. Although it was considered that the viability was lost on

freezing or on thawing, *Hufnagel* (1952) stated that, in rare cases, he had found cellular growth in tissue cultures of arterial segments which had first been frozen and thawed.

Vascular segments fixed in formalin or alcohol have been used as homografts by several authors. *Levin & Larkin* (1907, 1909) transplanted aortic segments fixed in formalin into the abdominal aorta in two dogs. Although the lumen was patent 10–17 days later, there was microscopic evidence of degeneration of smooth muscle. However, there was relatively good resistance of the elastic tissue. In a third case with a period of observation of two and a half months, the lumen was entirely obliterated and the transplant partly calcified. *Bode & Fabian* (1910) observed well-preserved structures six days after transplantation of a formalin-fixed segment of the femoral artery which had been implanted in the carotid artery of a dog. *Yamanoüchi* (1911) studied twelve devitalized arterial homografts (three of them boiled, three of them fixed in formalin and six in sublimate). The results were poor. Function was maintained in only one case in which the transplant had been fixed in formalin. After 24 days the specimen exhibited major histologic changes with loss of smooth muscle, fragmentation of the elastic substance, vigorous cellular proliferation in the adventitia, and loss of the intima except at the site of the sutures. Resorption had obviously taken place more rapidly than substitution. Consequently, there was considered to be grave risk of thrombosis, necrosis and hemorrhage.

Nageotte & Sencert (1919) examined a formalin-preserved carotid transplant artery three months postoperatively. The inner aspect was covered by a new intima originating from the endothelium of the host vessel. Muscle-like cells, "myome de régénération", were found near the suture lines and were assumed to have sprung from the media of the host artery. The muscle tissue of the transplant had completely disappeared, but the elastic tissue was still present. The wall was studded with fibroblasts and wandering cells. The adventitia was of relatively normal appearance.

The longest period of observation of a vessel transplant was reported by *Klotz, Permar & Guthrie* (1923). In their case a segment of the vena cava was stored in formalin for 60 days and was then implanted in the abdominal aorta of another dog. Eleven years later the specimen was found to be considerably dilated. The wall was thickened and calcified, and the smooth muscle had completely disappeared. Remains of the elastic structure were present in the form of small refractile bodies. Strong layers of connective tissue were seen on both sides of this central, partly calcified layer; the inside was

coated with endothelium. A small parietal thrombus was seen at a place which lacked endothelium.

Hosomi (1928) reported a series of 21 transplants devitalized in alcohol. In those the smooth muscle generally disappeared after 60 days. After 150 days, there was marked fibrosis with shrinkage and finally obliteration. *Hosomi* shared *Nageotte*'s & *Sencert*'s opinion that preserved dead vessels were not to be recommended for clinical use. *Paolucci & Tosatti* (1950), on the other hand, were more optimistic and apparently did not hesitate to use arterial segments preserved in alcohol for clinical purposes.

Also, *Peirce, Rheinlander, Moritz, Gross & Merrill* (1949) reported relatively good results with homologous aortic transplants, preserved in formalin, in which a special effort was made to minimize the irritant effect of the formalin on the host vessel. The period of observation was nine months. They found extensive histologic changes with degeneration of the media and fragmentation of the elastic tissue. Nevertheless, a "new intima" and a new adventitia had formed relatively quickly. Even if the results were not so good as with non-devitalized transplants, they still were a stimulation to further research. The authors did not appear to be entirely opposed to the idea of using transplants stored in formalin in human beings in emergency cases.

SUMMARY

It appears to be generally agreed that homologous transplants consisting of fresh arterial segments or arterial segments stored under special conditions may be used successfully. Opinions differ, however, concerning the processes that take place in the graft after transplantation. It is still not known whether or not the cells of the transplant survive, or whether they die and are totally replaced by new cells from the host. If the great majority of the cells in the graft are doomed to destruction and the graft is simply regarded as an organic tube or framework, the method of replacement by new cells is still not clearly understood.

Definitely devitalized and fixed arterial segments may become united in continuity and carry out the function for which they are intended, despite marked histologic changes.

It is probable that suitable transplants whether live or devitalized must fulfill the following two criteria. They must be capable of restoring the arterial pathway and they must allow the ingrowth of cells from the host for a gradual substitution.

CHAPTER 4

THE TENSILE STRENGTH AND ELASTIC PROPERTIES OF NORMAL AND SUTURED ARTERIAL WALL

The elastic properties of the arterial wall have been discussed in a number of earlier works, only a few of which will be mentioned in the following. Most of the studies concern the deformation of the vascular wall when loaded by a tensile force applied on excised strips or rings of vascular tissue or on the whole vessel. Another type of experimentation has dealt with intraluminal increase of pressure and recording of the increase in volume.

Definitions. Before referring to these earlier investigations, it would perhaps be advisable to define certain important concepts and at the same time to review a few theoretical facts concerning relevant aspects of the theory of strength of materials. "*Strain*" is the deformation (elongation) or change in linear dimensions per unit length which occurs in an object as a result of an outside force that has been applied to it. "*Stress*" is the internal resistance per unit area which opposes the deforming action of the outside force. Outside forces trying to pull apart the object produce, in other words, tensile strains and stresses. Stress and strain are related by a function of proportionality, called "*modulus of elasticity*", i.e. a measure of the resistance to deformation. If, for example, a homogeneous body of uniform diameter and breadth and with the cross-section A is loaded by the tensile force P, the internal stresses are assumed to be uniformly distributed over the cross-section and will balance P. The *specific tensile stress* (σ) then becomes $\sigma = \frac{d}{A}$. This tensile stress causes some lengthening of the object, at the same time as the cross-section decreases. The *unit elongation,* or *strain,* (ε) is defined as the ratio of the increase in length to the original length, i.e.

$$\varepsilon = \frac{\text{elongation}}{\text{original length}}$$

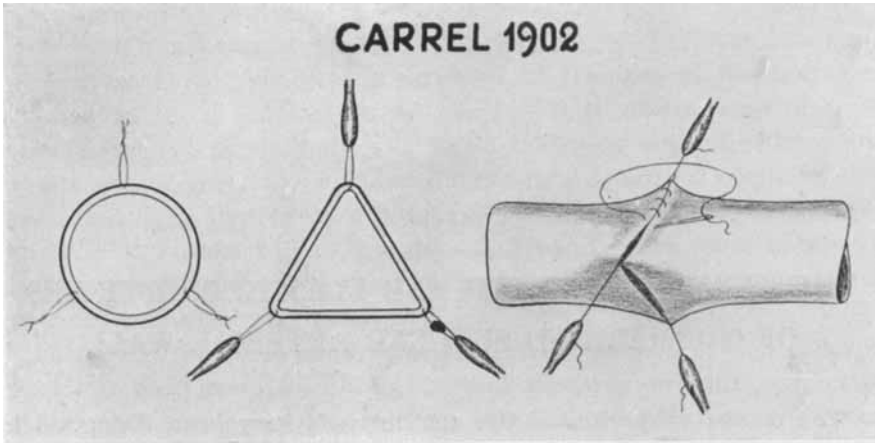


Fig. 4.
Techniques of vascular end-to-end anastomosis.

For many inorganic bodies there is, according to *Hooke*'s *Law,* a direct relation between tensile stress and strain (unit deformation). Consequently, when one is plotted against the other, a straight line is obtained (stress-strain diagram). It was long ago proved by *Wertheim*'s basic experiments (1847), for example, that the total stress-strain diagram for the wall of an artery was not a straight line. Since that time, several workers, notably *Triepel* (1902), *Frank* (1920) and *Ranke* (1925) have applied mathematical analyses to this field, thereby facilitating more exact computations applicable in the physiology of circulation.

In physics a body is called "*elastic*" if it returns to its original shape when the deforming forces (e.g., tensile force) are withdrawn. This may be true up to a certain load corresponding to the elastic limit of the material in question. If this limit is exceeded, *permanent deformation* (elongation) will be noted after unloading. Further increase in load will finally cause rupture, when the *ultimate stress* ($\sigma_{\text{ult.}}$) has been reached. If P_{max} denotes the ultimate load and A denotes the cross-section, we find $\sigma_{\text{ult.}} = \frac{P_{max}}{A}$. The elasticity of different bodies of the same size can be compared by comparing their elongation when exposed to the same load. If the differences are great, it is often sufficient to compare the elongation of the various bodies in diagram form, the elongation plotted as functions of the load, *load-deformation diagram.*

A numerically more exact evaluation is given by comparing the modulus of elasticity (E) of the bodies for the same load. The modulus of elasticity in tension is more accurately defined as the ratio of the increase in stress to the increase in strain for a specific load. The smaller the increase in strain for a given increase in load, the greater the modulus of elasticity will be. According to *Wertheim*'s experiments, E is not constant in tissues such as arteries. This was also noted by *Triepel* (1902) and *Frank* (1920). These workers reported the average of the modulus of elasticity within certain load intervals, calling it the modulus of interval, or MP, with P the mean load within the interval. M is clearly identical with E in the case of small intervals.

Krafka's (1939) figures for dog aorta may be cited as examples of the changes in modulus of interval that occur with increasing tensile stress. After conversion to grams per square millimeter, the following levels are obtained for his calculations of modulus of interval: $M^{100} = 15.7$, $M^{200} = 19.4$, $M^{400} = 27.3$.

The loads, 100, 200 and 400 g were applied to 5-mm wide specimen bodies removed longitudinally from the aortic wall, and the load was computed per true cross-sectional area.

The behaviour of the load-deformation and the stress-strain diagram for arterial wall in the case of circumferential stress have been the subject of considerable dispute. *Roy* (1880) found a hyperbola-like curve, while *Thoma & Kaefer* (1889) noted that the diagram corresponded to a parabola. *Triepel* (1902) did not consider it probable that a tissue of such complicated structure would yield a diagram of definite geometric form.

Some of these differences in results were explained by *MacWilliam*'s (1902) observation that the diagrams followed a different course if fresh vessels were used in the experiments instead of vessels several days old. The fresh vessels retained their contractility, but this quality was soon lost when the vascular tissue was stored after dissection.

The problem was treated in detail by *Reuterwall* (1921), who also conducted an exhaustive critical analysis of experimental methods used up to that time. He stressed the significance of what was known as accommodation. By accommodation is meant that, on repeated deformation, the initial stress-strain diagram differs from the following stress-strain diagram. After exposure to deforming forces a certain number of times, the vessel becomes accommodated, and thereafter the diagrams are identical. This is especially pronounced in the case of small loads, i.e., of a magnitude corresponding to a physiologic stress, in which the contractility of the smooth musculature is of decisive importance.

Taking these factors into consideration and using a refined technique, *Krafka* (1939) attempted to examine the different layers of the arterial wall separately, as will be described in some detail later on. Several other workers, however, pointed out the advantages of studying

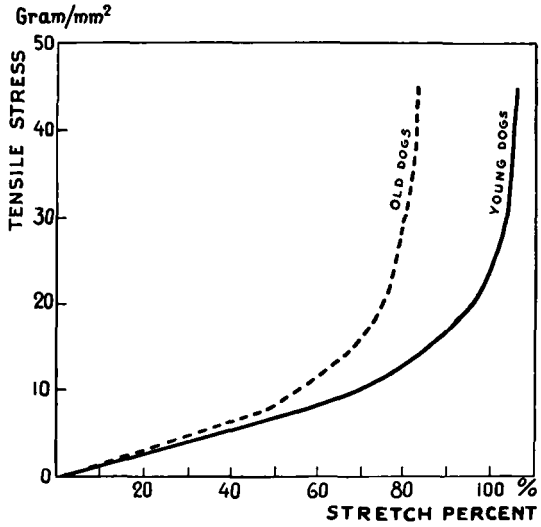


Fig. 5.

Tensile stress-strain diagram of transverse specimens of the dog aorta. Notice the decreased tensile strain (elongation) in old dogs. (Moissejeff, 1926).

the elastic properties of the arterial wall *in vivo* by means of investigations of the pulsations. The latter approach has practically dominated the literature in the field during recent years: *Bramwell, MacDowell & MacSwiney* (1923), *Wezler & Böger* (1939).

To return to tensile testing of strips of arterial wall, *Moissejeff* (1926) found greater elongation in specimens removed in the transverse direction from dog aorta than in longitudinal specimens. In both cases he found that the tensile strain was less in old dogs than in young dogs (Fig. 5), and that this decrease was more marked in longitudinal specimens than in transverse.

Fig. 5 also reveals that with very small loads, the elongation increased in direct proportion to the stress, i.e., the lower part of the diagram is a straight line. This interval of the diagram varied from case to case, but was always very short. In the transverse specimens, which are more interesting physiologically, the greatest elongation took place on loads corresponding to tensile stresses of 0 to 50 grams per square millimeter. Beyond this limit the curve tended toward an asymptotic course and scarcely changed until rupture finally occurred. *Moissejeff* also reported that the tensile stresses to which a functioning vessel is exposed are less than 50 g./mm². He further pointed out that the wall of the living vessel is always subjected to tensile stress because of the pressure of the blood within it. For the purpose of experimental studies, however, *Moissejeff,* considered it wise to proceed from zero.

He conducted his experiments on specimens of dog aorta stored for three to five days in a balanced saline solution. The ultimate stress or breaking load was not reported. The conditions under which the strips were attached can be criticized. The ends of the specimens were perforated with clamps and fastened with ligatures. This inevitably led to some damage to the tissue, which could result in premature rupture. Otherwise, the conditions of his experiments would appear to have fulfilled the most stringent requirements.

On the basis of tensile loading of specimens removed from dog aorta, amongst other materials, *Krafka* (1939, 1940) confirmed earlier observations that the stress-strain diagram consisted of an exponential curve. The course of the lower part of the curve was influenced by muscle tone, while the course of the upper part was not so affected. Calculations of the modulus of elasticity were made for the intact aorta, for the media and for the adventitia. These computations supported the hypothesis that the collagenous connective tissue was responsible for the upper asymptotic part of the stress-strain diagram of the aorta.

While the tensile strength of numerous other tissues has been the subject of study (cf. *Sandblom*'s, *The Tensile Strength of Healing Wounds*, 1944), the ultimate tensile strength of arteries has only been determined in a handful of cases.

Grenant & Quinquard (1885) determined the intraluminal pressure that had to be reached to bring about rupture of different arteries. The carotid artery in dog tolerated an intraluminal pressure 20 to 56 times that of the maximum blood pressure. This means that rupture did not take place until the pressure had been increased from 4 to 11 times the atmospheric pressure. As a general rule, they also found that the smaller the artery, the higher pressure it tolerated. If, in the dog, the aortic arch ruptured at twice the atmospheric pressure, the aorta would tolerate four times, the carotid artery seven to eight times, and the iliac seven to eleven times atmospheric pressure. This great increase in tensile strength of smaller arteries in comparison with that of the aorta was probably mainly due to the important influence of the diameter of the vessel on the tensile load acting on its wall. The tensile load would correspond to the tension produced in the wall by a certain intraluminal pressure.

Orth (1918) stated that an intact femoral artery in a human case tolerated a six-kilogram tensile load, while a sutured femoral artery tolerated four kilograms. The corresponding loads for the axillary artery were three and two kilograms, respectively.

Sako, Chisholm, Merendino & Varco (1949) tested different suture

methods and materials. They first divided and then sutured the aorta in dogs. They sacrificed the dogs after varying intervals of time up to one year, and then exposed aortic specimens to intraluminal pressure increase until leakage occurred. In a few cases the experiments were made *in vivo* on anesthetized dogs, but in most instances they were done on autopsy specimens. It was found that everting sutures of silk No. 6/0 were strong enough to stand up to considerably more than the physiologic requirements even after the period that corresponds to normal healing of a wound.

Lowenberg & Shumacker (1949) found that the ultimate tensile strength of sutured carotid artery in the dog was approximately the same as other sutured tissues during healing. Comparatively little force was required to rupture fresh sutures. Therefore, the authors recommended careful fixation during the period of the actual healing process. Experiments with intraluminal increase of pressure until leakage ensued revealed that even fresh sutures were able to resist pressure considerably higher than the normal pressure. After two weeks had passed, the anastomosis was able to stand higher intraluminal pressure than the normal artery. On the basis of their results, *Lowenberg & Shumacker* expressed the opinion: "If no leak is noted upon completion of an anastomosis, one need have no fear that it will leak subsequently if the blood pressure rises."

The ultimate tensile strength of arterial homografts does not appear to have been tested hitherto. The pronounced degeneration undergone by this type of graft was the reason the present investigation of the tensile strength in the operative area was undertaken.

PLAN

This experimental study had the following objectives:

1) To investigate the tissue respiration of arterial segments preserved in nutrient medium at +1° to +4°C.

2) To study the techniques of vascular surgery and to test homologous arterial transplantation by means of the reconstruction of experimental defects in the aorta and peripheral arteries.

3) To study the functional and morphologic fate of such transplants.

4) To determine whether or not the length of time a vascular segment is preserved is related to the process of healing or incorporation in the host, as determined by microscopic study.

5) To determine the tensile strength of the transplant, the anastomotic line and the host aorta.

CHAPTER 5

REMOVAL AND STORAGE OF ARTERIAL SEGMENTS

Segments of the aorta and the femoral artery were removed under sterile conditions from medium-sized, mature dogs which had been sacrificed because of accidental injuries and not infectious diseases. The segments were usually taken immediately after or within three hours of death. Two to five centimeter long arterial segments were freed from loose connective tissue, rinsed of blood in isotonic saline solution and placed in 50 ml. Jena glass tubes, containing a sterile modified Tyrode's solution, 10 per cent serum and penicillin in an amount of 1,000 units per milliliter. The tubes were sealed with a rubber cap and placed in an ordinary refrigerator at a temperature of +1° to +4°C.

The Tyrode's solution, which had proved satisfactory in the investigations by *Peirce, Gross, Bill & Merrill* (1949) mentioned on page 23, was buffered with sodium bicarbonate. Since solutions buffered with bicarbonate are not suitable in the Warburg test, a phosphate buffer was tried. The solution used for the blood vessel bank in the present investigation had the following composition:

NaCl	20.0 g
KCl	1.0 g
$MgSO_4$, 7 H_2O	0.2 g
$MgCl_2$, 6 H_2O	0.2 g
$CaCl_2$, 6 H_2O	0.7 g
Na_2HPO_4, 2 H_2O	0.2 g
KH_2PO_4	0.17 g
Glucose	2.5 g
Phenol solution 2%	5.0 g
NaOH N/1 q.s. ad pH 7.4	
Aq. steril.	2500 ml.

This solution was supplied by Messrs. L. Westman and S. Wahlqvist of the Military Pharmacy, Stockholm.

After sterilization, the pH was between 7.2 and 7.8. The buffer property was small. The pH was checked daily following the addition of 10 per cent serum, penicillin and the vascular segments. For the first few days, the pH dropped regularly to an average of 7.1, but never lower than 6.3. After three weeks, the average pH was 6.7 (maximum level of 7.5, minimum 5.5).

The technique of removal and storage of the segments proved satisfactory from the point of view of sterility. The sterility was checked by bacterial cultures and by the course of the Warburg test itself, bearing in mind a possible successive rise in the consumption of oxygen during the experiment. Only a few experiments had to be eliminated due to infection.

CHAPTER 6

INVESTIGATION OF TISSUE RESPIRATION

The tissue respiration of the arterial segments was studied by the Warburg method. In principle, this method consists of recording the pressure changes with constant volume and temperature in a closed system containing the specimen to be examined. A decrease in pressure during the experiment and under specified conditions is the expression of an oxygen consumption by the tissue studied. This method is in routine use in most biochemical laboratories and, therefore, need not be described in detail. A concise and instructive guide for Warburg investigations is found in *Manometric Techniques and Related Methods for The Study of Tissue Metabolism* by *Umbreit, Burris & Stauffer* (1947). The reader is referred to this book for details of the present experiments and also with regard to the sources of error in the method.

At first the arterial segments to be examined in Warburg vessels were cut into narrow strips, as suggested by *Warburg* (1923). This method also used by *Krebs & Henseleit* (1932) led both to traumatization of the tissue and to a loss of time. Control experiments with whole vessel segments were found to give the same results as with sliced specimens. From then on, therefore, the system of cutting the segments into narrow strips was abandoned. The vessel segments were split longitudinally and placed in Warburg vessels. The medium consisted of 3 ml. of saline solution. No substratum was added, since the specimens had already been stored in a nutrient solution with serum and were therefore assumed to contain a surplus of substratum. The consumption of oxygen was determined by Warburg's direct method (cf. *Umbreit, Burris & Stauffer,* 1947). The Warburg vessels were placed in a water bath with a constant temperature of +37°C. Fifteen minutes were allowed for the temperature to become balanced. Manometric readings were made at 15-minute intervals for two or three hours. The dry weight of each specimen studied was carefully determined. In general the dry weight amounted to 0.5 to 1 g per

specimen. The recovered oxygen consumption, i.e., the tissue respiration, is recorded as cubic millimeters per 100 mg. dry weight/hour (QO_2).

STATISTICS

Means (M), standard deviations (σ) and standard errors of the mean (ε_M) were calculated according to ordinary formulae (*Fisher*, 1947), x denoting a single value and n the number of variates.

$$(1) \qquad M = \frac{S(x)}{n}$$

$$(2) \qquad \sigma_X = \sqrt{\frac{S\ (x - \bar{x})^2}{n - 1}}$$

$$(3) \qquad (\varepsilon_M) = \frac{\sigma_X}{\sqrt{n}}$$

The experimental error (σ_X) of a single sample was determined from the standard deviation (σ_d) of duplicate samples, provided the mean difference (D) was not significant ($P > 0.05$):

$$(4) \qquad \sigma_X = \frac{1}{\sqrt{2}} \cdot \sigma_d$$

Level of significance:

$P > 0.05$: not significant.
$P < 0.05$: significant.
For reference see *Fisher* (1947) and *Cramér* (1949).

Estimation of Experimental Errors.

In order to estimate the experimental error involved in the Warburg technique *48 duplicate determinations* were performed on aortic samples. The duplicates were analyzed statistically. The values were grouped according to size (see Table 1, col. 1). The mean differences (col. 3) and the standard deviations of the different values were calculated (col. 4). The mean differences were not significant (col. 3), which indicates no systematic difference to exist between the first and the second sample.

The standard deviation of a single sample (σ_X) was calculated

(col. 5) and found to be 1.9–2.7 mm^3. This range, 1.9–2.7, indicates the experimental error involved in determining QO_2 of a single sample (QO_2 being between O and 34 mm^3).

Duplicates of samples of peripheral arteries showed the same experimental error.

TABLE 1

Duplicate Determinations of Respiration of Aortic Samples with the Warburg Technique. Respiration expressed as cubicmillimeters of O_2 per 100 mg dry weight per hour, denoted as QO_2.

Groups QO_2	Number of duplicates n	Mean difference between duplicates $(D \pm \varepsilon_D)$	Standard deviation of difference σ_d	Standard deviation of single sample (σ_x) $\sigma_x = \frac{1}{\sqrt{2}} \cdot \sigma_d$
1	2	3	4	5
0-9.9	20	0.49 ± 0.64	2.88	1.94
10-19.9	20	1.40 ± 0.85	3.80	2.70
20-ω	8	-0.50 ± 1.27	3.59	2.55

Level of significance.

In order to set a limit for a *significant value of QO_2*, 12 devitalized samples were tested. Before testing ten samples were treated with KCN, and two were treated with ethyl alcohol. The mean value for QO_2 of these specimens was 3.20 ± 1.06. The standard deviation was 3.68, indicating the variability *between* samples with no or only insignificant oxygen consumption. This value, 3.68, is thus higher than the experimental error of a *single* sample in the range of $QO_2 = 0$–9.9, which was ± 1.9 (Table 1, col. 5).

Significant consumption of oxygen ($P < 0.05$) was designated to be found over a limit of $3.20 + 2 \times 3.68 = 10.56$, which was approximated to $QO_2 = 10$.

This limit, $QO_2 = 10$, corresponds to the limit used for other work with the Warburg technique in the laboratory (*Lindberg*, personal communication).

CHAPTER 7

ARTERIAL HOMOGRAFTS FOR BRIDGING DEFECTS OF THE AORTA AND FEMORAL ARTERIES

The experiments were made on medium-sized mature dogs, weighing 15 to 38 kg., anesthetized with intravenous nembutal. A tracheal tube was inserted in most cases to ensure free passage of air.

The transplants consisted of segments of the aorta, carotid, femoral or brachial arteries, removed under sterile conditions from sacrificed dogs, according to the method described in Chapter 5.

Fourteen homologous transplants of aortic segments were made first. It is wise to select a large artery like the aorta in order to learn the technique of arterial anastomosis. Furthermore, a number of functioning transplants will be available for study after varying periods of observation, since the risk of thrombosis is much less with great vessels than with small.

In eleven cases the lower part of the abdominal aorta was exposed extraperitoneally by an oblique incision in the flank, approximately corresponding to a kidney incision. In three cases entry was made transperitoneally through a midline incision. Seven to ten centimeters of the abdominal aorta between the renal arteries and the bifurcation were freed from its bed, after ligating the intervening branches.

Proximally and distally the vessel was occluded with clamps covered with rubber (*Millbourn*, 1950). The aorta was divided midway between the two clamps. When the ends were retracted there remained a gap of three to five centimeters. Occasionally the size of the gap was increased by resection of a portion of the vessel ends. The defect was bridged by implantation of homologous graft of suitable diameter and length (Fig. 6). All the branches of the transplant were ligated close to the main trunk with fine silk. The ends were freed of loose adventitia. The anastomoses were made with continuous everting mattress sutures through all the layers of the wall, the cut surface having first been triangulated with the help of three stay sutures (Figure 4, *Carrel*). The contact between the transplant and the host

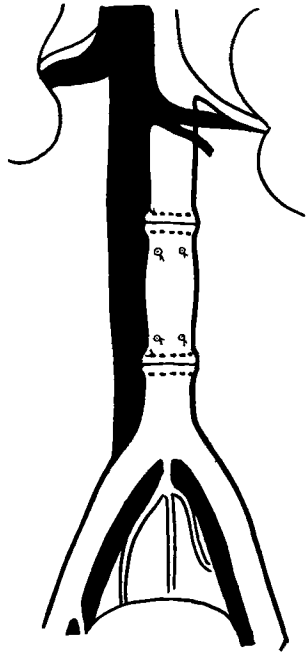


Fig. 6.
Diagram illustrating the portion of aorta in which the experimental defects were made, with a graft in place.

vessel was thus maintained by the everted intimal surfaces. Deknatel silk No. 5/0 or 6/0 on atraumatic needles was used. The transplant and host vessel were protected from drying and trauma throughout the operation, which took about one hour. When the vessel clamps were removed, hemostasis was ensured by a few minutes of mild pressure over the anastomoses and occasionally a few extra sutures. In general the animals withstood the operation and vascular occlusion without any difficulty. In one dog (No. 10) there was some rigidity of the back legs developing a week postoperatively. The cause of this complication was an infection in the wound, which led to a septic thrombosis in the transplant. None of the other cases showed any signs of paralysis following the occlusion of the vessels. Neither chemotherapy nor anticoagulants were used.

This series consisted of eleven homologous aortic grafts, removed under sterile conditions three hours after the death of the donor dog and stored in a blood vessel bank for 2 to 38 days. The series further included two transplants (Recipient Dog Nos. A12 and A13) of aortic segments taken from a donor dog 24 hours after its death. Finally, in one case (Recipient Dog No. 1F), an aortic segment was implanted

TABLE 2

Experimental Aortic Homografts.

	Recipient Dog No.	Duration Storage of graft		Postoperative period of observation	Pictured in report
a	8	2 days	at + 1 ° to + 4 ° C in 10 % homologous serum in modified Tyrode's solution	8 days	16
a	1	2 „		46 days	19
a	7	2 „		12 months	11 31
a	5	9 „		1 day	
a	9	9 „		14 months	
a	6	9 „		15 months	
a	2	9 „		81 days	15 17 18
a	3	14 „		15 months	20
a	10	18 „		12 months	
a	4	30 „		2 months	21
a	11	38 „		39 days	
b	A 12	24 hours	in the dead donor dog before transplantation	1 month	
b	A 13	24 hours	in the dead donor dog; then preserved in usual manner 7 days	2 months	
b	1 F	Devitalized	in absolute alcohol	14 1/2 months	12 22 32

a = respiring grafts; b = non-respiring grafts. (See Chapter 10).

after devitalization in absolute alcohol for two days. The details of these aortic transplants are tabulated in Table 2.

In 24 experimental defects in the femoral artery in dogs, the circulation was restored by homologous transplantation of peripheral vessel segments. Twelve dogs were operated on bilaterally under nembutal anesthesia with intubation. The femoral artery was dissected in the proximal portion of the thigh. The vessel was divided following temporary vascular occlusion. The vessel ends thereupon retracted one to two centimeters. A minor resection was done in a few of the cases. The resultant arterial defect, measuring one to three centimeters, was bridged with a vessel segment of appropriate diameter and length, which was either of fresh origin, had been stored in a blood vessel bank, or had been devitalized chemically. Anastomosis was done according to the same principles as the operation on the aorta. Because the femoral artery shrinks to a diameter of approximately three millimeters after transection, the anastomosis is technically difficult. Deknatel silk No. 6/0 on atraumatic needles was used for the sutures. The various grafts tested in this series are listed in Table 3.

Segments from both the aorta and the peripheral arteries retained their usual consistency and appearance during storage in blood vessel

banks. The aortic specimens removed 24 hours after the death of the donor did not appear to differ from those removed earlier. The specimens which were devitalized in absolute alcohol were of firmer consistency than the others. The peripheral arterial segments, which had been treated with KCN, did not differ in appearance from those stored in nutrient medium.

TABLE 3

Experimental Arterial Homografts in the Femoral Artery.

	Recipient Dog No.	Duration Storage of graft		Postoperative period of observation	Pictured in report
a	P 5 sin.	Fresh		12 days	26
	P 2 dx.	Fresh		14 1/2 months	
	P 1 sin.	1 day		4 days	24 25
	P 6 sin.	1 „		12 days	
	P 7 sin.	1 „		9 1/2 months	
	P 10 dx.	1 „		6 months	
	P 3 sin.	2 days		14 months	28
	P 3 dx.	2 „		14 months	
	P 4 sin.	2 „	at + 1 0 to + 4 0 C	5 months	
	P 4 dx.	2 „	in 10 % homologous	5 months	
	P 11 sin.	2 „	serum in modified	3 months	27
	P 11 dx.	2 „	Tyrode's solution	3 months	
	P 1 dx.	6 „		18 days	
	P 8 sin.	7 „		27 days	
	P 8 dx.	7 „		27 days	
	P 12 sin.	7 „		12 months	
	P 12 dx.	7 „		12 months	
	P 2 sin.	20 „		14 1/2 months	
b	P 9 dx.	36 „		14 months	13 29
	P 9 sin.	42 „		14 months	13
	P 10 sin.	61 „		6 months	
	P 5 dx.	One hour in KCN- solution		12 days	
	P 6 dx.	Devitalized in absolute alcohol		12 days	
	P 7 dx.	Devitalized in absolute alcohol		9 1/2 months	

a = respiring grafts; b = non-respiring grafts. (See Chapter 10).

CHAPTER 8

FUNCTIONAL INVESTIGATION OF THE GRAFTS IN VIVO

The function of the grafts was studied postoperatively by means of frequent determination of the pulsations in the femoral arteries and by evaluation of the general behavior of the dogs.

Routine roentgen examinations using contrast medium were made a few weeks after the operation and at the end of the period of observation in order to evaluate the anatomical and functional conditions of the grafts.

ARTERIOGRAPHY

The dogs were anesthetized with intravenous nembutal and were intubated. Blind puncture of the abdominal or thoracic aorta was tried in two cases, but abandoned thereafter as being too uncertain a method. In ten of the dogs, the carotid artery or the superficial femoral artery was exposed and a catheter was inserted in a retrograde fashion into the aorta. In the remainder, laparotomy was done and the upper part of the abdominal aorta was punctured under direct vision. The contrast medium, *Umbradil* (Astra), a water-soluble preparation, was used in a 35 to 50 per cent solution. For each exposure 20 to 30 ml. was injected.

An arteriographic technique was devised to test the distensibility of the transplants *in vivo*. Special arteriograms were made in ten cases (plus two control animals) in order to see whether the grafts showed the same variations in filling and diameter as the host vessels or whether they appeared as rigid, more or less unyielding tubes. For this purpose, the injection of medium and the exposure were made both with a rise in blood pressure (I) and with a decrease in blood pressure (II) (Figures 10–13). To achieve the increase in blood pressure, 5–10 μg of adrenalin per kilogram of body weight was given intravenously, while the decrease was brought about by the injection of 10–20 μg of histamine per kilogram of body weight.

The blood pressure was recorded on the kymograph via a glass cannula inserted in the carotid artery. The exposure was usually made when the drugs administered reached their maximal effect. The position of the roentgen tube was kept unchanged in order to compare the diameter of the vessel in the same dog in the two different exposures (I and II).

Even very small, unintentional changes in the position of the dog or of the roentgen tube between the two exposures can affect the diameter of the vessel as visualized on the arteriograms. In view of the difficulty of precise measurement, no numerical comparison between one object and another could be made. Only the diameter of the host vessel in relation to that of the graft in the same dog was evaluated.

CHAPTER 9

TESTS OF TENSILE STRENGTH

MATERIAL AND METHOD

Material. The thoracic and abdominal aortas were removed immediately after death from two dogs which had not previously been operated on (control material) and from six of the experimental dogs. In the latter, homologous aortic segments had been implanted in the abdominal aorta, as described on page 45. The grafts had been functioning for two months in one dog (No. A13) and for more than one year in the remaining five dogs (Nos. 3, 6, 7, 9, 1F).

The whole aorta was dissected and placed in isotonic salt solution immediately after the dog was sacrificed. (In the dogs operated on this was not done until after the operative field had been examined macroscopically and parts removed for histologic study.) A specimen corresponding in shape to that used in tensile strength tests of other materials was used. In order to obtain specimens of uniform size, a cutting tool was constructed. With this instrument both longitudinal and transverse specimens of the aortic wall were stamped out. The pattern and size of the specimens are illustrated in Fig. 7.

The thickness of the specimen was measured with a micrometer. It was frequently difficult to obtain the precise measurements due to variations in the thickness of the loose adventitial tissue.

The specimens were attached with clamps at their wide ends and were submitted to a longitudinal load in an apparatus, the principles of which appear in Fig. 8.

In order to measure the elongation of the specimens during a test, two India-ink marks, usually about 5 millimeters apart, were made on the intima. The position of the marks in relation to a millimeter scale immediately behind the specimen was noted through the telescope at the beginning of the experiment (load = 0 gram).

The load was increased in stages by 50 to 200 g until rupture occurred. The position of the India-ink marks in relation to the millimeter scale was read off at each increase of the load. The time interval

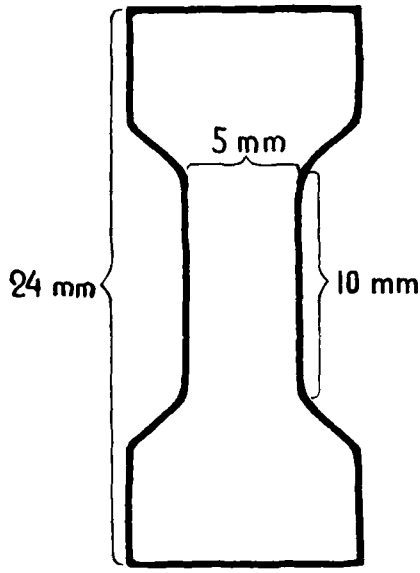


Fig. 7.
Size and shape of the aortic specimen stamped out from the wall by a cutting tool.

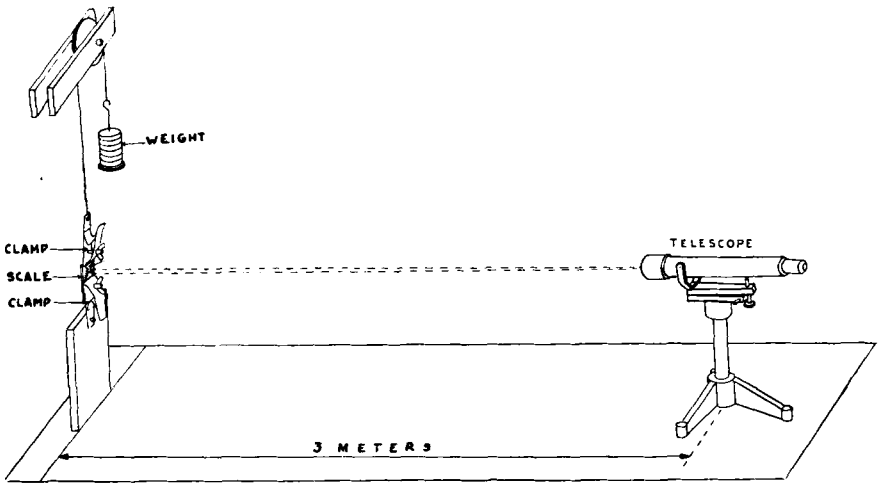


Fig. 8.
Schematic drawing of the apparatus used for the tests of tensile strength.

between the increases was kept as uniform as possible–about one minute. It was gradually learned that the increase in the load was small enough to permit differentiation between the strength of the various strips of tissue.

The specimens were sprayed with isotonic salt solution to prevent desiccation. The investigation was made at a temperature of about 18°C and took place only a few hours after removal of the specimens from the dog.

SYMBOLS AND FORMULAE EMPLOYED IN THE COMPUTATIONS

The strain (ε) was calculated as follows:

Gage length (= distance between the India-ink marks with zero load) = l_0 mm.

Length for a specific load = l_p mm.

$$\text{Strain } (\varepsilon) = \frac{(l_p - l_0).\ 100}{l_0}\ \%$$

The tensile stress may be expressed in force per unit area (σ) of the cross-section of the specimen or in *total load* (P) on the specimen. The structure of the arterial wall with its different elements of varying strength and elasticity, as well as the different orientation of the elements in the vessel wall, means that the stress distribution will vary in the cross-section. Of major interest from the physiological point of view is the total load on the whole vessel wall. *The tensile stress, therefore, was expressed in total load (P) per specimen 5 mm. in breadth.*

Given P and also the diameter of the aorta, it is simple to compute the corresponding tensile load on the whole aorta longitudinally and also the intraluminal pressure, expressed in mm. Hg., which produces the same tensile strain, as is illustrated in Fig. 9. Where the average aortic diameter is d mm. and the load on a 5-mm. longitudinal specimen is P, this corresponds to a longitudinal tensile force on the entire aortic wall of approximately $\frac{P \pi d}{5}$ g. The deduction is shown in Fig. 9a. An intraluminal pressure producing the same tensile force longitudinally on the aorta wall (Fig. 9b) would have the magnitude:

$$p = \frac{4\ P}{5\ d}\ g/mm^2 \text{ or } p \approx \frac{60\ P}{d}\ \text{mm Hg.}$$

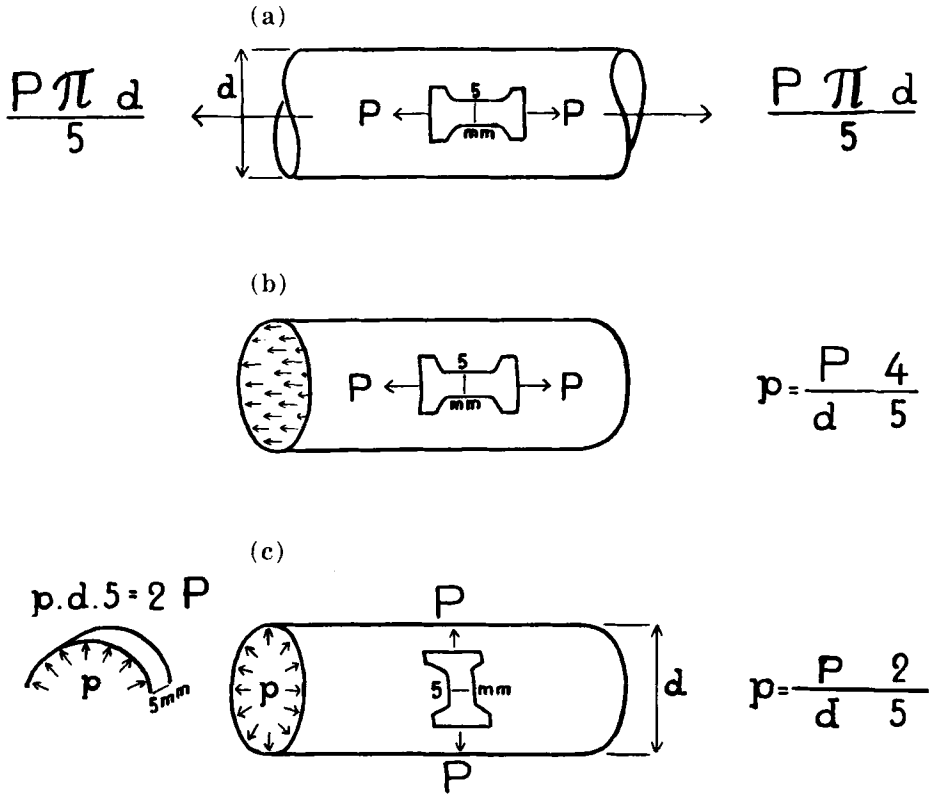


Fig. 9.

Formulae used for computing the tensile force on the whole vessel wall (diameter = d), when the tensile strain and breaking load (P) of the test specimen is known.

(a) The tensile force applied in longitudinal direction.

(b) The intraluminal pressure with the corresponding tensile force in longitudinal direction.

(c) The intraluminal pressure with the corresponding tensile force in transverse direction.

The internal pressure (p) which produces transverse tensile force corresponding to P g. per 5-mm. wide specimen, is shown in Fig. 9c, and can be computed from the following equation:

$$p = \frac{2\ P}{d\ 5}\ g/mm^2$$

$$= \frac{2\ P\ 1000}{d\ 5\ 13{,}6} \approx 30 \cdot \frac{P}{d}\ mm\ Hg.$$

SOURCES OF ERROR

1. Although the stamped-out specimens were all the same size, there is no guarantee that they corresponded to pieces of the living aorta of precisely the same size, because the postmortal degree of contraction might have varied.

2. The accommodation was not considered.

3. It was sometimes difficult to adjust the ocular hair-cross of the telescope to the India-ink marks, particularly in connection with considerable elongation when the contours of the marks were not sharply defined.

4. The readings depended on the ability of the examiner to estimate the position in relation to the millimeter scale. The maximal difference on repeated readings was $\pm$ 0.1 mm.

5. The fixing device sometimes broke under exceptionally heavy loads. In these cases it was not always possible to determine the ultimate load. However, the grips slipped at such heavy loads as constitute clear evidence of exceptional or superior strength in comparison with other specimens from the same vessel.

6. Since the specimens were not homogeneous bodies, the elongation of the intima could not be expected to correspond to the elongation of the other layers of the wall. As long as all the layers held together, the intimal elongation represented that of the total wall with good approximation. With heavy loads the intima sometimes ruptured between the ink-spots, which resulted in an apparent increase in the elongation.

7. A minor error regarding the magnitude of the load resulted from some friction in the pulley. This can be ignored, however.

It was not considered necessary to make a statistical analysis of the errors in the technique or of the results. For the purpose of the investigation was not to give a precise evaluation of elasticity, but to compare the tensile strength of different specimens.

CHAPTER 10

CHANGES IN OXYGEN CONSUMPTION OF ARTERIAL SEGMENTS STORED IN NUTRIENT SOLUTION AT +1° TO +4°C

The tissue respiration, which was measured by Warburg-technique as described in Chapter 6, was analyzed in 10 fresh and 149 preserved arterial segments.

TABLE 4

Respiration (QO_2) of Fresh Samples of Aorta and Peripheral Arteries.
QO_2 = Oxygen consumption in mm^3 of 100 mg arterial tissue (dry weight) per hour.

Vascular segments	Number of samples n	QO_2 Mean $M + \varepsilon_M$
Aorta	6	23.6 ± 2.7
Peripheral arteries	4	30.6 ± 6.9

A QO_2 level of 10 was considered significant to tissue respiration (page 44). As appears from Table 4, the mean QO_2 level for fresh aortic segments was 23.6 ± 2.7 and for fresh peripheral arteries 30.6 ± 6.9, and no fresh samples were found to have a QO_2 of < 10. In other words, definite respiration was recorded in the fresh samples. In occasional cases, this quality was lost after only a few days of storage in the blood vessel bank. On the whole, however, approximately half of the vascular segments continued to consume oxygen for three to four weeks of storage. The great majority, or nearly 80 per cent, still showed respiration after ten days in storage. This applied both to the aorta (Table 5) and to the peripheral arteries (Table 6). A few of these specimens exhibited significant consumption of oxygen after

TABLE 5

Respiration of Aortic Samples Preserved at +1° to +4°C in 10% Homologous Serum in Phosphate Buffered Tyrode's Solution.

Period of Storage (days)	$QO_2 < 10$			$QO_2 > 10$		
	Number of samples n	Number of samples in per cent of total (For each period of time)	Mean $M \pm \varepsilon_M$	Number of samples n	Number of samples in per cent of total (For each period of time)	Mean $M \pm \varepsilon_M$
1 — 5	2	9	5.4 ± 2.4	19	91	17.8 ± 1.4
6 — 10	5	18	7.5 ± 0.8	23	82	17.3 ± 1.3
11 — 20	7	58	2.6 ± 1.2	5	42	24.2 ± 4.0
21 — 30	20	59	4.6 ± 0.8	14	41	18.2 ± 2.0
31 — ω	5	72	0.6 ± 0.3	2	28	10.7 ± 0.6

QO_2 = Oxygen consumption in mm^3 of 100 mg. aortic tissue (dry weight) per hour.

TABLE 6

Respiration of Peripheral Arteries Preserved at +1° to +4°C in 10% Homologous Serum in Phosphate Buffered Tyrode's Solution.

Period of Storage (days)	$QO_2 < 10$			$QO_2 > 10$		
	Number of samples n	Number of samples in per cent of total (For each period of time)	Mean $M \pm \varepsilon_M$	Number of samples n	Number of samples in per cent of total (For each period of time)	Mean $M \pm \varepsilon_M$
1 — 5	0	0	—	18	100	25.5 ± 2.0
6 — 10	3	21	5.6 ± 2.6	11	79	22.9 ± 1.5
11 — 20	0	0	—	2	100	10.4 ± 0.5
21 — 30	9	75	1.9 ± 0.8	3	25	17.8 ± 6.0
31 — ω	1	100	7.2	0	0	—

QO_2 = Oxygen consumption in mm^3 of 100 mg. arterial tissue (dry weight) per hour.

four weeks. The figures representing the degree of metabolism in aortic segments do not indicate that tissue respiration decreased successively during storage. However, the possibility that the consumption of oxygen did drop successively in the peripheral arteries could not be definitely excluded.

It would therefore appear that the arterial segments which showed tissue respiration from the outset retained this manifestation of life relatively constantly for a number of days in storage in a nutrient solution with serum at a temperature of +1° to +4°C. The factors that cause tissue respiration to cease could not be analyzed in the present investigation.

CHAPTER 11

THE FUNCTIONAL RESULT OF ARTERIAL HOMOGRAFTS

Two of the dogs (Nos. 5 and 8) submitted to grafting of aortic segments died as a result of insufficiency of the suture line. Post-mortem aortography in both cases showed a small leak at the upper anastomotic line, but the lumen appeared patent.

Peritonitis developed in one case (Dog 11). The dog's general condition was poor, and the pulsations in the femoral artery were not clearly appreciable a few days after the operation. Aortography after 39 days showed complete obliteration of the lumen.

In two cases (Dogs 10 and 1F), the primary aortogram revealed small filling defects in the graft. These defects were interpreted as evidence of thrombi. When aortography was repeated at the end of the period of observation, the same defect was noted and the lumen was still patent in one case (Dog 1F), while in the other case (Dog 10) the thrombosis had progressed and caused total obliteration. The remaining dogs were in excellent condition throughout, and after the operation they showed normal activity and mobility. The pulsations in the femoral artery were strong. Fig. 15:I (Dog 2) is an example of an aortogram one month after segmental resection of the abdominal aorta and implantation of an aortic segment that had been stored in the blood vessel bank for eleven days. Thus, normal activity in the experimental animal and strong pulsations in its femoral artery constituted fairly reliable evidence of the function of the graft. *Neither secondary thrombosis nor obliteration was found in any of the cases in which the first aortogram revealed the absence of defects.* This fact appears in Table 7, which also shows the number of non-functioning grafts.

In studying the fate of peripheral arterial transplants, a high incidence of early thrombosis was seen (Table 8). However, as with the aortic grafts, if the lumen remained patent for the first few post-operative weeks it did so for the entire period of observation. There

TABLE 7

Function of Aortic Homografts.

Recipient Dog. No.	Treatment of graft before operation	First postoperative aortography		Second aortography	
		Time graft in recipent dog (days)	State of graft	Time graft in recipient dog (days)	State of graft
1	Stored 2 days	45	*Patent*	—	—
7	Stored 2 days	19	*Patent*	340	*Patent*
8	Stored 2 days	8	Died of hemorrhage. Small leakage of the upper anastomotic line. (Post mortem aortography.)	—	—
5	Stored 9 days	1	Died of hemorrhage. Small leakage of the upper anastomotic line. (Post mortem aortography.)	—	—
6	Stored 9 days	12	*Patent*	368	*Patent*
9	Stored 9 days	16	*Patent*	296	*Patent*
2	Stored 11 days	30	*Patent*	(81)[1]	*Patent*
3	Stored 14 days	27	*Patent*	462	*Patent*
10	Stored 18 days	30	Patent. Mural thrombi.	346	Completely thrombosed.
4	Stored 30 days	12	*Patent*	(68)[2]	*Patent*
11	Stored 38 days	39	Completely thrombosed.	—	—
A 12	In the donor dog 24 hours post mortem. Thereafter transplanted.	30	*Patent*	—	—
A 13	In the donor dog 24 hours post mortem. Then stored 7 days	59	*Patent*	—	—
1 F	Devitalized 48 hours in absolute alcohol.	24	Patent. Two small mural thrombi.	334	Patent. Two small mural thrombi.

[1] Distemper. No second aortography was performed.

[2] Died by an accident. No second aortography was performed.

was only one case of insufficiency of the suture line in the series of peripheral grafts (Dog P5 sin.). Here a large hematoma developed in the operative field after a few days, and the dog was therefore sacrificed on the twelfth postoperative day. On postmortem injection of contrast fluid, a leak at the distal anastomotic line could be observed, but otherwise no abnormalities were noted.

Because of the high incidence of thrombosis associated with suture

TABLE 8

Function of Arterial Homografts in Femoral Arteries.

Recipient Dog. No.	Treatment of graft before operation	First postoperative aortography		Second aortography	
		Time graft in recipient dog (days)	State of graft	Time graft in recipient dog (months)	State of graft
P 2 dx.	Fresh	13	*Patent*	14 1/2	*Patent*
P 5 sin.	Fresh	12	Died of hemorrhage. Small leakage from the lower anastomotic line.	—	—
P 1 sin.	Stored 1 day	4	*Patent*	—	—
P 6 sin.	Stored 1 day	12	Occluded	—	—
P 7 sin.	Stored 1 day	12	Patent. Mural thrombi	9 1/2	Completely thrombosed
P 10 sin.	Stored 1 day	29	Occluded	—	—
P 3 sin.	Stored 2 days	18	*Patent*	14	*Patent*
P 3 dx.	Stored 2 days	18	*Patent*	14	*Patent*
P 4 sin.	Stored 2 days	98	Occluded	—	—
P 4 dx.	Stored 2 days	98	Occluded	—	—
P 11 sin.	Stored 2 days	30	*Patent*	3	*Patent*
P 11 dx.	Stored 2 days	30	Occluded	3	Occluded
P 1 dx.	Stored 6 days	18	*Patent*	—	—
P 8 sin.	Stored 7 days	27	Stenosis; marked mural thrombi	—	—
P 8 dx.	Stored 7 days	27	Stenosis; marked mural thrombi	—	—
P 12 sin.	Stored 7 days	28	*Patent*	12	*Patent*
P 12 dx.	Stored 7 days	28	*Patent*	12	*Patent*
P 2 sin.	Stored 20 days	13	Occluded?	14 1/2	Partially thrombosed
P 9 dx.	Stored 36 days	48	*Patent*	12(-14)	*Patent*
P 9 sin.	Stored 42 days	48	*Patent*	12(-14)	*Patent*
P 10 dx.	Stored 61 days	29	Occluded	—	—
P 5 dx.	Stored 1 day devitalized in KCN	12	Occluded	—	—
P 6 dx.	Devitalized in absolute alcohol	12	Occluded	—	—
P 7 dx.	Devitalized in absolute alcohol	11	Occluded	—	—

of such small calibre vessels and the small number of transplants in each series, it is impossible to relate the method of preservation of the graft to the final functional result. It should be noted however, that

all transplants previously treated with KCN, or ethyl alcohol, became thrombosed.

It appears from the investigation that a partial thrombosis, observed within a few days or weeks of homologous arterial transplantation, is a bad prognostic omen. If, on the other hand, the graft is patent at the first examination, the end-results will probably be good.

Arteriography Performed Under Two Different Conditions of Blood Pressure.

Normal animals.

The results of this examination in the two control animals showed that both the aorta and the small vessels involved increased appreciably in diameter with increased blood pressure and decreased when the pressure was lowered. This was most clearly seen in the case of the aorta, as shown in Fig. 10 (Control Dog 2). At the top of the picture can be seen the kymographic recording of the pressure in the carotid artery. The injections of adrenalin and of histamine are noted on the curve. The exposure (↓) was made to coincide as closely as possible with the maximal effect of these drugs. The aorta was well visualized in both exposures, as will be seen from the aortograms. Variations in blood pressure of 250 to 35 mm. Hg. changed the diameter of the abdominal aorta in this case (measured on the plates) from approximately 10 to 7 mm. in the area between the renal arteries and the bifurcation.

Dogs with aortic grafts (Nos. 3, 6, 7, 9, 1F, A12).

Visualization of the operative field both with high (I) and with low blood pressure (II) in six dogs, submitted to aortic transplantation, revealed that the relationship between the diameter of the host vessel and that of the graft differed in exposures I and II.

Rather than a numerical survey, two typical aortographic series have been reproduced, arranged in the same way as for the control animals.

Fig. 11 consists of aortograms in Dog No. 7. They were taken 340 days after the implantation of an aortic segment which had been stored for two days in the blood vessel bank. Aortogram I corresponds to a blood pressure of 360 and No. II to a pressure of 60 mm. Hg. Comparison of aortogram I with II reveals that the relationship between the diameter of the host vessel and the graft differed. This is probably due to the fact that the graft did not conform to the changes

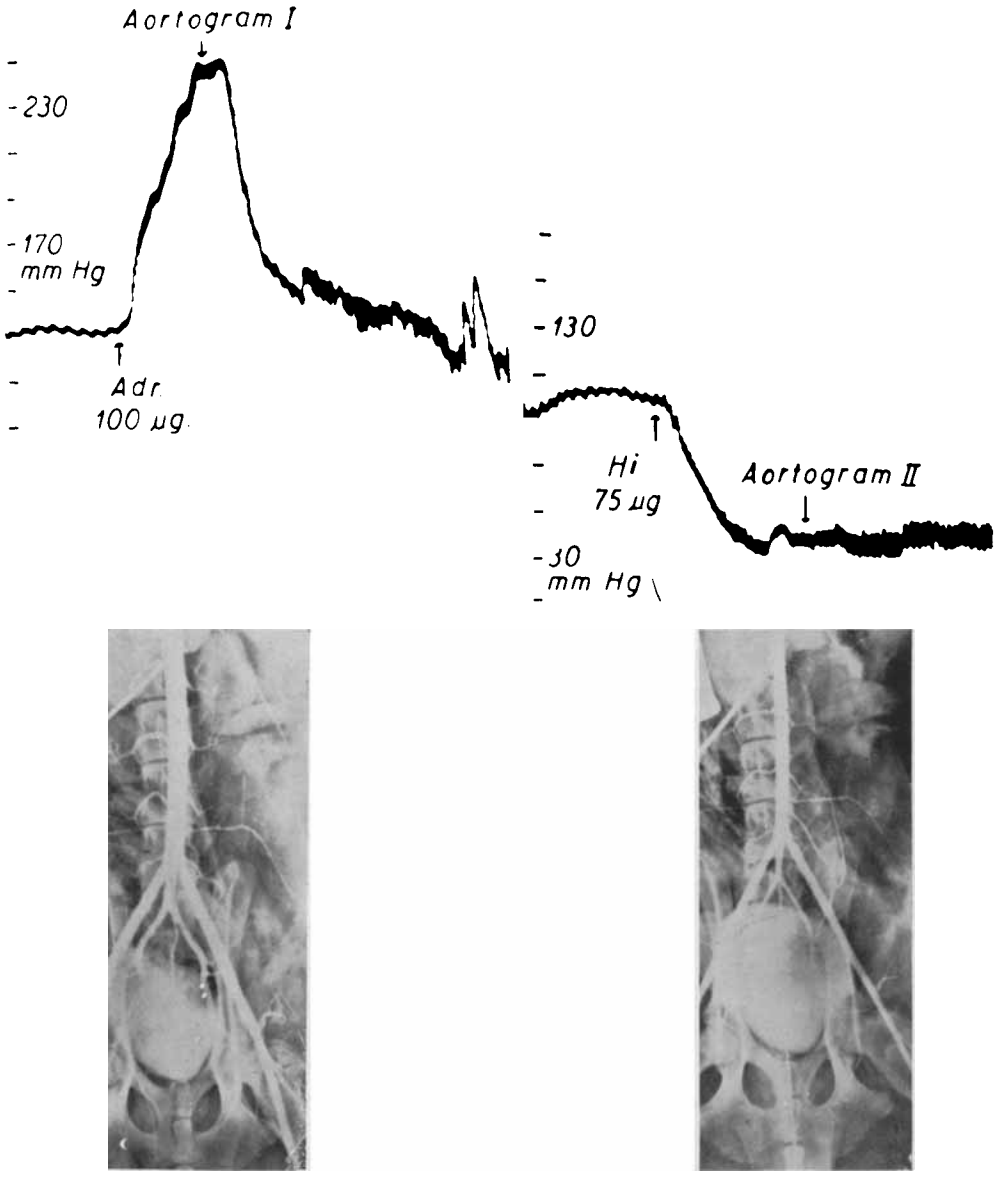
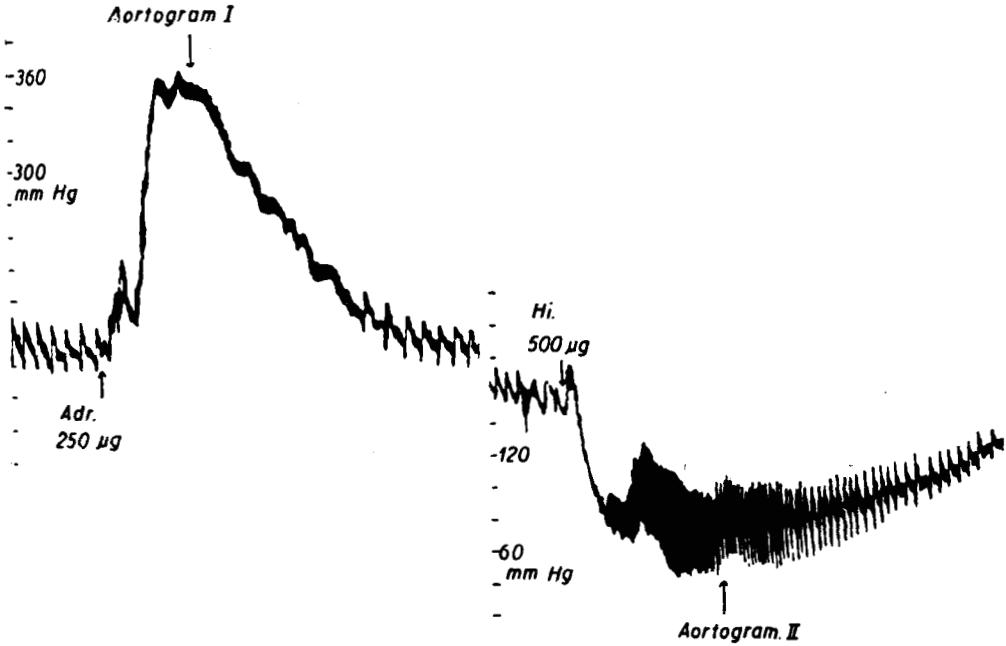


Fig. 10.
Aortogram in a normal dog under high (I) and low (II) blood pressure. Observe the difference in diameter of the visualized arteries under different blood pressures.



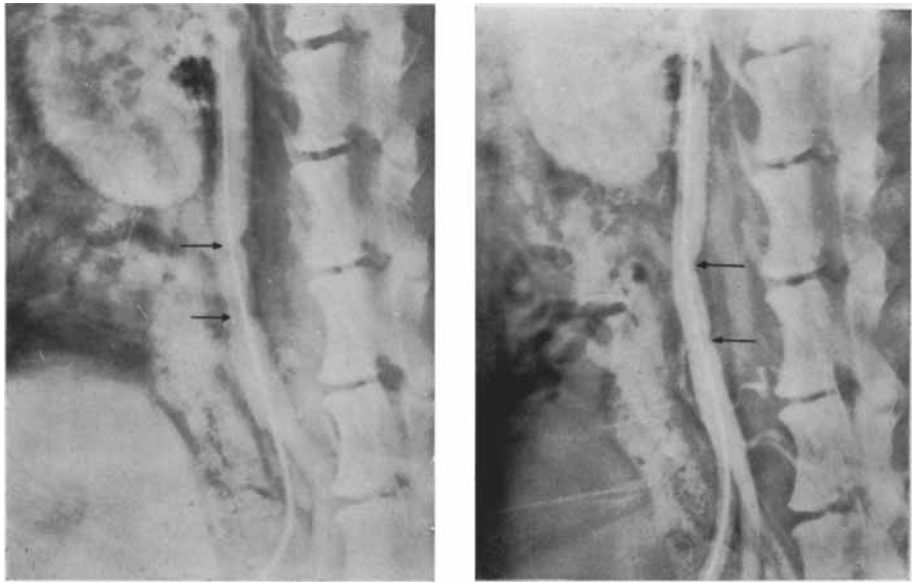


Fig. 11.
Visualization of aortic graft 340 days postoperatively under high (I) and low (II) blood pressure. The graft is patent but appears to act as a rigid tube.
Dog No. 7. Before implantation the graft was stored 2 days.

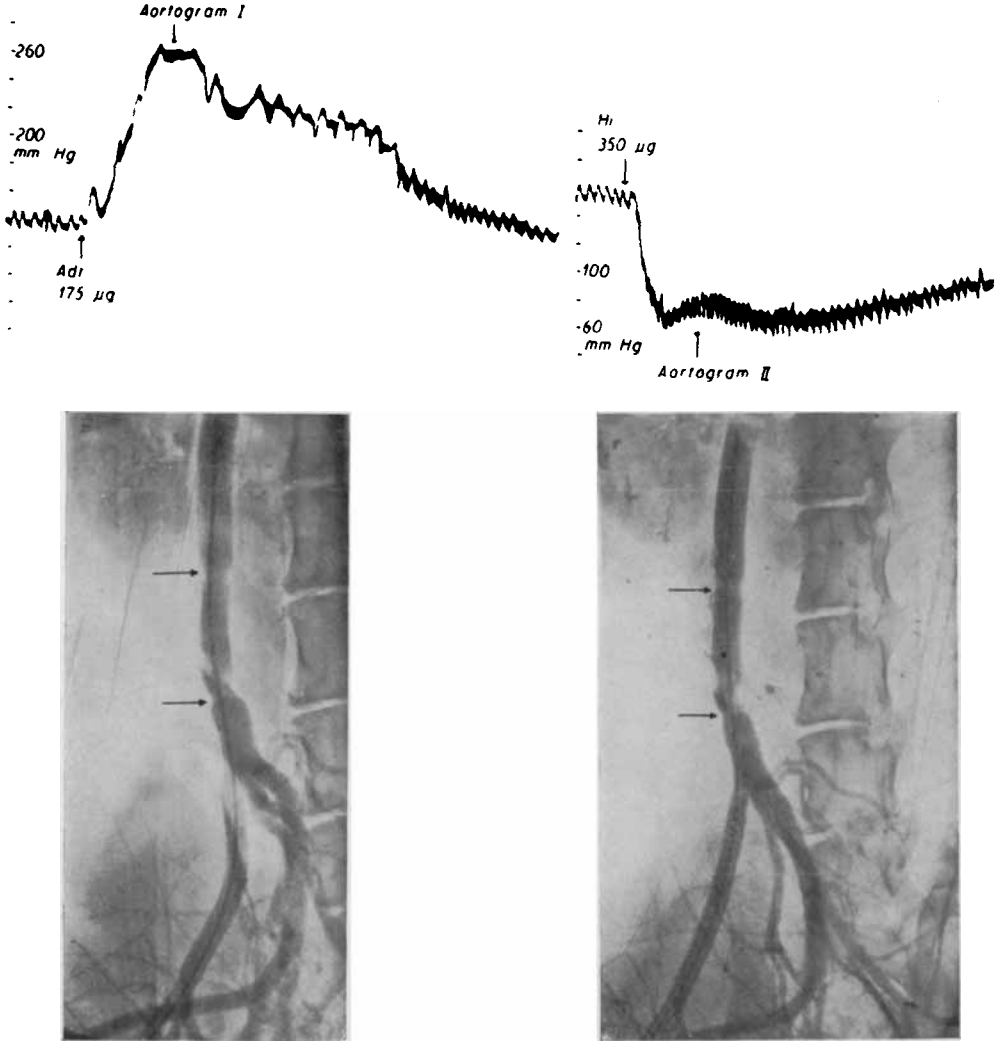


Fig. 12.

Visualization of aortic graft 334 days postoperatively under high (I) and low (II) blood pressure. Lumen is patent but there is evidence of mural thrombus. The graft does not expand and contract as much as the host vessel.

Dog No. 1 F. Before implantation the graft was devitalized in C_2H_5OH.

in the volume of the host vessel, but behaved more or less like an inelastic tube.

Fig. 12 (Dog 1F) illustrates approximately the same conditions. In this case the examination was made 334 days after implantation of an aortic segment fixed for two days in alcohol. The blood pressure was 260 in aortogram I and 74 mm. Hg. in aortogram II.

When the corresponding points on the host vessel and the graft had been marked on the film–as in the foregoing cases–and the diameter had been measured at several places, it was found that the relationship between the diameter of the host vessel and that of the graft was not the same in exposures I and II.

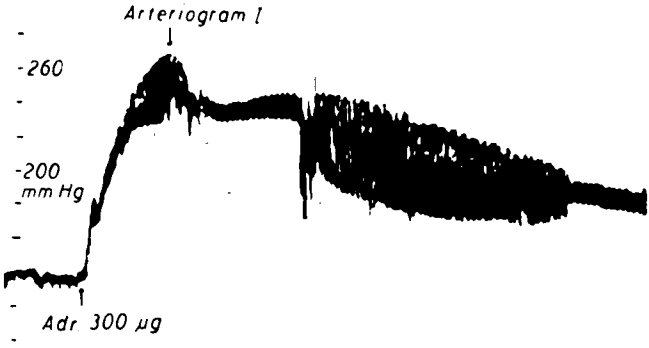
Irregularities in the contours near the upper and lower anastomotic lines are seen more clearly in aortogram I, in which the diameter of the host vessel is larger than in No. II. The anastomotic lines are less pronounced where the host vessel is contracted. In aortogram II, therefore, there appears to be a smoother transition of the outline of the contour from the host vessel to the graft, and consequently the mural thrombi are not so clearly visualized.

Dogs with peripheral arterial grafts (P 3 dx., P 3 sin., P 7 sin., P 9 dx., P 9 sin., P 12 dx., P 12 sin.).

The relationship between the diameter of the host vessel and of the graft differed from aortogram I to aortogram II in these cases in the same way as already described. Fig. 13 (Dog P9). The examination was made about one year after bilateral transplantation of a segment of the femoral artery. The segment implanted on the right side had been stored for 36 days and the one on the left side for 42 days in a blood-vessel bank. The blood pressure was 200 in arteriogram I and 69 mm. in arteriogram II. The anastomotic lines are scarcely visible with the lower blood pressure, while they are characterized by slight constriction in arteriogram I.

The variation in diameter was less for the femoral artery, for example, than for the aorta. The difference between the graft and the host vessel under the two conditions of pressure was also less pronounced. Nevertheless, all the cases examined exhibited the same tendency.

This examination, with visualization of the host vessel and the graft under different conditions of blood pressure, would indicate that the graft is relatively stiff and does not conform entirely to the contractions and dilations of the host vessel.



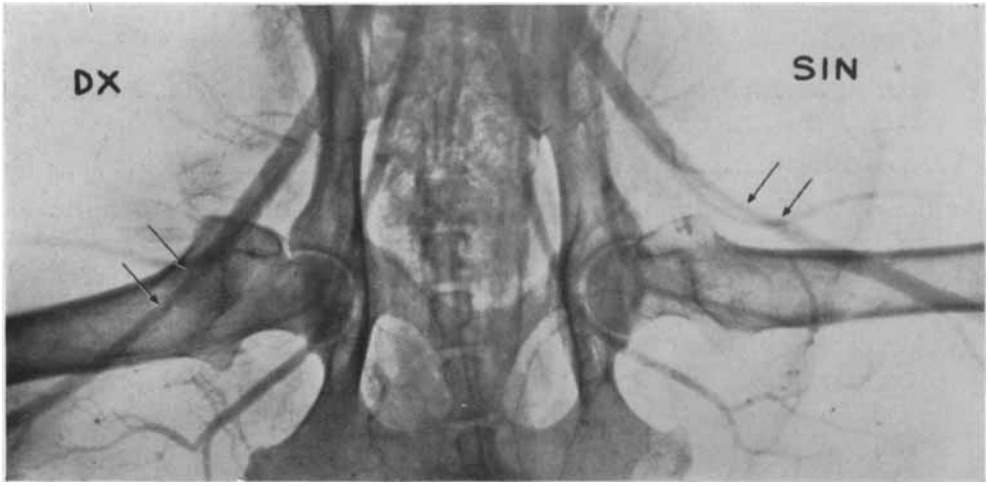
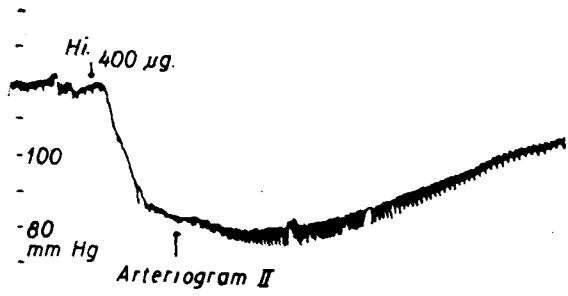


Fig. 13: I.



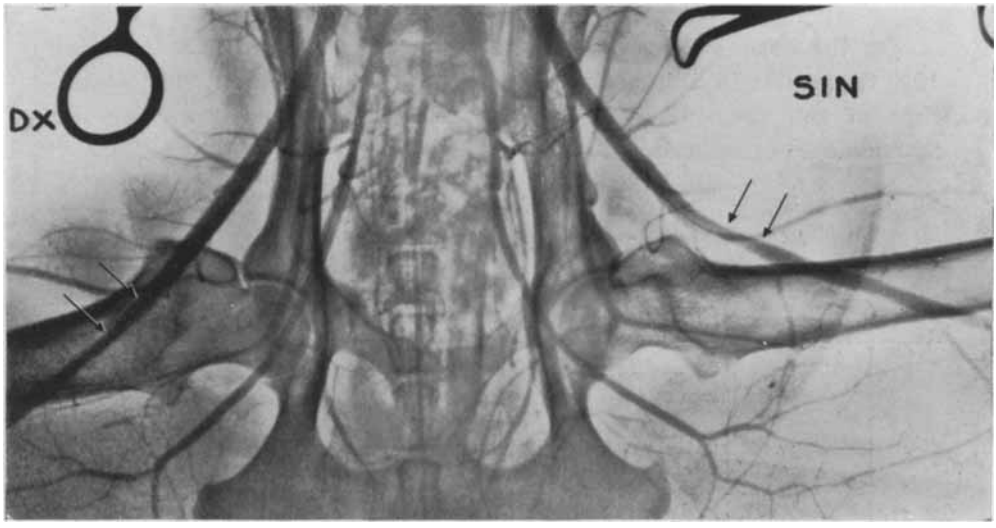


Fig. 13: II.

Visualization of femoral arterial grafts, bilaterally, one year postoperatively under high (I) and low (II) blood pressure. The lack of expansion and contraction of the graft is noticeable but less obvious than in the aortic grafts.
Dog. No. P 9. Before implantation the grafts were stored for 36 and 42 days, respectively.

CHAPTER 12

MORPHOLOGIC EXAMINATION

AORTIC GRAFTS STORED 1 TO 14 DAYS IN THE BLOOD VESSEL BANK

On the basis of the results of the Warburg experiments (Chapter 10), the grafts in this series were considered to be respiring at the time of the operation. The following specimens were used for the morphologic examination*.

TABLE 9

Patent Aortic Grafts, Respiring at the Time of Operation.

Dog No.	No. of days stored before operation	Period of observation			Results	Pictured in Report
		Year	Months	Days		
8	2	—	—	8	Faulty suture line	Fig. 16
1	2	—	1	16	Good function	Fig. 19
7	2	1	—	—	Good function	Figs. 11, 31
5	9	—	—	1	Faulty suture line	
9	9	1	2	—	Good function	
6	9	1	3	—	Good function	
2	11	—	2	21	Good function	Figs. 15, 17, 18
3	14	1	3	—	Good function	Fig. 20

GROSS EXAMINATION

Death, from hemorrhage, occurred in one case (Dog 5) on the first postoperative day, and in another case (Dog 8) on the 8th postoperative day. In each case examination revealed a leak at a suture line, apparently due to a technical error rather than to the nature of the graft.

The other six grafts in this group functioned well during varying periods of observation. In two cases, observed for short periods, the

* In Dog 10 was a contaminated graft (stored 18 days) implanted. Complete occlusion occurred as described on page 58.

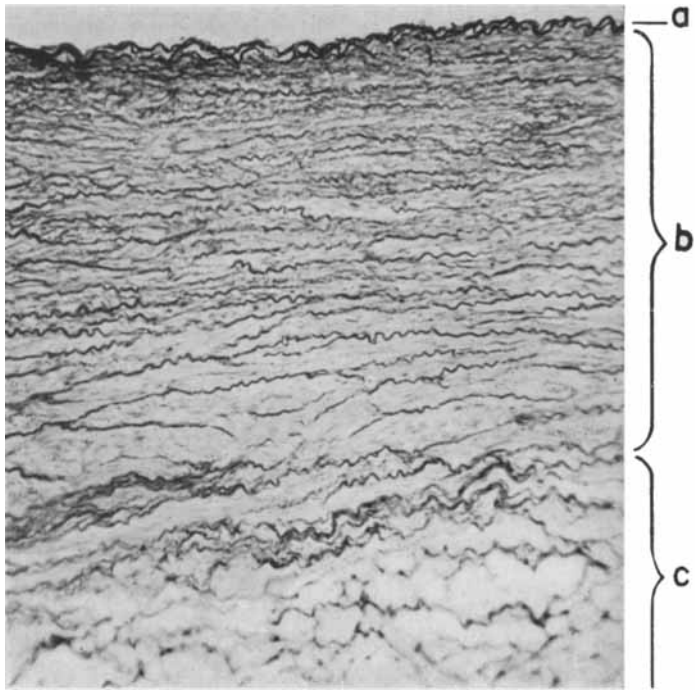


Fig. 14.
Photomicrograph of normal abdominal aorta of dog.
(a) Lamina elastica interna.
(b) Media.
(c) Adventitia.
Weigerts elastin stain × 130.

intima of the graft exhibited patches of a deeper pink color (Fig. 15: II). The histologic explanation of this was found to be that a "new intima" was in the process of development. The circumscribed patches were not observed in any of the four remaining cases in which the period of observation ranged from 12 to 15 months. Fig. 20 (Dog 3) is an illustration of this. In all four cases, the inner aspect of the graft was smooth and glistening with no signs of ulceration or thrombosis. The sutures were covered by a whitish membrane and did not protrude into the lumen. Neither narrowing of the diameter of the graft as compared with its original size nor secondary dilatation could be noted. The grafts were less elastic than the host vessels. There was always scar tissue surrounding the graft and part of the host vessel, which fact may explain the loss of elasticity.

HISTOLOGIC EXAMINATION

Fresh aortic segments.

Before describing the histologic results of homologous arterial grafts, it is perhaps advisable to consider the microscopic structure of a fresh vessel. Fig. 14 represents a photomicrograph of the abdominal aorta of a dog. Adjacent to the lumen can be seen a distinct intima, as well as a lamina elastica interna, patches of which are double. Next comes a strong media containing smooth muscle cells with well-stained nuclei. The elastic structure is concentrated in the media. Finally, we see the layer of connective tissue which constitutes the adventitia. A few thin elastin lamellae are normally found scattered through the adventitia. Most of the adventitia had been removed from the present specimen.

Aortic segments stored in the blood vessel bank.

In Chapter 10 it was demonstrated that with the method of storage used in this study, respiration studies indicated that almost all the grafts showed respiration for ten days and approximately 40% remained respiring up to three weeks.

Although the microscopic appearance of these stored vessels did not differ markedly from the normal, there were definite changes. After only a few days in the blood vessel bank, the vascular segments appeared to lose their normal intima. Traces of the original intima could be detected in some of the specimens, but it had invariably disappeared completely after longer periods of storage. Vessels stored for 14 days had an appearance similar to those stored for 30 days. Compared with fresh vessels, however, there was some degree of nuclear pyknosis in the media. The general structure, however, was relatively well preserved in vascular segments stored for several weeks in the blood vessel bank.

The Preoperative Duration of Storage and the Histologic Fate of the Grafts

Variations in the length of storage of up to 14 days appeared to have no effect on the histologic end-result. This was proved by examination of the grafts in Dogs 3, 6, 7 and 9.

The histologic examination took the form of a chronologic record of the changes in the operative field throughout the period of observation.

The reader is referred for orientation to a typical case (Fig. 15) of homologous transplantation of a segment of the abdominal aorta (Dog 2). Before the operation the graft was stored for 11 days in the blood vessel bank. It functioned in good order for 81 days. Fig. 15:I represents the graft *in vivo*, Fig. 15:II a photograph of the specimen viewed from the inside after the abdominal aorta had been split longitudinally. The darker patches represent deeper shades of color, not ulcerations. Fig. 15:III is a photomicrograph of a longitudinal section of the proximal anastomosis. The host vessel is to the left in the picture, the graft to the right. On the host side can be seen a silk suture with no signs of inflammation. The parts of the media of the host and the graft which are folded outward are clearly visible. On the inner aspect, toward the top of the picture the anastomotic angle has been filled in by a "new intima" or fibrocellular layer, which spreads over the graft. For the rest, one can observe the general structure of the host vessel and the graft, as well as richly vascular and cellular granulation tissue in the surroundings.

Even the cases that failed (Dogs 8 and 5) serve to some extent to illustrate the processes that take place in the anastomotic lines and the graft during the immediate postoperative period. In order to give a more comprehensive survey of the various components, it is advisable to describe the anastomosis and the rest of the graft separately.

Results

After only a *few hours* (Dog 5), there is already evidence of healing: a fibrin clot with no erythrocytes can be seen in the angle between the everted ends of the lost vessel and the graft. The folded parts of the media are altered and lack normal staining quality.

No trace of the original intima of the graft can be seen. Patches have been covered with a very thin layer of fibrin, while patches of lamina elastica interna are completely bare. The nuclei of the smooth muscle cells in the media show some pyknosis. The elastic structure appears to be intact. The very scanty adventitia is edematous, but shows no appreciable cellular activity.

After *one week* (Dog 8), fibroblasts have begun to appear in the thin fibrin clot (Fig. 16). A thrombus appears to be in process of organization. The connective tissue cells, which were immature at the outset, are growing in from the host intima and possibly also the media. They grow as in a tissue culture in the thin, uneven layer of fibrin lining the inner surface of the graft.

In the anastomosis, the everted pieces of the media of both the

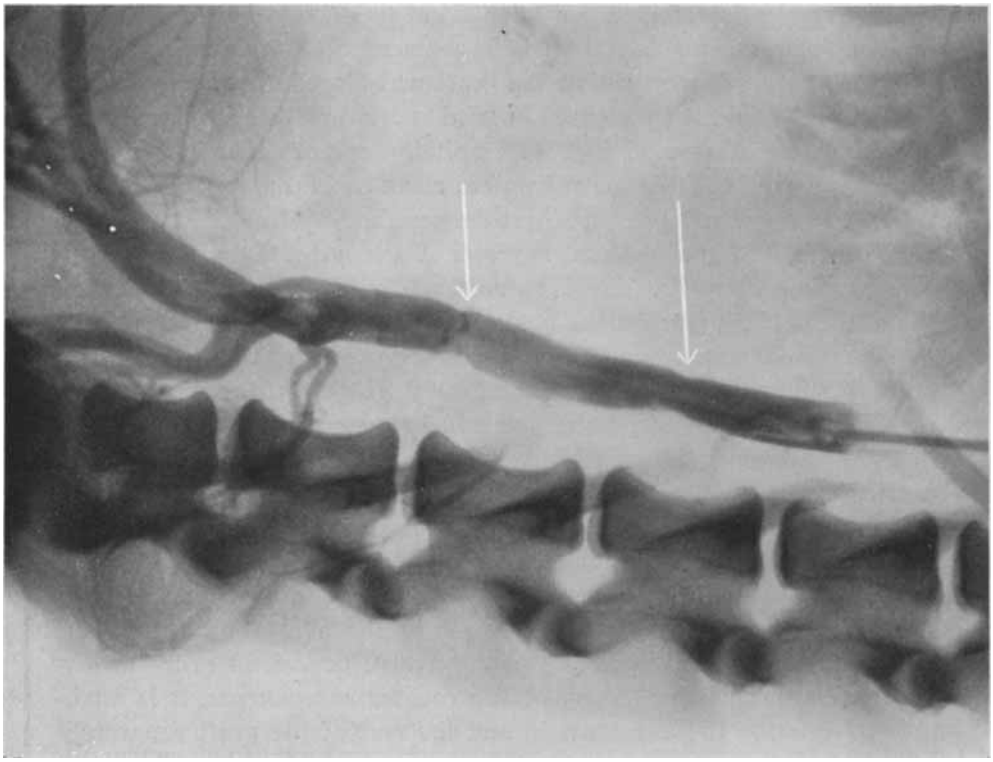


Fig. 15: I.
Aortic graft 81 days postoperatively. Dog No. 2. The graft stored 11 days before implantation.
I. Visualization of aortic graft *in vivo*.
II. Gross appearance.
III. Photomicrograph of the anastomosis. Hematoxylin and eosin stain × 130.

host vessel and the graft have taken on a hyalin appearance, probably a manifestation of a circulatory disturbance and necrosis. Surrounding the graft there is an inflammatory reaction.

Signs of degeneration in the media of the graft are now more pronounced. The nuclei of the smooth muscle cells have disappeared in some areas. This is especially true of the innermost areas. In the outer parts, collagenous fibers with normally stained connective tissue cells can be seen between the intact elastic structures. In this outer borderline layer, it is hard to determine whether the connective tissue represents a substitution of the external parts of the media emanating from without or if this area instead corresponds to the original adventitia of the graft with surviving connective tissue cells. If the latter were true, it would be in the process of becoming organized as a kind

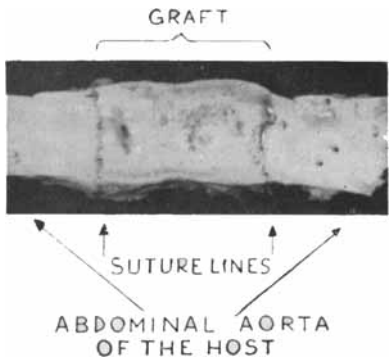


Fig. 15: II.

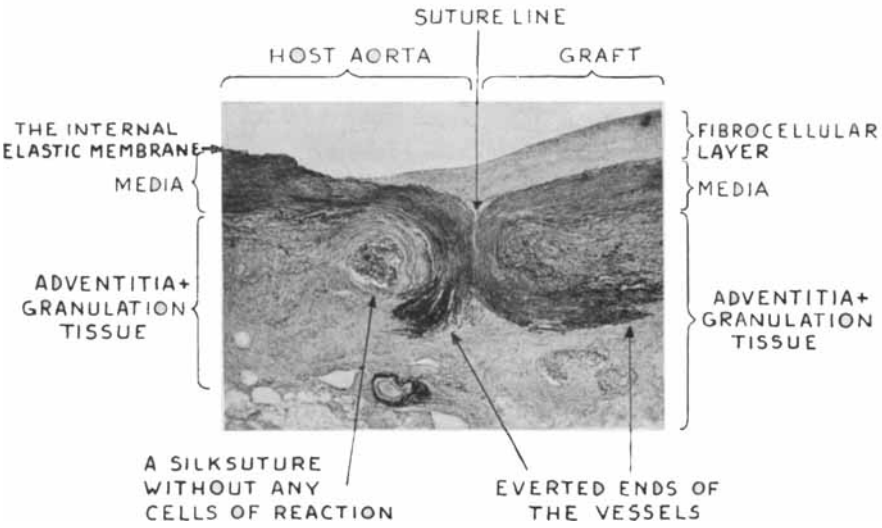


Fig. 15: III.

of media with the formation of new elastic fibers. Beyond this borderline layer we find ordinary granulation tissue rich in cells.

In the course of the first *three months,* major changes have taken place in the whole operative field (Dogs 1 and 2).

A tissue has developed in the transition between host vessel and graft, which, on the inner aspect, bridges and fills in the original eversion angle and spreads to a varying degree over the graft (Fig. 15: III). This tissue gradually becomes increasingly differentiated. The young fibroblasts turn into mature connective tissue cells, and colla-

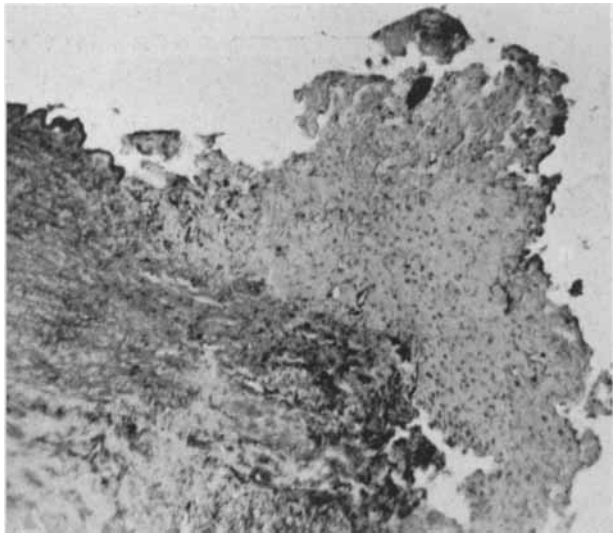


Fig. 16.
Young fibrin thrombus close to the suture line on the intimal surface of the graft. Notice numerous cell nuclei within the thrombus. This appearance represents an early stage of organization.
Aortic graft, 8 days postoperatively. Dog No. 8. Hematoxylin-v. Gieson stain × 70.

genous fibers appear. Flattened superficial cells and deep fibrocytes and collagenous fibers in various layers develop next to the lumen as a manifestation of functional adaptation. Fine elastic structures also make their appearance (Fig. 17). One of the grafts (Dog 2) also exhibited newly formed smooth muscle fibers in the inner membrane, concentrated in the central and deepest layer. In some places they were so dense that they formed a media-like layer within the new intima. This is illustrated in Fig. 18 (Dog 2), which gives a detailed picture of the fibrocellular layer with muscle cells. This newly formed, so-called fibrocellular layer extends only a short way into the host vessel and usually becomes merged with the normal intima at the level of the eversion. The fibrocellular layer grows out from the two ends of the graft as a substitution for the original intima of the graft, gradually covering more and more of the inner aspect. Its thickness and differentiation vary widely, and at a place corresponding to the center of the graft it appears as only a fibrin membrane with a few scattered fibroblasts, or a preliminary stage of the "new intima". Somewhat nearer the ends we see evidence of the development of the "new intima". The early fibrin clot appears as a substratum, into which young connective tissue cells are growing (Fig. 19). Still closer

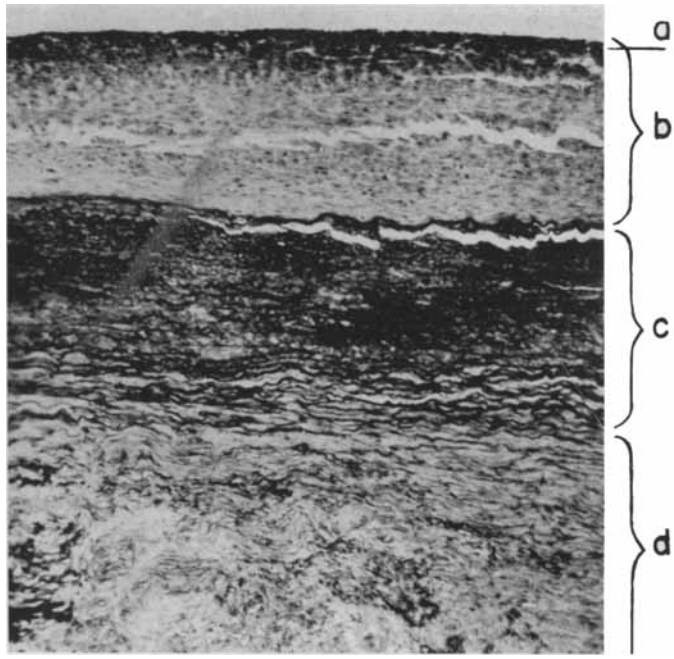


Fig. 17.

An aortic graft 81 days postoperatively. (a) Elastic tissue fibers; (b) Fibrocellular layer; (c) Media; (d) Adventitia and granulation tissue. The appearance of the fibrocellular layer (b) represents a later stage in the organization of the original fibrin clot (Fig. 16). Under higher power may be seen endothelial-like cells on the surface beneath which are connective tissue and smooth muscle cells as well as a layer of elastic tissue fibers (a).
Dog No. 2. Hematoxylin-v. Gieson stain × 70.

to the ends, this layer consists of denser collagenous tissue with cells resembling mature fibrocytes. The differentiation is most advanced at the angle between the host vessel and the graft.

The anastomosis is further characterized by the continued presence of the eversion. The folded parts of the media, which earlier showed signs of degeneration, have become completely hyalinized and acellular. The smooth muscle in the media of the graft appears to have almost completely disappeared, while the elastic structure seems to be well preserved. The granuloma formation beyond the anastomotic lines has progressed and now merges with an impressively thick connective tissue, which is rich in cells and vessels and contains collagen. This tissue extends over the whole graft and adjacent parts of the host vessel.

The reconstructive processes described above extend into the dif-

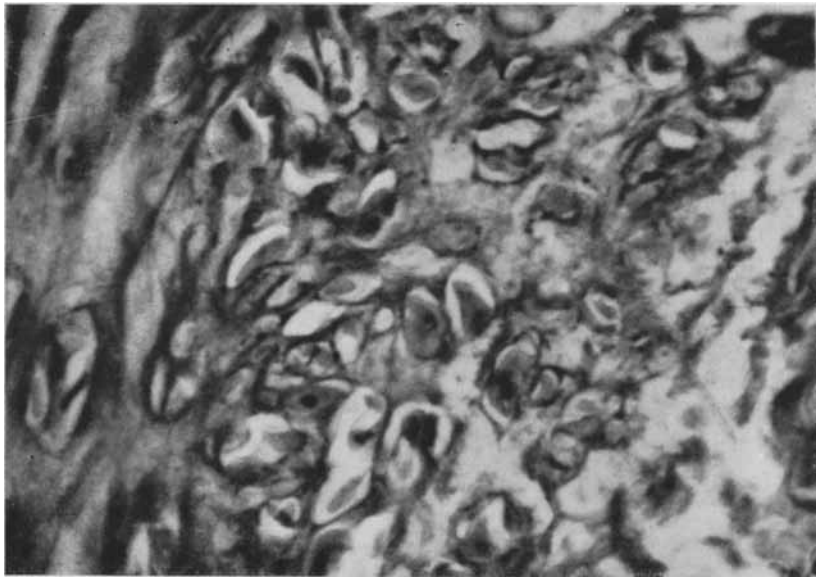


Fig. 18.
Higher magnification of the same specimen shown in Figs. 15 and 17. Young, smooth muscle cells in the new fibrocellular layer. Hematoxylin-v. Gieson stain × 800.

ferent parts of the operative field with certain minor individual variations, but on the whole in a uniform manner.

The picture in the grafts, which had been functioning for *12 to 15 months,* is quite different (Dogs 3, 6, 7, 9). The new fibrocellular layer in these cases is well developed and at the anastomotic lines looks like a thick scar membrane, extending over large areas of the graft. Smooth muscle cells are seen in several places near the host vessel. In one specimen it appears as if these cells emanate from the media of the host vessel. Not even at this late stage has the inside of the whole graft become lined with the fibrocellular layer. The thickness and cell content of this layer vary considerably.

The everted parts of the media in the anastomosis have not changed and are still hyalinized (Fig. 20). Beyond them the cellular granulation tissue has gradually turned into a less cellular, highly collagenous scar tissue of considerable thickness. It surrounds the whole graft and adjacent parts of the host vessel.

The smooth muscle tissue in the media of the graft has now disappeared completely. The degenerative process is on the whole not accompanied by ordinary inflammation cells. In general there is a noticeable lack of cell nuclei in the media of the graft. However, there

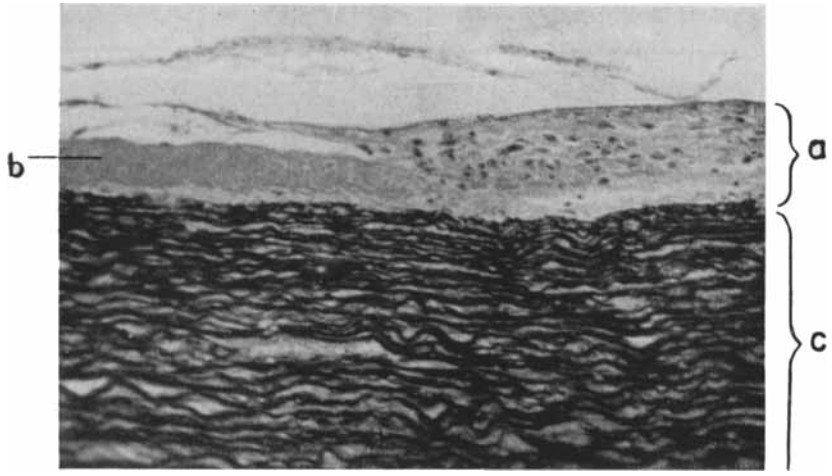


Fig. 19.
The transition between organized (a) and unorganized (b) parts of the "new intima" in an aortic graft intermediate in time between those of Figs. 16 and 17. Fortysix days after implantation the center of the graft is not completely covered by an organized fibrocellular layer. The invading connective tissue cells may be seen at (a) and the unorganized fibrin at (b). (c) Media of the graft. Dog No. 1. Before the implantation the graft was stored for 2 days. Hematoxylin-v. Gieson and elastin stain × 140.

are patches of cells resembling fibroblasts or fibrocytes. This may be a sign that the media too, is being organized by new connective tissue. This is especially true of the areas corresponding to the outer third of the original media of the graft. For there we can also observe coarse collagenous bundles of normally stained connective tissue cells between the elastic structures. This may mean that these parts of the media are becoming populated by connective tissue emanating from the host animal. The process most closely corresponds to what *Nageotte & Sencert* (1919) called "connective tissue reviviscence". It is also conceivable that some fibrocytes have survived in the original adventitia of the graft, or that secondary formation of elastin has taken place in this borderline layer. However, this is a rather far-fetched explanation of the fact that the area in question has taken on the appearance of a media.

The finding of collagenous connective tissue in the central parts, although in very small quantities, would indicate a successive substitution of the graft by connective tissue.

Due to the loss of the smooth muscle cells, the media of the graft has become thinner than that of the host. In the area of the grafted media there is a compact group of elastic fibrils.

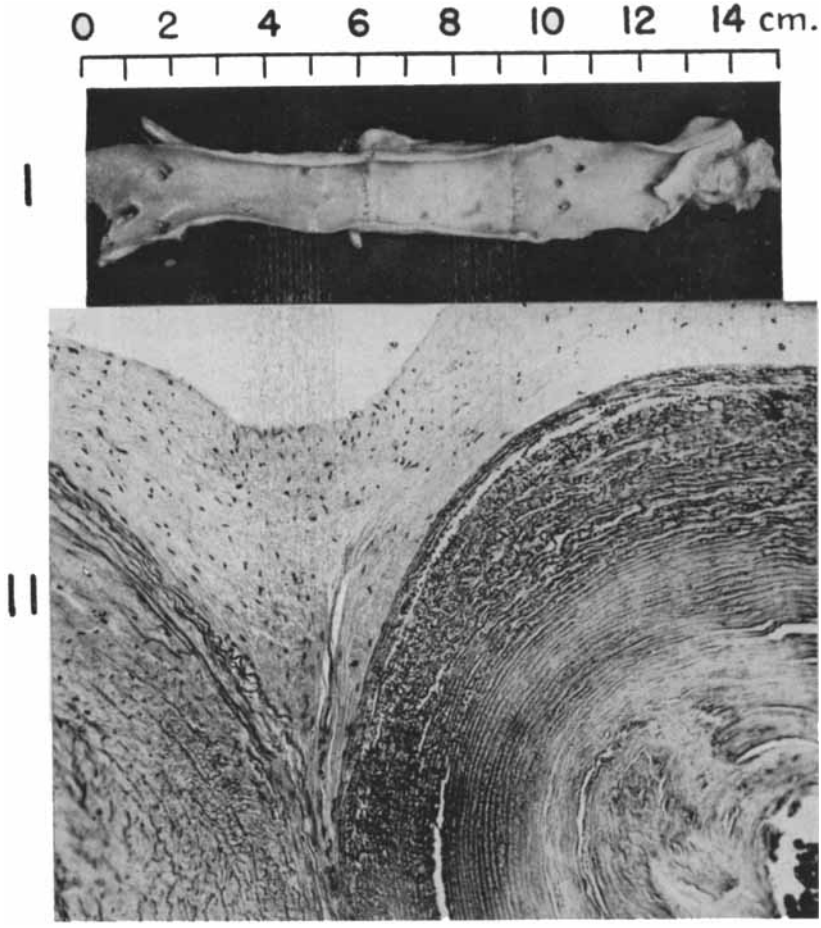


Fig. 20.

I. Photograph showing healthy appearance of the arterial lining 15 months post-operatively. Dog 3.

II. Photomicrograph of the anastomotic line. The eversion of the media is still present. The fibrous appearance of the media within the area of eversion is due to degeneration (graft at right). Hematoxylin and eosin stain × 132.

The elastic structure in the media of the graft shows normal staining quality in all the cases. It appears to be exceedingly resistant to degeneration however. There is some patchy fragmentation, mostly adjacent to the intima, but on the whole the elastic structure is well preserved in these grafts which have been functioning for as long as 15 months.

AORTIC GRAFTS CONSIDERED TO BE NON-RESPIRING AT THE TIME OF THE OPERATION

In two cases (Dogs 4 and 11), the grafts were stored for 30 and 38 days, respectively, in the blood vessel bank. Vascular segments preserved outside the organism for such long periods can, according to the results of the Warburg tests described on Page 56, be regarded as dead from the point of view of tissue respiration. One of these grafts was completely obliterated as a result of a septic infection (cf. Page 58). The second graft functioned well although its wall was calcified. In two cases (Dogs A12 and A13), the grafts were not taken until 24 hours after the donor dogs had been sacrified. These two were also considered to have lacked respiration viability. One graft was devitalized in absolute alcohol before implantation (Dog 1F).

Consequently, the following material (Table 10) was available for the morphologic examination of the functioning grafts in this group:

TABLE 10

Patent Aortic Grafts Non-respiring at the Time of Operation.

Dog No.	Preservation and Treatment of Graft Before Operation	Period of Observation (Months)	Results	Pictured in Report
4	Stored 30 days in 10 % serum in modified Tyrode's solution at + 1° to + 4° C°	2	Lumen patent. Calcified wall	Fig. 21
A 12	24 hours in dead donor dog	1	Lumen patent. Inflammatory irritation	
A 13	24 hours in dead donor dog; then preserved 7 days in 10 % serum in modified Tyrode's solution at + 1° to + 4° C°	2	Lumen patent.	
1 F	Devitalized in C_2H_5OH	14 1/2	Partial thrombosis	Figs. 22, 32, 12

The processes described in the previous group of aortic segments would also appear to have taken place in the grafts which lacked respiration. Due to the small number of cases in the latter category, none of the deviations observed could be definitely attributed to the preoperative treatment of the grafts. A brief morphologic description will now be given of these specimens, insofar as they differed from those in the previous group.

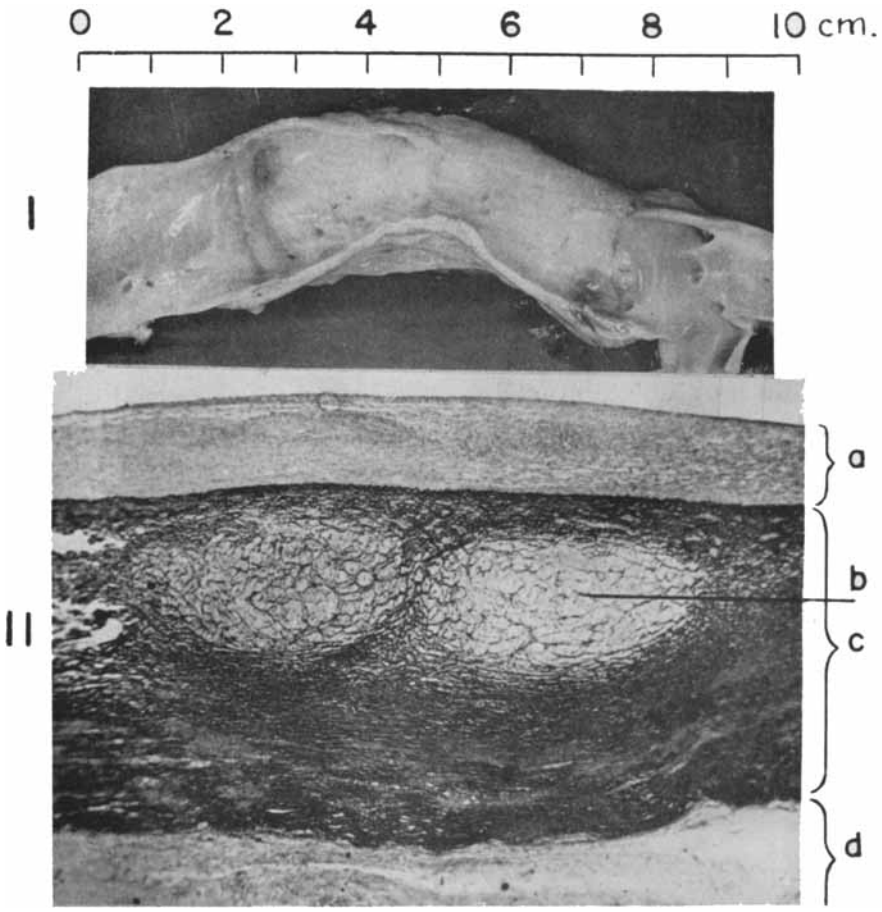


Fig. 21.

Aortic graft stored 30 days before implantation; in place for 69 days. Dog No. 4.

I. Gross appearance. The graft is patent. The luminal surface is smooth. Some areas of discoloration are noticeable. The wall is calcified and stiff.

II. Photomicrograph of the same graft near the center. Hematoxylin and eosin stain × 36.

(a) Fibrocellular layer.
(b) Patches of mucous degeneration in the media.
(c) Media.
(d) Adventitia and scar tissue.

Dog. No. 4: Aortic segment stored for 30 days prior to operation; no postoperative complications; period of observation 69 days.

Gross Examination:

In this case the graft consisted of a rigid tube which, when split, appeared to be calcified. The entire inner aspect (Fig. 21:I) was smooth and shining. The original sutures were scarcely perceptible inside the lumen. A smooth, glistening, white membrane covered the sutures. No thrombi or ulcerations were visible, but there were patches of a different shade than the surrounding graft tissue and host vessel. These are the patches that are prominent in Fig. 21: I. On the whole, the graft seemed to be too long and somewhat bent.

Histologic Examination:

The fibrocellular layer was well developed, corresponding to that of the anastomosis. However, this "new intima" and its precursor, the fibrin membrane, was very thin in the greater part of the graft, while it was completely lacking in patches.

The media of the graft was the site of calcific degeneration, very pronounced in spots. This was especially noticeable in the central parts, where both the smooth muscle and the elastic structure had disappeared. In other regions the elastic structure had been retained. Peculiar foci of myxomatous degeneration were observed in the central parts of the media (Fig. 21: IIb). Staining with toluidine of these areas produced a fine example of metachromasia. Substitution of connective tissue in the outer parts of the media in the graft was irregular.

Dogs Nos. A12 and A13: Aortic segments removed 24 hours after death of the donor dog; no postoperative complications; period of observation 1 and 2 months.

Gross Examination:

In Dog A12, the 24-hour-old segment was implanted directly, while in Dog A13 it was not implanted until after storage in the blood vessel bank for a further 7 days. Both dogs were normally active and the grafts functioned well. Gross examination 1 and 2 months later revealed the same picture as in the other cases of aortic grafts.

Histologic Examination:

The angle of the wound was filled with a relatively thin layer of connective tissue, in which flat cells resembling endothelium could be seen next to the lumen. The new intima was still very incomplete,

existing as a thin layer for a short distance from each suture line. The smooth muscle cells in the media of the graft had already disappeared. In one of the specimens there were distinct signs of inflammatory irritation with a violent infiltration of lymphocytes at the borderline between graft and granulation tissue. In the second specimen the histologic picture of the media was similar to that described for the respiring transplants relatively poor in nuclei and gradually merging with the granulation tissue of the host vessel.

Dog No. 1F: Aortic segments devitalized in C_2H_5OH; no postoperative complications; period of observation 14½ months.

Gross Examination:

There was just as much scar tissue surrounding this graft as the others described. No calcified patches could be seen when the aorta was split longitudinally. Both anastomotic lines were well healed and were bridged by a smooth, glistening membrane, which almost concealed the suture material. Two partly organized, relatively old mural thrombi were found in the lumen (Fig. 22: I). The larger one occupied an area of 25 × 12 mm. near the cranial anastomosis; the smaller an area 6 × 8 mm. near the caudal anastomosis. The thrombi were 1 to 2 mm. in height.

Consequently, the graft was able to function as a blood passage, as had already been shown in the aortogram (cf. Page 64). The rest of the inside of the graft was smooth and shining. Superficial ulcerations were seen at the site of the thrombi.

Histologic Examination:

The fibrocellular layer was only slightly differentiated and fairly thin (Fig. 22: II). Flat ulcerations, corresponding to the thrombi, could be seen in the media of the graft. These were covered by a layer of fibrin. The smooth muscle cells had completely disappeared from the media of the graft, as in all the other specimens with the same period of observation. The elastic structure was generally well preserved. In the outer parts of the media could be seen collageneous bundles with abundant normally stained fibrocytes; otherwise the media was poor in nuclei.

On the outside, the entire graft and the parts of the host vessel exposed at the operation were enclosed by thick scar tissue, poor in cells and rich in collagen.

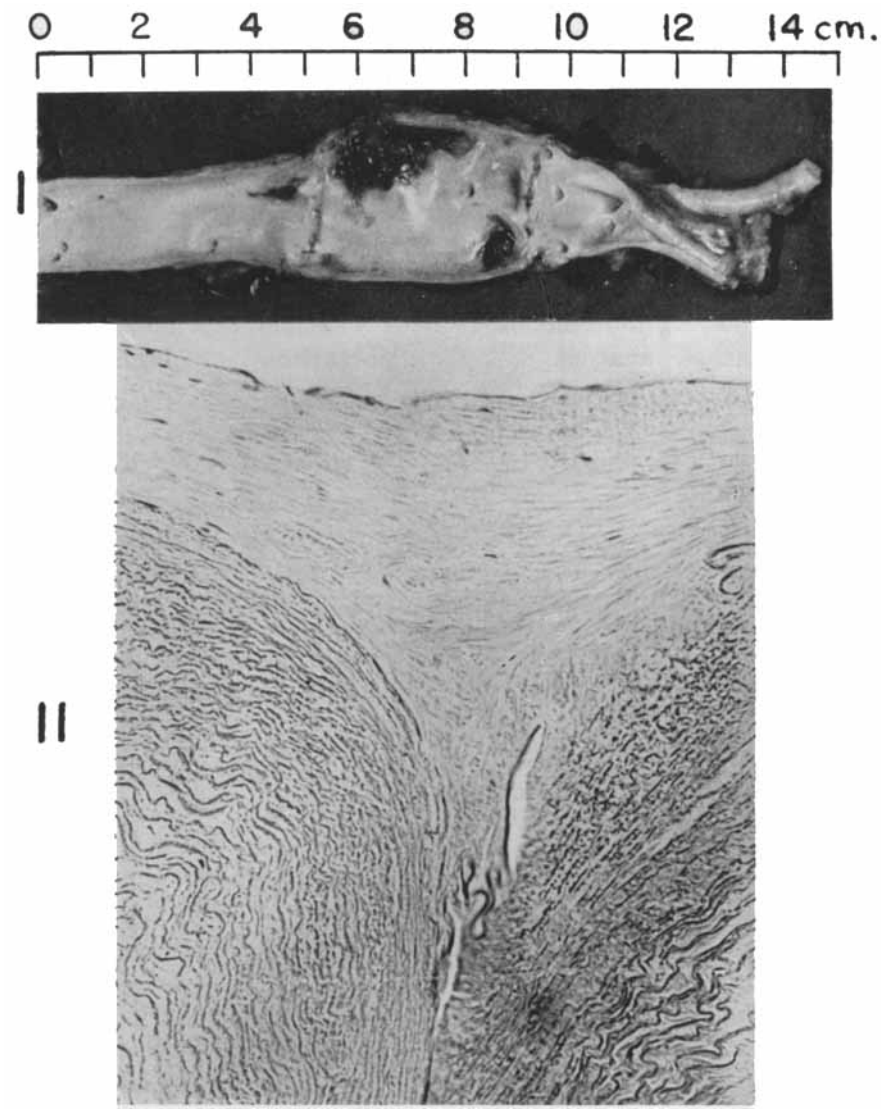


Fig. 22.

An example of a devitalized aortic graft functioning for 14½ months. Dog No. 1F.

I. Gross appearance. Most of the graft appears healthy apart from the two areas of mural thrombi.

II. Photomicrograph of the anastomotic line of the same specimen. Graft to the right. In general this resembles the picture in Fig. 20 of a graft preserved in Tyrode's solution. Hematoxylin and eosin stain × 190.

PERIPHERAL ARTERIAL GRAFTS RESPIRING AT THE TIME OF IMPLANTATION

On the basis of the results of the Warburg experiments (Chapter 10), the grafts in this series were considered to be well preserved and containing living cells from the point of view of respiration at the time of the operation. Eighteen of these grafts were implanted (Table 3). Six of them led to complete obliteration. This was observed by arteriography (Page 58). Morphologic examination of these non-functioning grafts revealed various stages of fibrosis, as well as organization of obstructive thrombi.

The following material of 12 dogs was thus available for gross and microscopic analysis of fully and partially functioning grafts (Table 11).

TABLE 11

Functioning Peripheral Arterial Grafts, Respiring at the Time of Implantation.

Recipient Dog No.	Length of time vessel preserved (days)	Period of observation			Result	Pictured in report
		Year	Months	Days		
P 5 sin.	Fresh	—	—	12	Leakage of the anastomosis	Fig. 26
P 2 dx.	Fresh	1	2	15	Lumen patent	
P 1 sin.	1 day	—	—	4	„ „	Figs. 24, 25
P 11 sin.	2 days	—	3	10	„ „	Fig. 27
P 3 dx.	2 „	1	2	6	„ „	
P 3 sin.	2 „	1	2	6	„ „	Fig. 28
P 1 dx.	6 „	—	—	18	„ „	
P 12 sin.	7 „	1	—	—	„ „	
P 12 dx.	7 „	1	—	—	„ „	
P 8 dx.	7 „	—	—	27	Mural thrombi	
P 8 sin.	7 „	—	—	27	„ „	
P 2 sin.	20 „	1	2	15	„ „	

GROSS EXAMINATION

One dog (Dog No. P5 sin.) showed a growing hematoma in the operative field, and was therefore sacrified twelve days postoperatively. This case was found to have limited insufficiency of the suture line, but otherwise the lumen was patent. Two of the functioning grafts exhibited numerous superficial mural thrombi (Dog Nos. P8 dx., P8 sin.), which did not obstruct the lumen. Another graft (Dog No. P2 sin.) showed a mural thrombus which left about half the graft as well as the suture lines free. The inner aspect of the other nine grafts was smooth and shining. A few cases had small patches of red or

yellow discoloration, but no ulcerations. The consistency of the grafts was usually somewhat tough and stiff. There were no calcifications. With the exception of the cases with thrombi, no stenosis occurred. In other words, the perfectly functioning grafts retained their original diameter. (One of the functioning grafts, 101 days postoperatively, is illustrated in Fig. 27 as an example: the inner surface is smooth and shining, and the even suture lines are covered by a whitish membrane. It resembles adjacent parts of the divided host vessel.)

HISTOLOGIC EXAMINATION

Normal structure of peripheral arteries

The structural arrangement of the femoral artery, for example, is fundamentally different from that of the aorta. In the femoral artery, the media is dominated by the smooth muscle cells. The vessels of that kind are therefore called *muscular arteries,* as contrasted with the elastic or central vessels. The media of the femoral artery contains very little elastic material. The elastic tissue is concentrated in a thin lamina elastica interna and in the adventitia (Fig. 23).

Segments of peripheral arteries stored in the blood vessel bank

Fig. 23 is a photomicrograph of a femoral artery which had been stored for seven days in the blood vessel bank. It illustrates well the normal histology of a peripheral artery, except for the intima which has practically disappeared. Remains of an intimal layer can be seen in the figure. The greater part of the media is made up of smooth muscle cells with easily stained nuclei. The most prominent feature of the elastic structure is the lamina elastica interna; otherwise, all that can be seen are occasional elastic fibers. The adventitia, on the other hand, contains abundant elastic tissue. It is about one-third as thick as the media.

After longer periods of storage, the intima usually had disappeared entirely. In these cases some degree of nuclear pyknosis could also be noted in the smooth muscle of the media. On the whole, however, the general structure of the small arteries was well retained during storage in the blood vessel bank.

THE PREOPERATIVE PERIOD OF STORAGE AND THE HISTOLOGIC FATE OF THE GRAFTS

It did not appear to make any difference to the histologic end-results whether the implanted segments were fresh or had been stored for one to seven days.

The histologic fate of the grafts is therefore described chronologically, depending on the length of the observation period.

As it was described for aortic grafts, the angle between the everted ends of the host vessel and the graft was filled by a small fibrin thrombus at an early stage in the case of the small vessels. After *four days* fibroblasts could be seen penetrating into the primary fibrin clot (Fig. 24).

The inner aspect of the graft, which had lost its original intima, was in the process of being coated by a thin irregular layer of fibrin. At the same time distinct changes were taking place in the media (Fig. 25). The nuclei of the smooth muscle cells were losing their normal staining quality. While some of them appeared to be swollen, others showed a varying degree of pyknosis. This explains the apparent nuclear pleomorphism in certain areas. In addition, patches were seen in which the staining quality had completely disappeared. This can probably be interpreted as an early stage of the progressive degeneration of the cellular elements of the media, which has been previously described in the aortic grafts.

Twelve days after transplantation, the formation of the fibrocellular intimal layer near the anastomosis was well under way (Fig. 26), but it had still only extended a short distance beyond the eversion. The progress of the degeneration in the media was particularly pronounced, notably in the outer parts next to the adventitia. Nevertheless, large areas which had retained their smooth muscle cells were still to be seen. This also applied to a specimen studied eighteen days postoperatively (Dog P1 dx.). Here the new formation of the "intima" had extended so far out that the center of the graft was coated by a thin layer of fibrin with scattered cells.

During the course of the *first three months,* a fully developed

---

Fig. 23.

Femoral artery, preserved 7 days in 10 per cent serum in Tyrode's solution at +1° to +4°C. This is an example of a muscular artery for comparison with the aorta, consequently the elastic fibers are mainly in the adventitia. Only traces of the normal intimal layer are to be seen on the intimal elastic membrane. The media looks entirely normal with well preserved nuclei in the smooth muscle.

I. Hematoxylin and eosin stain × 130.

II. Weigerts elastin stain × 130.

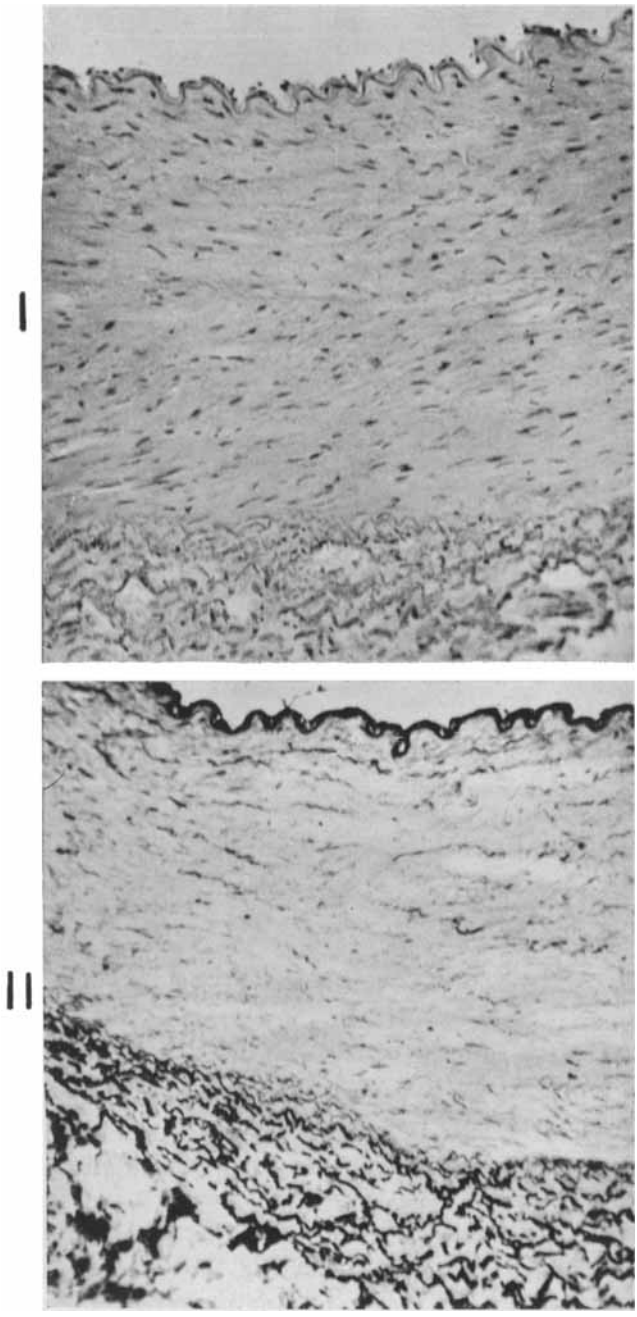


Fig. 23.

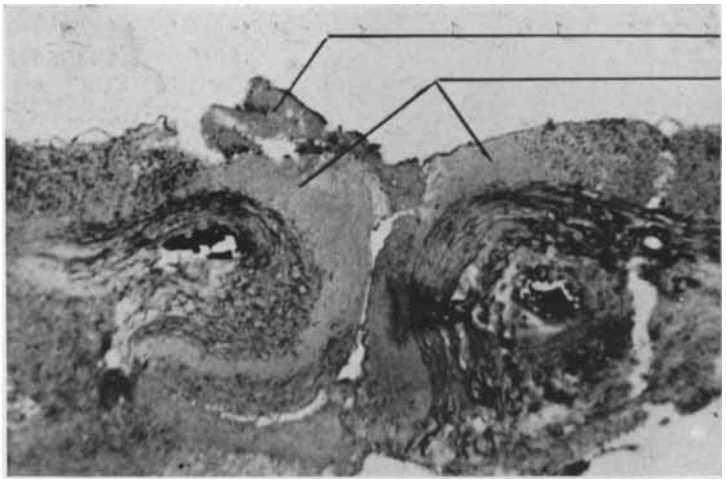


Fig. 24.

An early stage of repair in a femoral graft (4th day) comparable to that seen in the aorta in Fig. 16. Fibrin, which is just becoming organized by connective tissue cells, lies between the everted luminal edges of the suture line.

(a) Young fibrin thrombus close to the suture line.

(b) Necrosis of the media of the host vessel and of the graft (graft to the left). Dog No. P1 sin. Hematoxylin-v. Gieson stain × 40.

fibrocellular layer had been formed, covering and levelling the anastomotic lines and constituting a sort of "new intima" over the graft (Fig. 27). As in the aortic grafts, the new intima was still irregular with regard both to extent and to thickness. Practically all the smooth muscle cells in the media had degenerated. Their nuclei could no longer be identified. The whole media had taken on a hyalin appearance and looked somewhat compressed compared with that of the host vessel. Scattered cells, interpreted as histiocyte elements, could be seen in this hyalin media. Otherwise the degenerative process was not accompanied by inflammatory cellular infiltration.

After *twelve to fourteen months,* the "new intima" had turned into a thick, firmer membrane, which even exceeded the media in thickness (Fig. 28) (Dogs P2 dx., P2 sin., P3 dx., P3 sin., P12 dx., P12 sin.). In one case, smooth muscle cells were distinctly visible in the "new intima".

Beneath the fibrocellular intimal layer, the lamina elastica interna was generally clearly discernible. While the elastic structure was scarcely perceptible in the media in small arteries, the grafts often appeared to have a high elastin content in the media, due to the loss of the smooth muscle cells (Cross-sections of such elastic structures should not be confused in the Figures with cellular nuclei). Only in

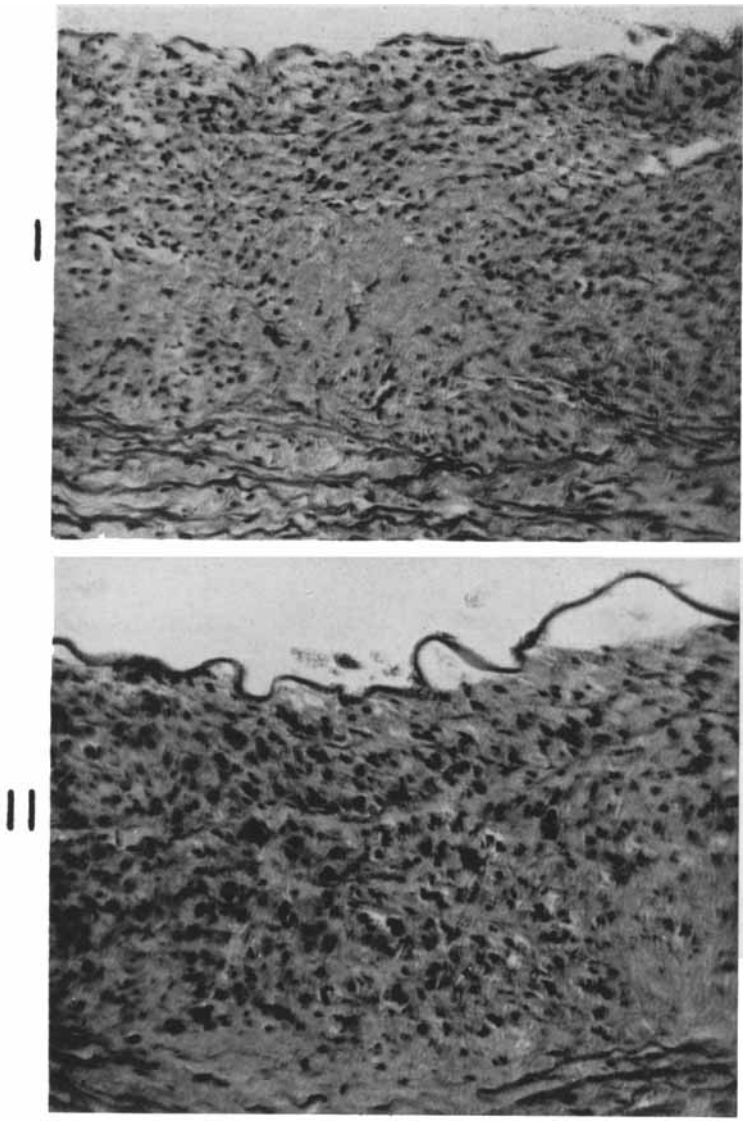


Fig. 25.

Higher power of the graft shown in Fig. 24. No trace of the original intima is visible. The nuclei of the smooth muscle cells begin to disappear. In some areas there are pycnotic nuclei, in other the nuclei are swollen.

Hematoxylin and eosin stain.

I. Acellular areas in the media × 140.

II. Nuclear pleomorphism × 160.

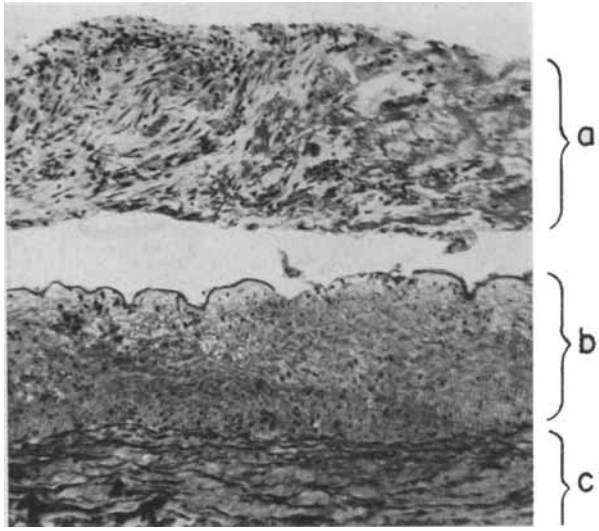


Fig. 26.
Femoral graft 12 days postoperatively. Dog No. P5 sin.
(a) The new fibrocellular layer with fibrin and fibroblasts (separation is an artefact).
(b) Degenerating media.
(c) Adventitia.
Note the early invasion of connective tissue cells and compare with the appearance at 46 and 81 days for the aortic grafts (Figs. 18 and 19 resp.).
Hematoxylin-v. Gieson and eosin stain × 90.

one case were scattered foci of calcification seen (Dog P12 sin.). Here, too, the elastic structure was somewhat fragmented. On the whole, the elastic structure appeared to be highly resistant, which was also true of the aortic grafts.

After twelve to fourteen months, the adventitia contained some thick, well-preserved elastic lamellae, although others had undergone some degree of fragmentation. The adventitia and periadventitia had normally stained nuclei, and the collagenous connective tissue resembled that described in the aortic grafts. Here also, there seemed to be some emigration of fibroblasts to the borderline area between the graft and the scar tissue of the host vessel. It is often difficult to draw a clear line of demarcation in this area. Easily stained elastic lamellae and connective tissue with normal cell nuclei form an unbroken natural transition from the original graft to the host vessel. The transition of the first, loose, cellular granulation tissue to scar tissue poor in cells is also identical in both groups.

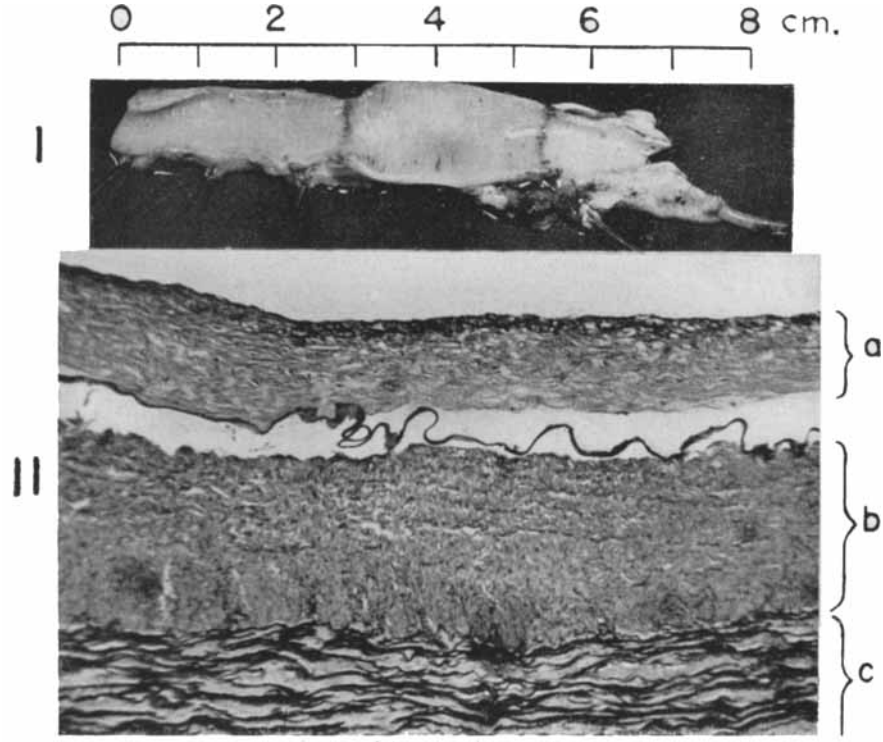


Fig. 27.

Femoral arterial graft 101 days postoperatively. Before implantation the graft was stored 2 days. Dog No. P11 sin.

I. Gross appearance.

II. Photomicrograph of the center of the same graft.

(a) New fibrocellular layer (separation is an artefact). Observe dark upper margin due to newly formed elastic tissue.

(b) Media.

(c) Adventitia.

Compare with Fig. 17 for the appearance of the aortic graft at a similar stage. Hematoxylin-v. Gieson and elastin stain × 30.

PERIPHERAL ARTERIAL GRAFTS NON-RESPIRING AT THE TIME OF IMPLANTATION

In three cases (Dogs P9 dx., P9 sin., P10 sin.), the grafts had been stored for 36, 42, and 61 days, respectively, before operation. One dog (P6 dx.) was given a segment devitalized in KCN, and in two cases (P6 dx., P7 dx.), the grafts consisted of segments of the femoral artery that had been fixed in C_2H_5OH. The grafts became obliterated with thrombi in all but two of this series of six dogs (cf. Page 59).

Only two specimens were available for morphologic investigation of functioning grafts in this series (Table 12).

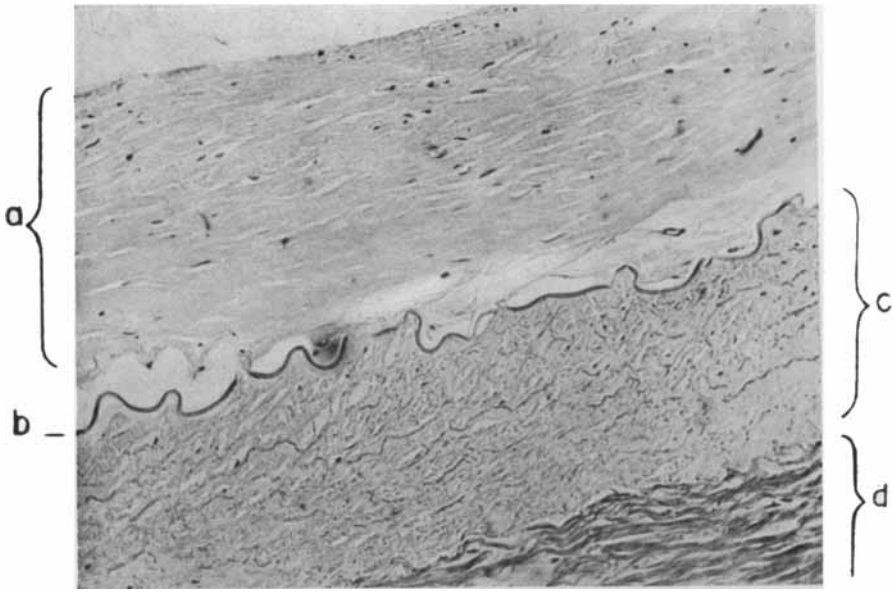


Fig. 28.

Femoral graft 14 months postoperatively. The fibrocellular layer (a) is thick and covers the internal surface of the graft. The internal elastic membrane is well preserved (b). No normal nuclei of the smooth muscle cells are to be seen in the media (c). Adventitia (d).

Dog No. P3 sin. Before implantation the graft was stored for 2 days.
Hematoxylin and eosin stain × 190.

TABLE 12

Functioning Grafts of Peripheral Arteries, Non-respiring at the Time of Implantation.

Recipient Dog No.	Length of time vessel preserved	Time of observation	Result	Pictured in report
P 9 dx.	36 days	14 months	Lumen patent	Fig. 29, 13
P 9 sin.	42 days	14 months	Lumen patent	Fig. 13

MORPHOLOGIC EXAMINATION

In both cases the inner aspect of the graft was smooth and glistening without signs of ulceration or thrombosis (Fig. 29). The sutures were scarcely perceptible. The anastomotic lines were covered by a whitish membrane. There was no stricture or dilatation. The walls of the grafts were tough. On the outside the grafts were surrounded by scar tissue.

The picture here was in general the same as already described. In

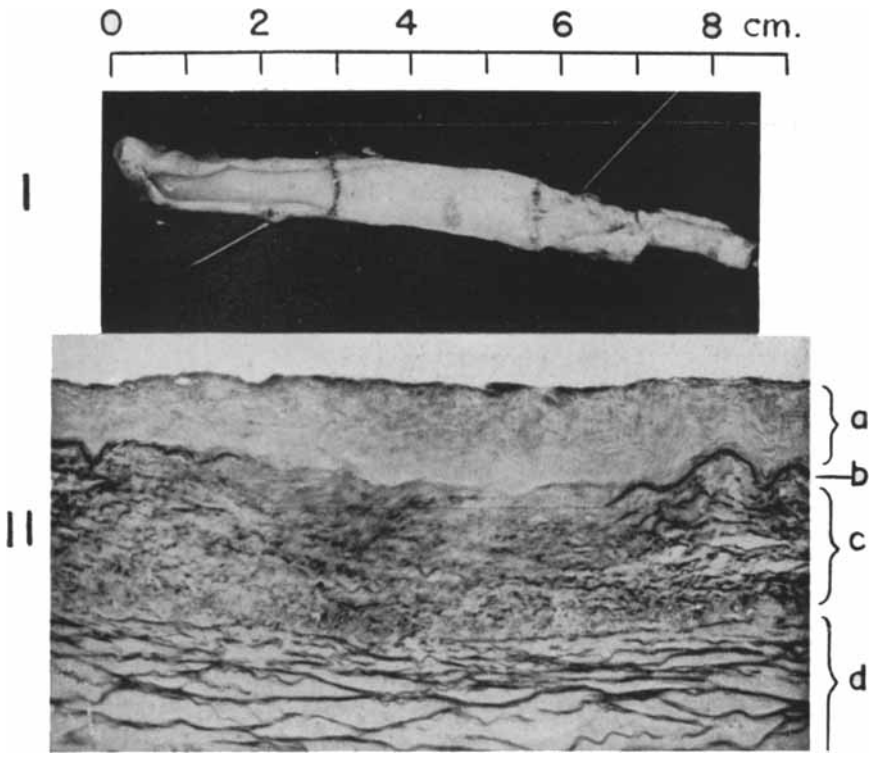


Fig. 29.

An example of a femoral graft preserved for over a month before implantation. Time of observation 14 months. Dog P9 dx.

I. Gross appearance. Note the healthy appearance of the luminal surface, except for the small area of discoloration.

II. Photomicrograph of the graft near the center. (a) Fibrocellular layer; (b) the elastica interna, which has disappeared in the center, probably due to necrosis of the media; (c) media; (d) adventitia.

Hematoxylin-v. Gieson and elastin stain × 150.

these cases also, the "new intima" consisted of a strong membrane of uneven thickness. The degeneration of the media was of the same degree after 14 months, although the media appeared to be somewhat more compressed. Between the elastic lamella in the outer parts of the adventitia could be seen collagenous bundles with well-stained connective tissue cells, which were interpreted as immigrant connective tissue cells in the outer parts of the graft.

One observation should perhaps be cited as evidence of the unsuitability of grafts that have been stored for such a long time in the blood vessel bank: These cases (Fig. 29: II) showed breaks in the lamina elastica interna which were not seen to the same extent in the other cases.

CHAPTER 13

RESULTS OF TESTS OF TENSILE STRENGTH OF SPECIMENS REMOVED FROM THE AORTIC WALL

Controls

Let us first consider the dogs not submitted to operation, in order to evaluate the tensile strength and the elastic properties of their vessels and to compare the findings with those in which grafting operations were performed. The control material consists of nineteen specimens submitted to tensile tests.

The results of the tensile tests are shown in the diagram in Fig. 30. The lowermost graph reveals the variations in thickness of the aorta. Above we see a schematic representation of the aorta, divided approximately according to dimension, as well as examples of transverse and longitudinal specimens. The other main diagrams show, for transverse and longitudinal samples of the aorta, the *ultimate, or breaking, load* (P_{max}) expressed in grams per specimen width (5-mm.) and *maximum strain* (ε_{max}) expressed in per cent for different parts of the aorta. The small diagrams represent typical load-deformation diagrams for specimens removed at the points where the respective graphs are plotted. In these the load (P) is marked on the ordinate and the strain (ε) in per cent on the abscissa.

The thickness of the aorta decreases caudally from the aortic arch. With the relatively rough method of measurement used, the thickness

---

Fig. 30.

Diagrams of tensile strength of aortic specimens. The lowermost graph reveals the variations in thickness of the aortic wall. Above is a schematic representation of the aorta and examples of transverse and longitudinal specimens stamped out. The main diagrams show for the transverse and longitudinal samples the ultimate load (P_{max}) expressed in grams per specimen width (5 mm.) and maximum strain (ε_{max}), expressed in per cent for different parts of the aorta. The small diagrams represent typical load-deformation curves for specimens removed at the points where the respective curves are plotted. In these the load (P) is marked on the ordinate and the strain (ε) in per cent on the abscissa.

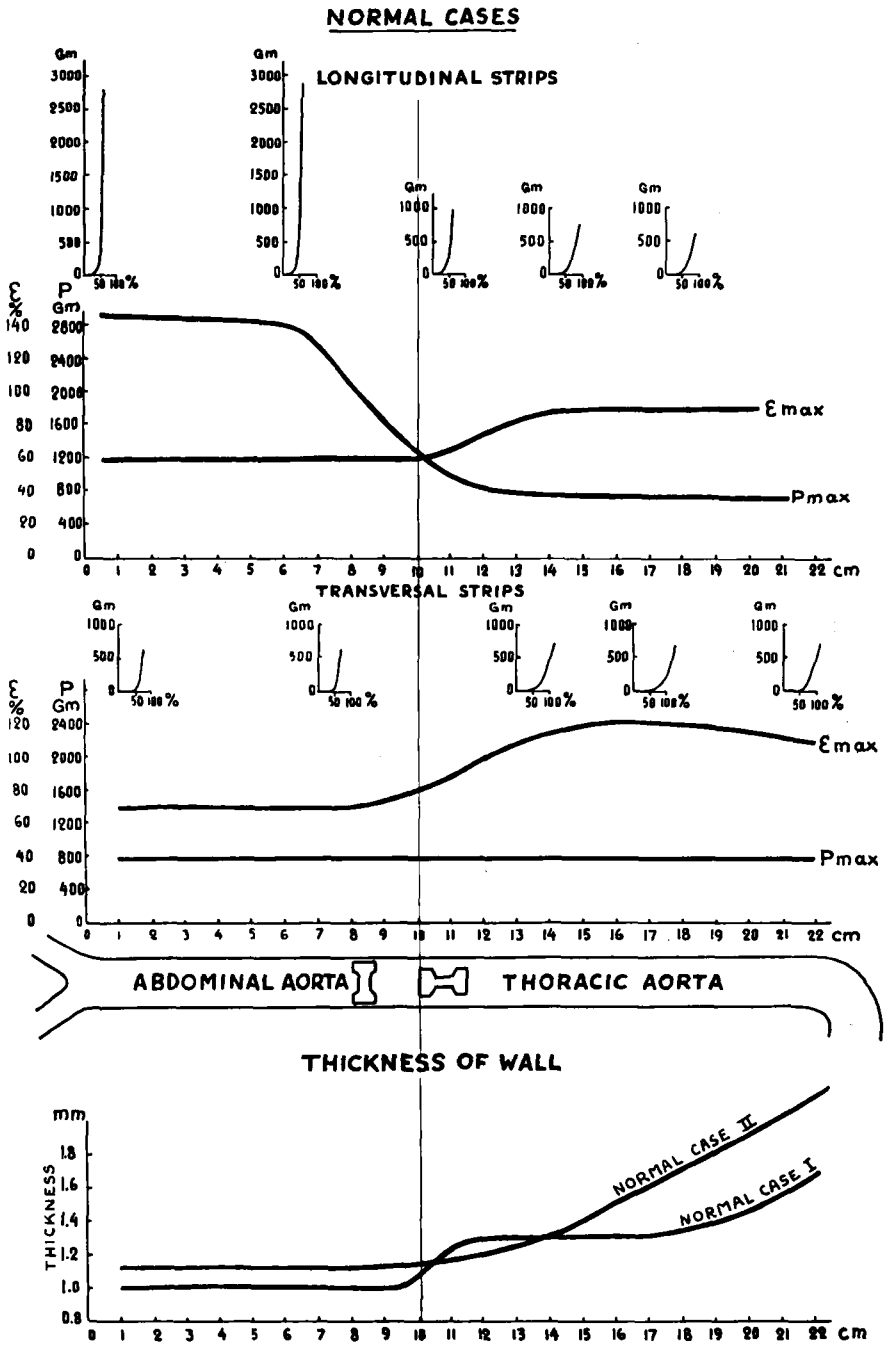

NORMAL CASES
LONGITUDINAL STRIPS
Gm
3000
2500
2000
1500
1000
500
0
50 100%
ε %
P
Gm
140
120
100
80
60
40
20
0
2800
2400
2000
1600
1200
800
400
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 cm
ε max
Pmax
TRANSVERSAL STRIPS
ABDOMINAL AORTA
THORACIC AORTA
THICKNESS OF WALL
mm
THICKNESS
1.8
1.6
1.4
1.2
1.0
0.8
NORMAL CASE II
NORMAL CASE I

Fig. 30.

was found to be constant in the abdominal aorta and somewhat less than in the inferior part of the thoracic aorta. (The two graphs represent normal dogs Nos. I and II, respectively).

In the *transverse specimens*, the ultimate, or breaking, load = (P_{max}), was relatively similar in the different parts of the vessel, or about 800 g. per specimen. Occasional deviations acounted to a maximum of 150 g. The maximal tensile strain (ε_{max}), was the equivalent of 100 to 130 per cent for the thoracic aorta and 65 to 75 per cent for the abdominal aorta. The load-deformation diagrams for individual specimens were the same when taken from various segments of the thoracic aorta. The various segments of the abdominal aorta show the same curve when compared to each other. There was, however, a distinct difference between the curves for the thoracic and for the abdominal aorta.

Longitudinal Specimens

The ultimate, or breaking, load (P_{max}) for the thoracic aorta and the upper part of the abdominal aorta corresponded to that for the transversal tests. This load was considerably higher in the inferior part of the abdominal aorta, as appears from the top section of Fig. 30.

The maximal tensile strain (ε_{max}) was fairly constant for the entire thoracic and abdominal aortas. The latter showed somewhat less tensile strain than the former. The comments regarding the load-deformation diagrams of the individual transverse specimens also were applied to the longitudinal ones.

Experimental Material

The results of the tensile tests were reproduced in diagram form in the same way as the control material. Two such typical diagrams are shown in Figs. 31 and 32 (Dogs Nos. 7 and 1F).

Thickness

The variation in thickness of the entire thoracic aorta and the upper part of the abdominal aorta was the same as in the control material. The thickness increased near the operative field, and at the site of the anastomosis it was 0.6 to 0.7 mm. greater than in the intact abdominal aorta. This applied to all the specimens examined. The explanation is to be found in the heavy formation of scar tissue around the whole graft and parts of the host vessel.

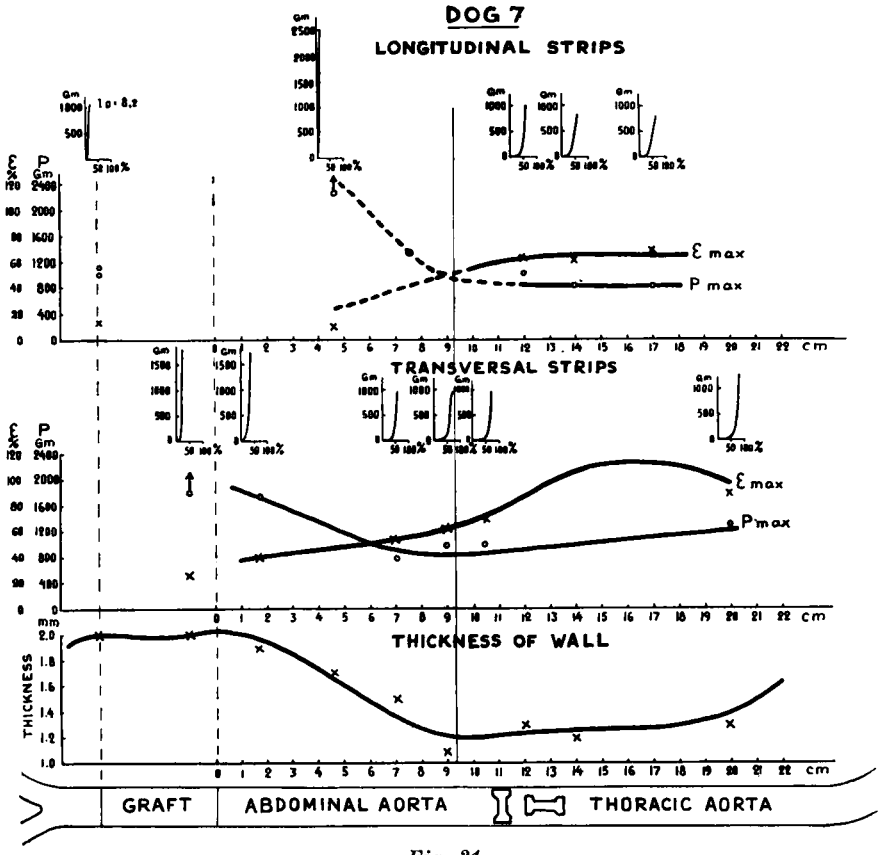


Fig. 31.

Diagrams of tensile strength tests of aortic specimens (Dog No. 7). For explanation and comparison see Fig. 30. In the schematic representation of the aorta, the graft has been marked. Note the increase of thickness of the aortic wall in the operative field. ♂ = the grips slipped at that level.

The graft itself was generally 0.5 to 0.6 mm. thicker than an intact abdominal aorta. The figures for thickness cannot be regarded as completely accurate, due to the impossibility of dissecting every specimen in precisely the same way. They do, however, express a definite trend.

Transversal Tests

The ultimate, or breaking, loads in the thoracic aorta and the upper part of the abdominal aorta lay within the same limits as in the control material, which was to be expected. The ultimate loads (P_{max}) increased at a distance of 4 to 5 cm. from the anastomosis.

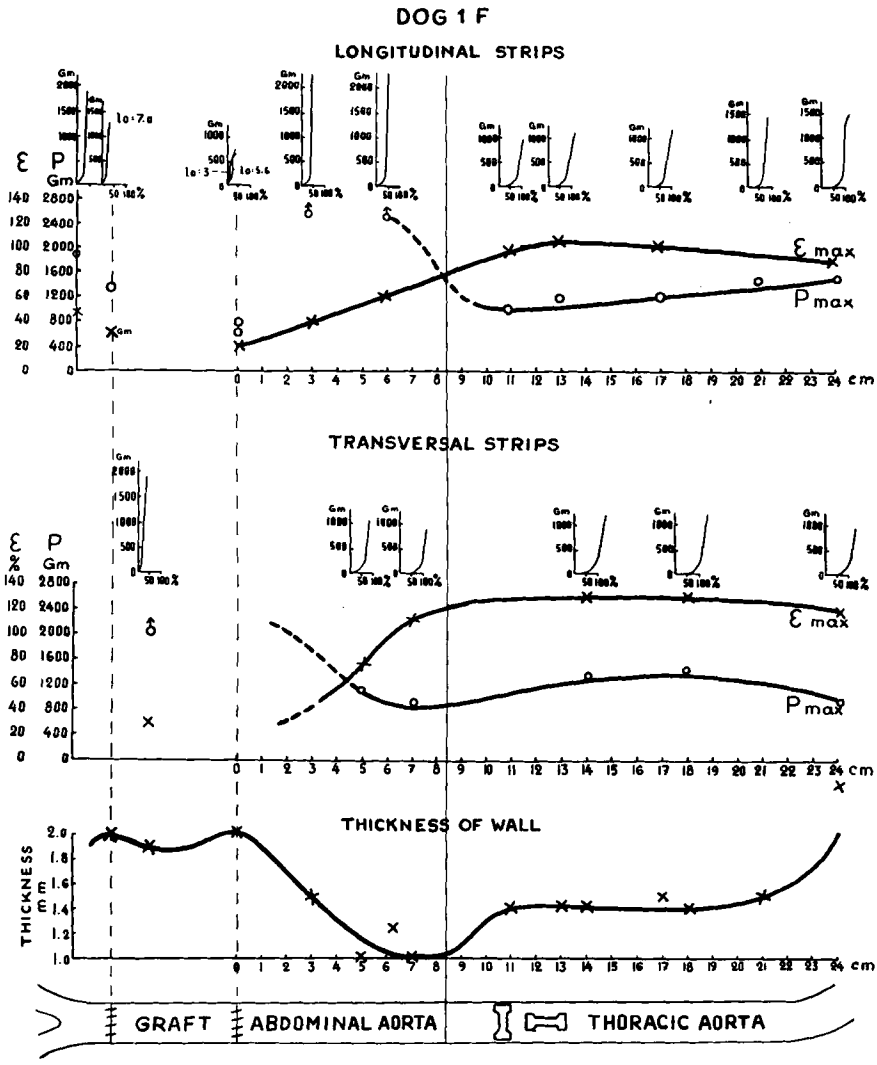


Fig. 32.
Diagrams of tensile strength tests of aortic specimens (Dog 1F). For explanation see Figs. 30 and 31.

This increase appeared to follow a parallel course with the increase in thickness. Next to the anastomosis the ultimate load was approximately 2000 g. per specimen, i.e., about twice as much as for the intact abdominal aorta. The grafts themselves also proved much more resistant to tensile force than the intact abdominal aorta. The ultimate loads for transverse specimens of the grafts were of the same magni-

tude as for transverse specimens from the abdominal aorta in the area of the anastomosis.

The maximal tensile strain (ε_{max}) in the thoracic and the upper part of the abdominal aorta was the same as in corresponding parts of the control material, and the load-deformation diagrams for the individual specimen also corresponded with the control material. Closer to the anastomosis, the tensile strain decreased at about the same rate as the thickness increased. The transverse tensile strain of the graft amounted to 25 to 35 per cent in four cases and to as much as 55 to 60 per cent in two cases (Dogs 3 and 9). The material is too small to permit of any conclusions regarding the effect of factors such as the length of observation and the viability of the graft. After only one month had elapsed, however, there was a considerable increase of thickness over the anastomotic lines due to the formation of scar tissue. No transverse specimens could be obtained from the anastomotic line because of the small size of this area.

Longitudinal Tests

The longitudinal tensile strength of the thoracic aorta and an intact portion of the abdominal aorta corresponded on the whole with the control material. The tensile strength was impressive near the anastomosis–in no case was it possible to obtain precise figures for the ultimate load (P_{max}), since the equipment for fastening the specimens was not adequate for such heavy loads. No longitudinal specimens were taken from the grafts themselves because of the small size.

Longitudinal specimens, removed over the anastomosis (= the suture line across the specimen) varied in tensile strength. The levels for ultimate load (P_{max}) were between 600 and 1500 g. per specimen. The lower figure is only slightly smaller than the lowest levels for the control material. However, these figures probably do not represent the true strength of the anastomosis, since the loading must be regarded as very unfavorable to specimen because of its irregular shape, taken from across the suture line. A regular specimen from an intact vessel, for example, would be much more uniformly loaded than a specimen with local deposits of scar tissue. Furthermore, a specimen containing sutures which may pass somewhat obliquely across it, will be much more sensitive to tensile force than a complete vessel with a circular suture. It would therefore seem reasonable to regard the suture lines as approximately of the same strength as the intact host aorta.

The maximal strain (ε_{max}) in the thoracic aorta and the intact

part of the abdominal aorta were of the same magnitude as in the control material.

It is interesting to observe the individual load-deformation diagrams for the various specimens. The curves in Figs. 31 and 32, for example, reveal clearly that the course became steeper the closer to the anastomosis and the scar tissue the specimen was removed.

DISCUSSION

The tensile strength of the vascular wall in the operative area appears to be intimately connected with the quantity of the scar tissue. On the whole, the effect of the scar tissue may be said to be in proportion to its thickness. Thanks to the high tensile strength of the scar tissue, the anastomoses compare favorably in strength with the intact host aorta–even though the configuration of the anastomoses is very unfavorable from the point of view of tensile strength to tensile loading across the suture lines.

The grafts themselves, which are also surrounded by a strong rind, show greater tensile strength and less tensile strain than the intact host vessel. The part played by the newly formed intima and by the elastic structures of the original media has not been analyzed in the present investigation.

As a result of the effect of the scar tissue on the elasticity of the vascular wall, the tensile strain of the operated vessel is probably less in response to physiologic loading than that of the normal vessel. It would seem that this would be especially marked in the anastomotic lines, partly because of the strong scar formation and partly because of the accumulation of mural tissue in those areas. This assumption is borne out by observations in connection with the visualization of grafts *in vivo* under different conditions of pressure (Page 61). The effect of the thickness of the scar tissue on the load-deformation diagrams of the different specimens may be likened to the effect of age on the elastic properties of the vascular wall. The load-deformation for specimens removed within and beyond the scar tissue area are somewhat similar to the stress-strain diagrams which *Moissejeff* (Fig. 5) gave for young and old dogs respectively. In this instance thickness of the vessel wall instead of age seems to be the factor involved. The more this factor increases, the steeper the curve becomes.

That the vessel wall decreases in elasticity with age is a generally

accepted fact and has been proved by *Moissejeff* (1926) and *Krafka* (1940), among other workers. *Krafka* (1940) was of the opinion that this decrease in elasticity is twenty per cent due to fibrotic degeneration of the vessel wall. In the experimental dogs, each factor, which might be expected to affect the remarkably great tensile strength and the relatively insignificant strain in the area of the anastomotic lines, was not investigated separately. However, the gross and microscopic observations and the failure to contract and expand when visualized *in vivo* under different conditions of pressure, indicate that the outer and inner scar tissues constitute the most important factors from the point of view of strength.

As far as the grafts themselves are concerned, it is probable that the often well preserved elastic structure also makes an important contribution to the tensile strength.

In view of the foregoing, the risk of aneurysmatic dilatation following homologous arterial transplantation appears to be insignificant. It seems that the view expressed by *Schloss & Shumacker* (1950) and *Sako, Chisholm, Merindino & Varco* (1949) regarding circular arterial sutures to the effect that, "if no leak is noted upon completion of anastomosis, one need have no fear that it will leak subsequently if the blood pressure rises", is equally applicable to homologous arterial grafts.

SUMMARY

The need for a method of bridging gaps in the major arteries in various injuries and diseases has long been felt. Although it was proved as early as the beginning of this century by *Carrel* and others that preserved arterial segments could be successfully used as homologous vascular grafts, the practical clinical use of arterial homografts was not demonstrated until recently.

The availability of arterial segments of different calibers and lengths, preserved in a blood vessel bank, appears 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 those patients with incapacitating pain in the extremities due to segmental arterial obstruction may be submitted to radical removal of the obliterated area and bridging the resultant defect with a vascular graft.

It appeared from previous investigations that arterial tissue, removed aseptically within a few hours of death and refrigerated in nutrient medium, maintained living cells for days or even weeks. Opinions differ, however, about the importance of using such viable instead of nonviable grafts. It is still not known whether or not cells of a viable transplant do survive or whether they all die and are totally replaced by new cells from the host. If it is true that the great majority of all cells of the graft are doomed to destruction, and the graft is simply regarded as an organic tube or framework, then the method of replacement by new living cells from the host, which is not clearly understood, must be studied more carefully. Definitely devitalized arterial segments are also reported to be successful as homografts. As with the viable grafts, the nonviable may carry out their function in spite of marked histologic changes.

While the early results of arterial grafting have been good, there have been no adequate long-term follow-up studies. When we consider the histologic changes which occur early, it is conceivable that late complications may occur, i.e., a progressive degeneration of the graft, late thrombosis, rupture or aneurysm formation.

This experimental study had the following purpose:

1) To investigate the tissue respiration of arterial segments preserved in nutrient medium.
2) To study the techniques of vascular surgery and to test homologous arterial transplantation by means of the reconstruction of experimental defects in the aorta and peripheral arteries.
3) To study the functional and morphologic fate of such transplants.
4) To determine whether or not the length of time a vascular segment is preserved is related to the process of healing or to successful grafting.
5) To determine the tensile strength of the transplant, the anastomotic line and the host aorta.

1. An experimental blood vessel bank was established by removing aortic and peripheral arterial segments under sterile conditions from recently sacrificed dogs and preserving the specimens at $+1°$ to $+4°C$ in sealed Ehrlenmeyer flasks containing 10% homologous serum in phosphate buffered Tyrode's solution. The pH dropped regularly from 7.4 to 7.1 within a few days. After 3 weeks the average was 6.7. No attempt was made to maintain the original pH.

The oxygen consumption was studied by the Warburg technique in 10 fresh normal arteries and 149 segments stored in the blood vessel bank for 1 to 53 days. Oxygen consumption equal to and above 10 mm. per hour per 100 mg dry weight was shown to be significant. Only a few of the segments lost their tissue respiration within the first few days of storage. Most of them, or 80%, were respiring for 10 days. About 40% maintained a significant tissue respiration for 3 to 4 weeks. These figures correspond well with results of vitality tests used by other authors.

2. Experimental defects in the abdominal aorta (14 cases) and in the femoral artery (24 cases) were bridged with homografts of various kinds (Tables 13 & 14). Hemorrhage due to leakage from the anastomosis was noted in three cases. Heparin was not used. From the technical point of view, it was found to be of importance to use a careful atraumatic technique and 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.

3. The functional fate. It was usually possible to determine whether the grafts were patent by observing the dogs' general activity and by palpation of the femoral pulse. The transplantation of peripheral

arteries was frequently complicated by thrombosis, regardless of the length of the storage period (Tables 8 & 14). The chances of a good functional result were much better when the larger calibre arteries were used (Tables 7 & 13). Arteriography both early and late was used to visualize the presence or absence of a satisfactory lumen. No secondary dilatation of the graft was seen. If the lumen remained patent for the first few postoperative weeks it did so for the entire period of observation. The longest period of observation was 15 months.

The morphologic fate. Histopathologic study of 22 (26) patent homologous grafts of aortic and peripheral arterial segments revealed certain typical reconstructive processes common to both the viable and the nonviable 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 appeared so 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. In these cases the collagenous bundles of apparently normal connective tissue cells visible between the elastic lamellae of the graft must have arisen from the host.

It was exceedingly interesting to study the attempts made by the host animal to build up a new vessel wall inside the graft. This process seems to start by the precipitation of fibrin in the region of the original intima. Fibroblasts then grow in, beginning at the sites of the anastomoses, and collagenous and elastic fibers are eventually formed. The surface cells tend to become flattened and resemble endothelium. The process is a slow one, and several months generally pass before a real membrane has been developed. After a year, however, the fibrocellular layers, or 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 the fibrocellular layer. What little evidence is available from this study suggests that they are not transformed fibroblasts but migrant muscle cells.

In general there was no great difference in the histology of implanted respiring and nonrespiring homografts. It should be pointed out, however, that the latter showed occasional thrombi, areas of myxomatous degeneration, calcium deposits and also breaks in the

TABLE 13

Aortic Homografts–Functional Results.

Type of graft	Number of transplantations	Patency			Occlusion
		Good function	Early hemorrhage from anastomosis	Partial thrombosis	
I *Removed under sterile conditions within 3 hours after death of donor dog:*					
a. Stored 2-18 days in nutrient medium at $+1^0$ to $+4^0$ C.	9	6	2	0	1
b. Stored 30-38 days in nutrient medium at $+1^0$ to $+4^0$ C.	2	1	0	0	1
II *Removed under sterile conditions 24 hours after death of donor dog.*	2	2	0	0	0
III *Devitalized in absolute alcohol.*	1	0	0	1	0

lamina elastica interna. It seems, therefore, 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 of a pipeline.

4. It was, however, impossible exactly to relate the method of preservation of the graft or the length of time the graft was stored to the process of healing or to the final functional result. Because of the high incidence of thrombosis associated with suture of the small calibre vessels only a relatively small number of functioning transplants in the various series were available (Tables 13 & 14). It should be noted, however, that all peripheral transplants treated with KCN or C_2H_5OH became thrombosed.

5. After the period of observation had come to an end and the dogs had been sacrificed, a number of tests of tensile strength were made on strips of the aortic wall in and beyond the area of the operation. Load-deformation curves provided an explanation for the apparent rigidity of the grafts shown in the arteriograms at different blood pressures. The tensile strength of the anastomoses compared favorable with that of the intact host aorta. The grafts themselves possessed

TABLE 14

Arterial Homografts in Femoral Arteries–Functional Results.

Type of graft	Number of transplantations	Patency			Occlusion
		Good function	Early hemorrhage from anastomosis	Partial thrombosis	
Fresh	2	1	1	0	0
Stored 1-20 days in nutrient medium at $+1^0$ to $+4^0$ C.	16	7	0	3	6
Stored 36-61 days at $+1^0$ to $+4^0$ C.	3	2	0	0	1
Devitalized in KCN or C_2H_5OH.	3	0	0	0	3

considerable tensile strength. The risk of aneurysmal dilatation or rupture in the area of transplantation would therefore seem to be small.

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