STUDIES OF THE VASCULARISATION OF BONE GRAFTS

Gabriele Stringa, Florence, Italy

Formerly Foreign Research Scholar, Nuffield Orthopaedic Centre, Oxford

The purpose of the present work was to obtain data on the vascularisation of bone grafts, with particular reference to the vascular pattern and rate of vascular penetration into the implants. Autogenous, homogenous and heterogenous bone was studied simultaneously to allow these two factors to be compared in the three types of graft.

REVIEW OF LITERATURE

A copious literature, including many accounts of experimental studies on bone grafting and on the different behaviour of autogenous, homogenous and heterogenous implants is already available. Among these contributions only a few are dedicated to describing the vascular pattern of the bed and graft and most of these give only scanty or unsupported evidence of the vascular behaviour in bone grafting.

Marchand (1901) seems to have been the first to study the vessels in transplanted bone; only a few isolated sections of each graft were studied in dogs, and no photographic records were obtained.

Läwen (1909) described the vessels in a human tibial graft after excision of a sarcoma of the humerus, and showed a drawing of the specimen.

Nemilow investigated the part played by the periosteum in the early vascular penetration of the graft; so far as we know from the quotation of Petrow (1914), the most important result of his eight experiments in rabbits was to show that in the first six days the transplanted periosteum was vascularised, whereas no vessels were to be seen in the bone without periosteum. Bertini (1926) used only autogenous bone to study the behaviour of the vessels near and in the holes he had made in the bone of rabbits. Unfortunately only four photomicrographs with very little magnification were given in his paper. Rubaschewa and Priwes (1932) studied the macroscopic modifications of the vascular network around the graft in dogs of various ages, particularly in the periosteum; no account was given of any fine vessels or of the microscopic characteristics of the graft. May (1937) observed the macroscopic modifications of the vessels in a radius experimentally transplanted, without giving any details of the vessels.

The two main contributions based on recorded experimental data will now be considered. Hancox (1947) observed directly the vascularisation of small grafts transplanted to the chorio-allantoic membrane of the chicken; the size and the type of the fragments (frontal bone) and of the bed used seem too far removed from clinical practice in orthopaedic surgery to allow any definite conclusion to be drawn from this work. Kiehn et al (1952) followed the evolution of the vascular pattern in grafts transplanted in transparent chambers; here also, as the authors pointed out, the bed and the size of the fragments were quite unusual and this may explain the different rate of vascular penetration compared with that which we have found.

Apart from these few papers, some hundreds of the two thousand odd works relating to bone grafts mention the vessels; yet only from a few of them is it possible to gain any information on the vascular pattern and the rate of penetration. Trying to assess from these investigations the rate of penetration of the vessels in autogenous grafts we find the following.

Hancox (1947) saw functioning vessels in a graft after only five hours from its implantation; Nemilow (quoted by Petrow 1914) found the injected substance in the vessels of periosteum transplanted two days previously, and Gallie and Robertson (1919) after the
same time saw new capillaries in the opened Haversian canals. Bertini (1926) and Urist (1953) could only observe the earliest signs of revascularisation at the fifth day. Kiehn et al. (1952), working with very small fragments, could not find vessels inside the graft before the eleventh day but found almost complete vascular penetration after two weeks. Marchand (1901) also saw many injected vessels in the grafts of the skull after two weeks.

Kreuz et al. (1951) saw evidence of vascularisation of the homogenous graft after thirty-five days, and the greatest penetration after sixty to ninety days. Siffert (1955) could not see any vascular invasion after six weeks. Wilson (1951) reported good vascular penetration in twelve weeks.

The great variation between these results seems to be due to such causes as the different type and size of the transplant and bed, the technique followed in grafting, and particularly the method employed to show the vessels. With the available material it is impossible to acquire a documented view of the process of revascularisation in the three different types of graft commonly used in bone surgery. Furthermore, there has been no accurate three-dimensional visual representation of the vascular pattern of the graft and the bed, because this cannot be seen in simple histological preparations; it may however be obtained from a technique which combines microradiography and microphotography of transparent specimens.

**METHODS**

Young rabbits and guinea pigs towards the end of the growth period were used. The rabbits were not only of the same race but for several years they had been inbred, and this must be borne in mind in the interpretation of the results of the homogenous grafts. Both cortical and cancellous bone was transplanted. It was obtained from the distal shaft and metaphysis of rabbits and guinea pigs. The grafts were placed in the lower femoral metaphysis and on the surface of the kidney, under the renal capsule. The technique was as follows. Under general anaesthesia with nembutal-ether the lowest third of the rabbit's femur was exposed through a medial incision. The periosteum was detached from the bone, care being taken not to disturb its connection with the surrounding tissue. A rectangular fragment 2.5 centimetres long and 0.75 centimetre wide was cut with an electric saw from the anterior femoral surface, just below the inferior epiphyseal plate and reaching proximally to the cortical bone of the shaft; the marrow space was thus opened. A chisel was used instead of the saw in a small number of experiments. Part of the bone obtained was put in the bone bank refrigerator for use as a homogenous graft in another rabbit. After waiting some minutes in order to stop any bleeding, a fragment 1 centimetre by 0.5 centimetre was turned 180 degrees through its length and was reimplanted in the same place, the upper part now being the lower, but deep into the marrow cavity. Adjacent to it two other fragments of the same size, of homogenous and heterogenous bone, which had been kept in the refrigerator for a time varying from five to sixty days, were also implanted. Some fragments included articular cartilage, and some the thin cortex of the metaphysis, and some, from young animals, contained part of the epiphyseal plate. The position of these structures was recorded exactly, because the main purpose of the work was to study the rate of vascular penetration into the three types of bone implants, and the position of the graft in the bed may influence vascular penetration. In other cases fragments of cortical bone, 0.5 centimetre by 0.5–1 centimetre, were used instead of the metaphysial cancellous bone. The skin was sutured with silk; no plaster immobilisation was used. Next, a laparotomy was performed through the linea alba; the capsule of the kidney was incised; an autogenous bone fragment, like that already grafted in the femur, and refrigerated homogenous and heterogenous bone fragments, were lodged between the capsule and the parenchyma. The use of an electric saw, and of fresh autogenous grafts and of refrigerated homogenous and heterogenous grafts, was made in order that the technique should conform to the procedures prevailing in man.

The animals were killed at times varying from two to ninety days after the grafting.
For the study of the blood vessels, techniques used in previous investigations in this Centre were employed (Trueta, Barclay, Daniel, Franklin and Pritchard 1947; Trueta; and Harrison 1953). Under general anaesthesia the chest was opened, a needle was introduced into the thoracic aorta, and about 100 cubic centimetres of 2 per cent Berlin Blue solution was injected; in cases for radiological analysis barium sulphate suspension (Micropaque) was mixed with Berlin Blue in a proportion of 1:1. Femora and kidneys were removed after varying periods, freed from the surrounding tissue, fixed in 10 per cent formalin and radiographed. They were then decalcified and made transparent by the Spalteholz technique so that the injected vessels could be studied in detail.

After the preliminary examination each specimen was repeatedly sectioned in various planes, and studied under the dissecting microscope. Photographs were taken of most sections. Microradiographs by the technique described by Barclay (1951) were also obtained of the specimens that had been injected with the radio-opaque medium. Other specimens were prepared for histological study.

Altogether 119 bone grafts were studied: thirty-eight were autogenous, thirty-nine homogenous and forty-two heterogenous. Fifty had been implanted into the femur and sixty-nine under the renal capsule. The autogenous grafts were all fresh; the homogenous and heterogenous grafts were preserved by refrigeration.

RESULTS

AUTOGONOUS GRAFTS

Marked differences in the vascular behaviour were found between cortical and cancellous grafts, and according to whether the bed used was femoral or renal.

Cancellous bone—The fragments implanted in the femur were mostly placed with the articular cartilage superficial in order to give the best conditions for a quick penetration through the intertrabecular spaces opened towards the bed.

At the end of the first week the specimens of grafts placed in the femur were found to be already adherent to the deep structures of the medullary cavity; only slight elastic displacement was possible and in the numerous sections made with razor blades the autogenous fragment never became detached from its bed, giving evidence of an already well established organic connection. This was not always found with the same type of fragments placed under the renal capsule, even if the latter appeared very well vascularised with newly formed vessels. The Spalteholz technique is particularly suited for this study because the limits of the graft are easily seen owing to differences of light refraction.

Two types of vascular pattern were found penetrating the graft from the bed. In one, single vessels or groups of vessels were seen passing from the bed to the graft (Fig. 1); the normal circulation, as existing in the bed, extended into the transplanted bone almost without modification. It was, for instance, possible to follow for a long distance a relatively large vessel, coming from the surrounding bone and crossing over the border to enter deeply into the graft. Sometimes three or four narrow vessels of the bed would join together close to the border to form a larger vessel which then ran into the graft. The other type of pattern appeared as a thick network formed by many small branches anastomosing with each other, and extending as a vascular belt directly over the graft (Fig. 2). This network was derived from large vessels dividing into numerous diverging branches; in the kidney these vessels often came from the thin subcapsular network superficial to the glomeruli. The network adhered to the graft and followed its boundaries but did not penetrate it. Single vessels emerged from the junction of many others and penetrated into the graft only in a few isolated points; perhaps no more than one for an area of a square millimetre.

These two different patterns were explained by the structure and position of the transplant. If the medullary side of the graft was directly facing the vascular network of the bed, and its
Autogenous cancellous graft seven days after transplant on to a femur. \((\times18.)\) The graft is already vascularised for half its thickness (2.3 millimetres).

Autogenous cancellous graft six days after transplant on to femur. \((\times35.)\) There is a network of vessels lining the cortical surface of the graft. In the lower part of the photomicrograph the open intertrabecular spaces are already well penetrated.
trabeculae were perpendicular to the surface of the bed, the vessels penetrated it easily. If, however, the trabeculae of the graft were placed parallel to the surface of the bed, and were rather large and strong, or if, moreover, some other obstacle existed such as a thin metaphysial or epiphysial cortex, or articular cartilage, a network was first formed superficial to the graft and initially only isolated vessels were able to penetrate the graft, through pre-existing channels or after osteoclasis.

The course of the penetrating vessels of either pattern was sinuous, the vessels clearly adapting themselves to the shape of the trabeculae. They advanced through the medullary spaces within the trabecular frame and turned around them when they became an obstacle to their progress. It was noted in many cases that the vessels were adherent to the trabeculae, and advanced in close contact with them (Fig. 3). When a graft 0.5-0.6 centimetre thick had been placed in the best conditions to favour vascular penetration it was found already vascularised for half of its thickness after only seven days. A fragment 0.3 centimetre wide placed on the kidney was found completely penetrated by many vessels after six days (Fig. 4).
At this initial stage the vessels were often fairly big in calibre and ended rather abruptly, after two or three divisions, still keeping their large size; small capillaries branched from them but soon ceased. It is possible that for some reason the whole of some capillaries could not be perfused by the injection mass, perhaps from lack of sufficient pressure of the injected fluid; but it seems more likely that the penetrating vessels become wider towards their termination because of the peculiar attraction that the invaded tissue exercises on the vessels of the bed. This phenomenon is being studied in this laboratory at present.

The pattern of an isolated, rather thick vessel with few collateral branches and an abrupt ending was commonly found situated deeply in the graft. A rich network, perhaps arranged as a candelabrum, with many big branches diverging from a single stem, seemed to appear when there was marked resistance to the vascular penetration.

During the second week the vascularisation gradually progressed. The border of the transplanted bone in the femur could still be outlined but it was now losing its sharpness.

At this stage the usual pattern in the femoral specimens was of numerous, even if not very crowded, vessels, forming a band just across each border of the graft. In this area the vascular arrangement no longer followed the structure of the transplanted bone; as could be seen in the histological sections the vascular band corresponded to the layer of hyperaemic granulation tissue which was already far advanced in removing the dead trabeculae and in laying down new bone. There were gaps in the vascular penetration in some isolated places; these corresponded to the presence of a barrier to the progress of the vessels from the bed, and it was here that the thick capillary network, which has already been described, was to be found.
Inside the graft the vessels continued their penetration. They seldom formed definite groups because they were quite widely spread throughout the graft; isolated vessels could be seen very deeply, having left other vessels and the granulation tissue far behind. Whether this feature may be considered indicative of a recanalisation of the old vascular system or not will be discussed later.

In the kidney the pattern was not so uniform. Some fragments showed a marked vascular penetration for a few millimetres, even although not always as rich in vessels as in the femur (Fig. 5). In certain other fragments only a single, not very deep, vascular tuft was found and in others only a few isolated capillaries crossing the bone for about two millimetres.

![Autogenous cancellous graft ten days after transplant on to kidney](image)

**Fig. 5** Autogenous cancellous graft ten days after transplant on to kidney. (×100.) The black, lower part of the photomicrograph represents the subcapsular network of the kidney, with the superficial glomeruli, from whose capillaries the vessels which penetrate the graft directly originate.

were noted. These variations may be explained by the different characteristics of the blood supply of the renal capsule as compared with that of the lower end of the femur, particularly with reference to the distribution of the vessels. Commonly the main vessels of the subcapsular area of the kidney are situated somewhat apart from each other.

*At the end of the third week* the autogenous grafts in the femur were found vascularised for five-sixths of their depth (Fig. 6); only the areas more remote from the bed usually were not reached. Some vessels inside the graft, surely newly formed, were about eight millimetres long. The general arrangement was as follows: it was no longer possible to recognise the borders of the graft, but some increase of the vascular network still persisted in that area. The centre of the graft was penetrated in many directions by vessels of different sizes and
Autogenous and homogenous cancellous grafts twenty-four days after transplant on to femur. ($\times$10.) Four-fifths of the autogenous bone has been invaded by new vessels. The area at the summit is not reached by the vessels because a portion of the epiphysial plate had detained the vessels coming from the right. The smaller homogenous fragment has in this case a fairly good vascularisation. *Top*—Injected specimen. *Bottom*—Histological section.
irregular disposition. But again, in the more distant parts of the graft, the advancing vessels followed paths which are indicated by the architecture of the graft.

Histological examination at this stage showed young mesenchymal tissue invading the dead trabeculae in the regions nearest to the bed, with extensive osteoclastic reaction and appositional bone growth here and there. A new tissue was being laid down but the dead trabeculae far from the bed were still unaltered and were reached only by a few vessels.

An interesting relationship was noted between the vessels and the transplanted cartilage of the articular surface and the epiphyseal plate. Cartilage represented an insurmountable obstruction for a long time when it lay across the line of penetration of the vessels (Figs. 6 and 7), and it was only after thirty days that evidence of its perforation by isolated vessels was found (Fig. 8). Next to them was the usual pattern of a delicate network from which a penetrating branch was seen at only two or three points. In contact with the articular cartilage sinusoids were to be seen forming broad juxtamedullary capillary loops which have been
Autogenous cancellous graft on to kidney after thirty days. (×40.) Penetration of vessels through transplanted articular cartilage covering the bone graft. The vascular network of the renal capsule has been removed; in only three places a small number of vessels were able to reach the underlying cancellous bone.

Fig. 8

Autogenous cancellous graft thirty-five days after transplant on to femur. (×35.) Subchondral sinusoids and capillary loops are seen at the end of a large vessel. The darker, narrow layer in the upper part of the photomicrograph is the articular cartilage of the transplanted fragment.
Autogenous cortical graft twenty-one days after transplant on to kidney. \((\times 45,\) At the lower part of the microradiograph \((\text{top})\) vessels coming from the subcapsular renal network have penetrated deeply on to the graft. The histological section \((\text{bottom})\) shows the dilated Haversian canals with newly formed vessels and young connective tissue. Osteoclastis and bone apposition are occurring at the same time.
described in normal bone by previous investigators (Trueta and Harrison 1953) (Fig. 9). We will return again to the interpretation of these findings.

A venous system inside the graft was now clearly recognisable; it drained into the large veins of the middle of the marrow cavity, with larger vessels often characterised by their elliptical appearance in section.

In the kidney no uniform results were found. In one case only a very superficial vascularisation was obtained; in another a few isolated capillaries through the bone graft were seen; and in a third an almost complete penetration was obtained (Fig. 10).

After one month the autogenous graft in the femur was revascularised throughout (Fig. 20), although certain areas, such as the more distant or those protected by some barrier, were less rich in vessels. The borders of the graft were no longer visible, but they were indicated by an abnormal vascular arrangement. In the depth of the marrow cavity there appeared a pattern of scattered irregular opacities representing the sinusoïds of the red marrow, crossed over by a few important arteries and veins. These opacities, resembling those normally observed in the metaphysis near the epiphysial plate, which correspond to venous enlargements, were also noted against the transplanted epiphysial plate within the graft.

The graft on the kidney showed an almost complete vascularisation at this stage (Fig. 21).

After forty days the number of vessels in the graft decreased. In a longitudinal section of the femoral specimen, the trabecular structure of the graft could no longer be distinguished from its bed by naked eye, which had been possible two weeks before. The only remaining portion was the articular cartilage and a layer of dense bone beneath it. The femoral metaphysis appeared normal except for the peripheral area.

Histological sections showed remnants of necrotic trabeculae with poor appositional growth, with scattered areas of bone marrow and sparse connective tissue. The cortex was in the midst of marked osteoclastic and osteoblastic changes.

On the kidney the vessels were also fewer in number than ten days before; because of reabsorption the fragment was no more than half its original thickness and the capsule over it was still well vascularised.

After ninety days the fragment transplanted in the kidney showed very few small narrow vessels connected with those of the capsule; the histology demonstrated haemopoietic living marrow, as already observed by Lacroix (1949), in a framework of dead trabeculae which was being reabsorbed.

Cortical bone—Before the third day the fragments of cortical bone were still completely avascular. In one of the grafts in the kidney hyperaemia was noted in the capsule and in the subcapsular network of the renal parenchyma outside the glomeruli. The capsule was detached quite easily from the bone. A fine vascular network was to be seen also around the fragment of the femur. The histological sections showed a thin layer of non-organised tissue separated from the bone by the newly formed vascular network.

After seven days the capsule was still more hyperaemic and was more difficult to detach from the graft. Three or four narrow branching vessels, coming from the capsule, ran through the whole thickness of the graft from one surface to the other (Fig. 11). They were newly formed vessels penetrating pre-existing canals; in one specimen this was clearly seen (Fig. 12).

At the end of the third week some of the specimens showed a pattern quite like that of the previous week, the graft being pierced by only a few capillaries. Others, on the contrary, were completely penetrated by numerous vessels, establishing an intricate network with branches and anastomoses (Fig. 13). We were unable to find the reasons for such a difference.

On the fiftieth day the fragment placed in the femur at the same level as the normal cortex was found firmly fixed to the underlying bed. Under the dissecting microscope it appeared perforated by many narrow vessels, mostly from the marrow cavity (Fig. 14). It seemed that some superficial areas were not yet reached. In the kidney the vascularisation was less good; one graft was penetrated by capillaries to half its thickness at only four or five points.

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FIG. 11
Autogenous cortical graft eight days after transplant under renal capsule. (×10.) It has been already completely pierced by some vessels coming both from the capsule and from the subcapsular renal network.

FIG. 12
Autogenous cancellous and cortical graft twenty-five days after transplant under renal capsule. (×45.) A capillary is piercing the cortex through a pre-formed canal. The capillary network outside the bone has been removed. The graft was both cancellous (with thin metaphysial cortex), on the left, and cortical, on the right.
Autogenous and homogenous cortical grafts twenty-four days after transplant under the renal capsule (× 5). No vessels penetrated in the homograft, whereas the autograft was largely vascularised and well on the way to reabsorption. The capsule had been detached.
At the ninetieth day three specimens were removed. One of the grafts in the femur was still recognisable because of its different refraction, yet it was extensively penetrated by new vessels, and under the microscope extensive osteoclastic and osteoplastic changes were seen. The limits of another graft had already disappeared in the transparent specimens, and were only revealed histologically by a layer of small fragments of old and newly formed bone. This difference in the behaviour of the grafts may be related to their position in the bed: the first graft was implanted near the cortex whereas the second one was buried deeply in the bone marrow.

In the kidney graft a few isolated vessels were found; the histology demonstrated fragments of dead bone with scanty new bone delimiting living haemopoietic marrow.

Summary of the vascularisation of autogenous grafts—If the architecture of the cancellous graft had a relation to the bed which favoured vascular penetration, a substantial number of vessels might be seen as far as three millimetres inside the implant after seven days; if there was an obstacle to the vascular penetration a delicate network of vessels appeared in the bed and at first only some isolated vessels penetrated the graft at this stage. At one month grafts five millimetres thick placed in the femur were reached everywhere by the new vessels. Towards the thirty-fifth day, with the exception of the regions situated farthest from the bed, they showed a vascular pattern already almost indistinguishable from normal. Gradual reabsorption of the necrotic bone and some appositional growth, together with the replacement of the granulating tissue by means of haemopoietic marrow, completed the normal picture. The cortical bone was completely pierced at the twenty-first day and disappeared at the ninetieth. Inconstant results were obtained with the renal grafts probably due to the different circulatory patterns of the two beds.
HOMOGENOUS GRAFTS

Cancellous bone—Caution was necessary in sectioning the specimens after one week because the bone was so weakly adhered to its bed that it became easily detached; it appeared uninjected and had very sharp borders in contrast with the intensely injected surrounding tissues. Very delicate capillary networks limited the periphery of the graft (Fig. 15). The networks were close together, some anastomosing with neighbouring groups and others remaining totally independent. They appeared in intimate contact with the transplanted bone, exactly following its surfaces but even if the intertrabecular spaces were open towards the bed no vessel was ever seen inside the homogenous graft at this stage.

On the kidney a richly vascularised capsule covered the graft but no vessels were seen to penetrate the implant.

At the end of the second week the graft became less easily detachable during its handling in the laboratory. Twenty to a hundred isolated capillaries and arterioles were found per square centimetre in both femoral and renal grafts. They were coming from the network lining the surfaces and proceeding into the graft for 100-200 microns, and sometimes for half a millimetre. The arterioles frequently divided terminally into two or three branches. On the kidney they were coming either from the capsule or from the subcapsular capillary network.

The homogenous grafts examined from the twentieth to the twenty-fifth day were firmly fixed to the bed. Their borders could not be seen by the naked eye. The vascularisation had penetrated two to three millimetres, which was sometimes as much as half of the thickness of the graft (Fig. 16). In other areas the delicate vascular network was still delimiting the
shape of the graft. This had decreased in size when compared with that of the previous examination and already showed a tendency to fibrosis. Large vessels of venous type were observed ending in sinusoids.

On the kidney, a few penetrating vessels were seen in one case and a continuous capsular system of vessels in another, without in either case entering deeply into the bone. *After thirty to thirty-five days* some of the grafts placed on the femur were vascularised for three-fifths of their thickness. The regions nearest to the bed had already taken up the normal vascular pattern; elsewhere the vessels showed a tendency to diminish both in number and in calibre. But other areas were completely avascular and appeared encapsulated by fibrous tissue.

![Image of bone grafts](image)

**Fig. 16**

Homogenous and heterogenous cancellous grafts twenty-four days after transplant on to femur. (×10.) The homogenous graft is vascularised for nearly half of its thickness. The network which encircled the borders still persists. The heterogenous graft has been penetrated by only two vessels, supplying a limited peripheral area; here too it is possible to see the lining network.

On the kidney two of the four grafts were almost entirely crossed by new vessels, but the other two had only a single vessel inside them. *At the forty-fifth day* nearly the same pattern was seen. At least two-thirds of the graft showed evidence of a normal vascular arrangement. In some other areas, on the contrary, no vessels were seen, the histological sections demonstrating only necrotic bone and marrow. Sometimes the explanation could be found in a mechanical obstacle due to the position of the graft in the bed; in other cases the reason remained obscure.

More than once the graft was found supplied only by two or three vascular networks (Fig. 17) coming from as many large vessels, which alone penetrated its surface. *After three months* both the implants in the femur and the kidney appeared revascularised,
and on histology granulating tissue was found widely distributed with a great deal of dead bone still present.

**Cortical bone**—*In the first week* a profuse vascular network was noted around the graft. In numerous sections of four specimens, only two narrow capillaries 100 microns long coming from the bed were observed.

*In the second week* no vessels were seen in the grafts.

*After three weeks* it was only possible to see six vessels in each graft, placed widely apart from each other. They had penetrated for about 500 microns, approximately one-fourth of the total thickness of the bone graft. A long vessel was found running for about a millimetre parallel to the main surface of the implant, probably following a Haversian canal.

![Image](image-url)

**FIG. 17**

Homogenous cancellous graft forty days after transplant on to femur. (×45.) The graft occupies all the field. A single vessel pierces the graft and supplies alone a fairly extensive area. This is a common finding in invaded homogenous transplants.

In a graft of the femur *after fifty days* isolated arterioles with few branches were running through the bone from one surface to the other. No vessels were found in the kidney graft. *After three months* the findings were similar. The grafts were still clearly visible in both the femur and the kidney, but they had become narrowed here and there; a poorly vascularised network was present where an osteoclastic process was active. About five vessels were seen to penetrate the implant in the femur; none was seen in the kidney graft which was slightly eroded in a few superficial places.

**Summary of the vascularisation of homogenous grafts**—*At the first week* the cancellous graft was surrounded by a vascular network from which isolated vessels supplying limited areas had penetrated about 0.5 centimetre in depth after *thirty-five days*. In other areas this network was found gradually to decrease, corresponding to the establishment of a front of osteoclasia.
which seemed to slow down the penetration; moreover it was seen to disappear when a fibrous covering encircled the graft, which suggested that there was only slight possibility of further vascular penetration.

Vascularisation of the cortical fragments was always slower and more incomplete.

On the kidney the results either resembled those on the femur or showed even more limited vascular penetration.

HETEROGENOUS GRAFTS

Cancellous bone—At the end of the first week the bone was almost entirely free from any organic connection with the bed, an observation which was often made in this type of implant even after more than six weeks. The border of the graft was very clearly seen just where the injected vessels ended and where they formed the capillary network already described for the homograft. Here, however, this network appeared even denser and surrounded all the deeper surfaces of the graft (Fig. 18), and it did not touch the bone, being separated from it by a narrow layer of 50–100 microns of exudate and fibrin. In the kidney the same local hypervascularity was found around the grafts and a serous fluid collection was found between the capsule and the graft.

At the second week the findings were the same; a dense network of vessels encircled the graft, but none had succeeded in penetrating its borders.

After twenty to twenty-five days the surface was crossed at a few isolated points in the femoral graft. Where the vascularisation of the bed was profuse some vessels, no more than four or
Fig. 19
Heterogenous cancellous graft twenty-one days after transplant on to femur. Top—Transparent injected specimen. (× 40.) Bottom—Histological section. The upper curved line corresponds to the border of the graft; a peripheral and definitely limited area is vascularised, the vessels getting round the trabeculae. This area corresponds to a massive invasion of young mesenchymal tissue, as the histological section shows.
five in each implant, were seen to penetrate the graft from the femoral bed for a maximum depth of half a millimetre. Each vessel was large in calibre, as it supplied a substantial segment of bone. The vascular tree occupied definite areas spreading round the old dead trabeculae (Fig. 19). Histologically this vascular zone corresponded to a massive penetration of young connective tissues, which succeeded in crossing the border at a single point, while everywhere else the limits of the implant were lined with fibrous tissue spreading inside the graft.

In the renal graft no vessels were found.

After thirty to thirty-five days the findings were similar. An isolated vessel entering for one millimetre, or even less, was seen in two grafts, but none was seen in the others (Figs. 20 and 21).

At forty-five days slightly greater penetration was seen, although it was always restricted to very limited areas (Fig. 22). If the cancellous bone of the graft was covered by a thin cortex a definite capsule of fibrous tissue surrounded the bone, and no vascular network was ever seen.

After three months, in the more favourable grafts, the vascular penetration could be seen to cover from about one-third to half of the entire graft, together with a gradually invading connective tissue. Nevertheless, one graft on the femur was seen to be completely deprived of vessels at this stage.

Cortical bone—After ten, twenty and thirty days there was no, or very slight, adherence of the implant to the bed; the usual vascular network was found around the graft in the three first weeks, but no vessels had penetrated inside it.
After two months the radiographs showed all the three fragments intact, with a surrounding osteoporosis of the bone bed; in the femoral grafts the implants were excluded by a fibrous capsule, without any peripheral vascular network.

After three months, still no vessel was observed inside the graft, and only a few vessels around it, corresponding to a limited area of osteoclasis.

Summary of the vascularisation of heterogenous grafts—After twenty to twenty-five days some isolated vessels coming from a peripheral dense capillary network penetrated the cancellous graft for half a millimetre. In the following months the vessels proceeded for a further half millimetre in a few areas; while the greatest part of the graft was not vascularised, and often definitely excluded by a layer of fibrous tissue. No vessel was found inside any of the cortical grafts even as long as three months after their implantation.

DISCUSSION

Some important differences were found to exist in the form of vascular penetration of autogenous, homogenous and heterogenous transplanted bone, both as regards the rate of penetration and the pattern of the new vessels (Figs. 20 and 21).

A cancellous autogenous fragment five millimetres thick may be completely vascularised in twenty to twenty-five days whereas the homogenous grafts of the same thickness showed at the most a vascularisation of only three-fifths after thirty to thirty-five days. Moreover the rabbits used for this investigation have been inbred for a long time and may have attained
a certain amount of tissue affinity. The heterogenous grafts followed up to three months after implantation never showed any appreciable vascularisation.

The pattern of the new vessels is likewise quite different in the three types of implant. A capillary network surrounds the implant during the first week in the homografts and the heterografts but is seen only very occasionally and for a very short period in the autograft. It represents the vascular response to a mechanical barrier comparable to that of the articular or epiphyseal cartilage implanted with the graft. The capillary network is directly attached to the bone in the autografts and homografts; in the heterografts it is separated from it by a layer of fibrin and inflammatory cells and becomes a sort of "no man's land" which is very difficult for the vessels to cross.

![Image](image.jpg)

**FIG. 22**

Heterogenous cancellous bone forty days after transplant under renal capsule. (×70.) From the capsular vessels running on the surface of the graft one isolated branch penetrates perpendicularly into it for about 400 microns.

The present findings emphasise once again the importance of the "schleichender Ersatz" claimed first by Barth (1895) and later by Phemister (1914) in its literal translation of "creeping substitution." But while this process plays a most important part in the rehabilitation of the autogenous graft, where the vessels usually penetrate singly accompanied by few connective mobile cells and frequently rest directly on the dead bone (Fig. 23), its importance decreases in the homogenous, and even more in the heterogenous, implants. In the homogenous graft many areas seem resistant to the penetration of isolated vessels from the bed, and a frontal invasion is often required; the latter is almost the rule in the heterogenous graft, where the piercing vessel must be accompanied by a considerable amount of granulation and bone-removing tissue. In the autograft penetration is immediate, following the entire surface of contact; in the homograft it takes place after a quiescent period of ten to fifteen days, and only in selected regions; in the heterogenous graft the latent period is of twenty to twenty-five
days, but it is not uncommon to find that, after three months, only a small vessel penetrates the implant for a total surface of two square centimetres.

Already after twenty days it is possible to see the initially invaded areas of the autogenous bone return to a normal vascular pattern. At least fifty days are necessary before this happens in the homogenous bone, and it never was noted to occur in the heterogenous implants.

To summarise: vascularisation is quick and complete when the graft is placed in a good bed such as the femoral metaphysis; it is two to three times slower and incomplete in the homograft; it is extremely slow (six or more times) and absolutely limited in the heterograft.

We paid a good deal of attention to the possibility, so often mentioned by many investigators from Phemister (1914) and Albee (1915) to Nageotte (1922), of an immediate recanalisation of the pre-existing vascular system of the implant, at least in the autograft. We could not find any indisputable evidence of this process. A cortical fragment, two millimetres thick, was found after seven days completely perforated from one surface to the other by some vessels; but the smallness of the distance covered does not exclude the possibility that it was in fact a new advancing vessel, because the average speed of penetration for an autograft in good position was several times proved to be about two to three millimetres a week; in that case a pre-existing canal may have contributed to this penetration. The same may have happened with the three millimetres thick fragments found completely vascularised, one after ten and the other after twenty-one days. Unfortunately we never found an injected vessel at the first week at a distance greater than three millimetres from the bed. A broad
capillary network was observed more than once in the subchondral space of grafted bone that included some joint cartilage just beneath the calcified layer of the cartilage; the network was quite far from the bed and from the granulating tissue and was surrounded by trabeculae with some definitely living cells. Considering all these facts together, and particularly the finding at an early stage of such a differentiated vessel as a capillary juxtachondral loop, recanalisation of the pre-existing vessels could be assumed. But, on the other hand, if the suggestion by Abbott et al. (1947) that a cell of a graft could survive for fifteen days without blood supply is true, we may assume that a new vessel could have reached the pre-existing bone canal, and been transformed into a sinusoid in time to allow the local osteocytes to recover.

SUMMARY

1. The rates of vascularisation in 119 autogenous, homogenous and heterogenous bone grafts, placed in the femoral medullary cavity and under the renal capsule of rabbits, were studied.
2. Substantial differences have been found in the speed of vascular penetration and arrangement among autografts, homografts and heterografts: penetration of the heterogenous implant was six or more times slower. Moreover, large areas of the homografts and heterografts were often totally excluded from the circulation for as long as the research was continued (up to three months). Revascularisation of the cortical bone was slower and less profuse than in cancellous bone, keeping always the same respective proportion between the three types of bone we have described. The results on the kidney were much less constant, and I attribute this to the vascular peculiarities of the bed.
3. Vascular patterns peculiar to the time of implantation and type of graft are described.
4. Suggestive, even if not totally convincing, evidence was found of recanalisation of old vessels inside the graft by advancing vessels from the bed.
5. There is striking correlation between the rate of vascular penetration of the bone implants and their ultimate "take" or incorporation in the bed.

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