VASCULARISATION OF BONE GRAFTS IN THE ANTERIOR CHAMBER OF THE EYE

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The interest shown in the mechanism of incorporation of bone implants has produced a copious literature of which an incomplete survey has shown to be well over 400 papers in the last ten years. Most of the contributions distinguish between three types of implant according to their individual or species identity with the host. For experimental purposes the isograft from animals inbred over a long time is now being used as a near equivalent of an autograft. The readiness of auto-implants to become incorporated is believed by some authors to be caused by the early penetration of the implant by vessels from the host (Maatz, Lentz and Graf 1954; Hurley, Stinchfield, Bassett and Lyon 1959; Trueta 1960; Bassett, Creighton and Stinchfield 1961). The delayed incorporation of homografts is thought to be the result of an antibody reaction which disturbs the initial vascularisation (Zeiss, Nisbet and Heslop 1958; Chalmers 1959; Sabet, Hidvegi and Ray 1961). Finally, the rejection of heterografts is attributed by some to the inability of the vessels of the host to advance into the implants, which are excluded and isolated either by fibrous tissue or occasionally by new bone (Stringa 1957) or which, more frequently, are removed.

The part that the implant plays in its incorporation or rejection has been the object of several investigations, and the views are now prevalent that, whatever its action upon the host, there is little or no active participation of the implant in the osteogenetic activity, even if some of its osteocytes do survive (Nishimura, Yaeger and Sabet 1962).

To elucidate some of these facts, methods which prevent the close connection of the implant with a vascular bed have been used, such as the Algire millipore chamber which allows the passage of fluids but prevents, if intact, the penetration of vessels and cells. With the millipore chamber Goldhaber (1958) was able to obtain new bone but it has been objected that this might have resulted from a very slight rupture of the chamber which allowed some undetected vascular penetration of the implant to occur.

In the laboratory of the Nuffield Orthopaedic Centre a series of investigations has been undertaken regarding the osteogenetic capacity of the vascular wall. The origin of the osteoblast, indispensable for implant incorporation as for all other forms of osteogenesis, is still under discussion, and the nature of the stimulus which brings it into activity is still undetermined.

In the present state of knowledge it seems impossible to demonstrate the osteogenetic power of any graft while isolated from the cells and vessels of the environment. Most authors describe the earliest fusion between graft and host as of a fibrous nature (Axhausen 1908; Phemister 1930, 1947; Castiglioni and Venturini 1954). The first cells proliferating around and in the graft are regarded by most authors as young mesenchymal cells or fibroblasts whose multiplication and differentiation Carrel and Baker (1926) attributed to proteins and " split " products from the dying cells. The origin of these so-called fibroblasts is still open to debate. That some cells may remain alive in the graft was stated by Dobrowolskaja as early as 1917, and that these cells are not osteocytes was suspected by Macewen (1912),

Keith (1927), Bloom, Bloom and McLean (1941) and Mowlem (1941). It has also been postulated that undifferentiated cells of the marrow may become osteogenetic (Bloom 1937; Weiss 1939) or that cells of the reticulo-endothelial tissue may be responsible for the new bone (Keith 1927). Trueta (1958, 1963) believed that the osteogenetic cells descended from the endothelium.

Maximow (1926) thought that injury caused the production of granulation tissue, which is accompanied by a growth of connective cells that invade and fill the gaps and work their way into all available spaces and " tow " behind them the tip of the capillary. To know whether the connective cells provide the invading force and the vascular buds subserviently follow them, or whether the opposite is what occurs is of more than academic importance, for vascularisation is an unavoidable premise to graft incorporation and the understanding of why and how it occurs seems essential. Resorption of dead bone takes place by the enlargement of the Haversian canals and Howship's lacunae in the presence of mononuclear cells and polynuclear osteoclasts.

It is recognised that new bone formation in bone implants occurs only in the presence of vascular invasion, new bone being laid down on some parts of the walls of the large canals at the time absorption is proceeding in other areas. Using vascular perfusion, a number of authors have studied the relationship between the vessels and the osteogenetic-osteolytic process (Clark and Clark 1939; Odell, Mueller and Key 1951; Maatz *et al.* 1954; Stringa 1957; Trueta 1958, 1961, 1963). Despite the use of these methods the part played by the invading vessel in the process of implant incorporation is still under discussion. As early as 1901 Marchand had observed the vessels in the implant and these were again studied by Läwen (1909). Mosiman (1950) pointed out that the greatest formation of new bone occurred in the most vascularised areas.

Most authors believe that the vessels in the implant originate from the host and penetrate the graft after covering its surface. Studies on skin grafts suggest that in autografts, at least, revascularisation occurs as an end-to-end anastomosis between the vessels of the graft and those of the host (Bert 1865; Billingham and Medawar 1951; Converse and Rapaport 1956). Hancox (1947) and Zeiss *et al.* (1958) reached the same conclusions. The main evidence in support of the end-to-end anastomosis mechanism was the short time required by the vessels to invade the graft, much shorter than the normal progression as calculated by Clark in his transparent chamber studies, which was about 0·22 millimetres per day. Moreover, if such an anastomosis were to exist, the quantitatively different rate of osteogenesis observed between autografts and homografts by Heslop, Zeiss and Nisbet (1959) would need to be explained by some differences of milieu between the two. Billingham, Brent and Medawar (1956) disagreed with these views, and believed that vascularisation is achieved by vessel ingrowth.

Disagreement is also found in the interpretation of the role of vascularisation in the different types of implants. The varying selection of site, size and nature of the implant may be responsible for the diversity of opinion among workers.

Stringa (1957), in an extensive study in the rabbit by perfusion methods, found in autografts a penetration rate of three millimetres at seven days and five millimetres at one month, while in homografts the vascular invasion was substantially delayed. Siffert (1955) had already seen this to occur. Kiehn and Gutentag (1955) could not see their homograft penetrated at all by vessels.

Zeiss *et al.* (1958) reported that in fresh homografts the vascularisation which occurred in the first week regressed afterwards, which suggested that an immunity reaction had taken place; the vascularisation started again later by a slower, new ingrowth of vessels from the bed. Anderson, Schmidt and Clawson (1959) confirmed these findings. Hammack and Enneking (1960) also found an immunity reaction with grafts of homologous periosteum after the first week, which caused the vascular occlusion and subsequent death of the recently laid down bone. Sabet *et al.* (1961) concurred in most of these findings.

Heterografts were found by Stringa (1957) to have been penetrated one millimetre by vessels after several months, and this only in a few areas.

The rate of vascularisation has been found also to depend on the animal used for the experiments. In rats, using tiny bone fragments, vascularisation of autografts has been seen to occur after only four days (Chalmers 1959); in guinea-pigs it takes six days (Mosiman 1950). Stringa (1957) using slightly larger grafts in rabbits saw a penetration of three millimetres in seven days.

The nature of the graft also influences the vascular penetration. Thus, Maatz, Lentz and Graf (1953) established the time relationship of one to ten between cancellous and cortical bone, and Holmstrand (1957) said that cortical bone offers the Haversian canals as the only pathway for vascular penetration. Stringa (1957) showed the great superiority of cancellous over cortical bone to invite vascular penetration.

From the many points still under investigation three are of special interest. These concern 1) details of the vascular reaction of the host related to the nature of the implant; 2) the readiness with which vessels penetrate the different types of implant; and 3) the part the vessels play both in the formation of new bone and in the removal of the old bone according to the nature of the graft.

The investigation described here was planned in an attempt to contribute to the understanding of these three phenomena. For this work the anterior chamber of the eye of the guinea-pig was used as host. Polacco (1929) first used the anterior chamber for bone graft studies, and Moro (1947) showed the osteogenetic potency of the uveal tract. Many other authors have also employed this method, among them Ray, Degge, Gloyd and Mooney (1952), Urist and McLean (1952), Danis (1956) and Anderson, Schmidt and Clawson (1959).

MATERIALS AND METHODS

Ninety-eight albino guinea-pigs six to eight weeks old and twenty-four three to four weeks old albino Wistar rats were used. Under general anaesthesia with ether the upper part of the tibia 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, five millimetres long by three millimetres wide, which included some cortical bone, was cut with an electric saw from a site just medial to the anterior tibial crest, below the epiphysial cartilage. The fragment was placed in normal saline solution containing 20,000 units of penicillin per millilitre and cut into tiny pieces of either cancellous or cortical bone one to two millimetres square. The skin was sutured with silk. No plaster immobilisation of the limb was used.

Preparation—The fragment was either transplanted directly to the anterior chamber as a fresh implant, or it was boiled in tap water for three or ten minutes, or it was stored in a bone refrigerator at a temperature of −20 degrees Centigrade for at least forty-eight hours.

Grafting—With a corneal knife an incision was made at the upper margin of the cornea and the small graft was introduced with a fine pair of forceps deep into the anterior chamber in close contact with the iris.

The autografts, homografts or heterografts (rat to guinea-pig or vice versa) were either fresh or had been boiled or refrigerated. The frozen grafts were allowed one hour to reach room temperature before they were used.

The experiments were ended at intervals varying from two to forty-five days. Under general anaesthesia with ether the chest was opened and the posterior parts of three or four left ribs were excised. The thoracic aorta was freed from its surrounding connective tissue, severed, and a substantial amount of blood allowed to pour out. A catheter was then introduced upwards and, with the animal still alive, a mixture of equal parts of 2 per cent Berlin blue solution and barium sulphate (Micropaque) was injected slowly under constant

pressure. At the end of the perfusion the animals were all dead. The grafted eye was then removed and, after opening the anterior chamber, it was submerged in 10 per cent formalin for at least forty-eight hours.

Each specimen was decalcified and made transparent by the Spalteholz method so that the perfused vessels could be studied in detail. The graft was then removed from its bed and the vascular pattern was photographed.

As no scientifically accurate method to measure the degree of vascularisation of any tissue is known, the authors adapted the technique of estimation under either the dissecting or optic microscope. The proportion of the total tissue mass or section which was occupied by the perfused vessels to that which was not, was estimated. It is realised that for such a method to be of any value the details of the vascular perfusion should be constant and that the perfusion should be carried out by the same worker in all the experiments. It is obvious that this procedure " measures " vascularity in a way that includes both the number of vessels and their size but it is realised that, in a study of vascular invasion, the number of vessels is more

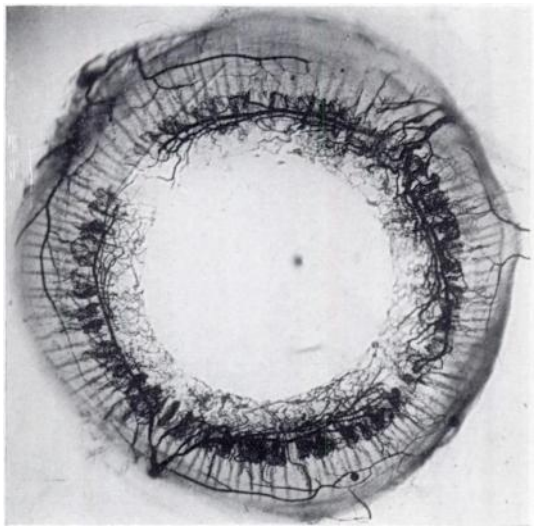

FIG. 1

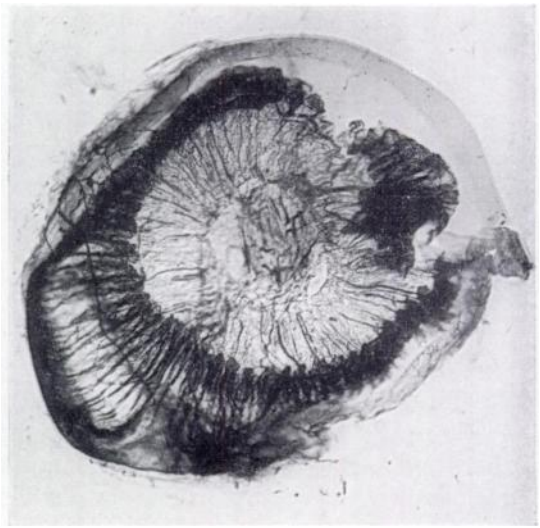

FIG. 2

Figure 1—The normal vascular pattern of the guinea-pig's eye showing the short ciliary arteries which are branches of the ophthalmic artery; the two long ciliary arteries which enter the sclera laterally and medially; and the anterior ciliary arteries anastomising and forming the main arterial circle. Figure 2—The vascular reaction promoted by the auto-implant which can be seen to have been penetrated deeply by vessels.

important than their size. In the experiments reported in this paper the authors were helped by the smallness of the implants which made total examination easy. The gradation of vascularisation given in the results is from none to complete in which all the bone marrow spaces examined contained perfused vessels, the intermediate grades being slight, moderate or substantial vascularisation.

The specimens were embedded in paraffin wax or in methacrylate. Sections of 5–7 μ were obtained and stained with haematoxylin.

The normal vascular bed of the host—Three kinds of vessels are observed in the guinea-pig's eye: 1) short ciliary arteries which are branches of the ophthalmic artery and which perforate the sclera around the optic nerve; 2) two long ciliary arteries which enter the sclera lateral and medial to the optic nerve; and 3) anterior ciliary arteries anastomosing with the choroidial arteries and the main arterial circle. All these vessels contribute to the constitution of a capillary network which, in a radiating form, is orientated towards the inner border of the iris (Fig. 1). Any inflammatory reaction increases the size of the vascular tufts of the iris and straightens the radiating capillaries to give them the appearance of vascular loops (Fig. 2).

These vessels develop concentrically as soon as the implant is inserted. As will be shown later, in fresh as well as in boiled or frozen auto-implants, this vascular reaction of the host tends to diminish from the second day. Hetero-implants cause a great vascular reaction while the implant, however, remains undisturbed. In the fresh homo-implants the vascularity of the bed intensifies after the fifteenth day, but without ever attaining the proportions of the hetero-implants.

The implants—Ninety-eight bone implants were studied in the guinea-pig, consisting of twenty-nine fresh autogenous, fourteen fresh homogenous, six fresh heterogenous, seventeen boiled autogenous, sixteen boiled homogenous, eight refrigerated autogenous and eight refrigerated homogenous grafts. Twenty-four bone implants were studied in the rat, consisting of four fresh autogenous, sixteen isogenous and four heterogenous grafts.

The vascular reaction of the host—Any type of implant elicited an almost immediate vascular reaction in the host. This increased vascularity varied with nearly every type of implant used.

The hetero-implant, either cancellous or cortical, caused the greatest vascular reaction of all. Auto-implants and homo-implants, either fresh or frozen, elicited a much less profuse host vascularity, while boiled implants were responsible for the least vascular reaction. There seemed to be no relationship between the reaction of the vasculature surrounding the implant and the ease with which the implant was revascularised. A poor or limited vascular reaction was not an indication of poor vascularisation, neither was a severe vascular reaction suggestive of good vascularisation of the implant. There were seven varieties of reaction. 1) Early and abundant vascular reaction of the bed, followed by early and deep vascular penetration, was found with fresh cancellous auto-implants. 2) Early and abundant vascular reaction of the bed, followed by delayed vascular penetration of the implant, was found with fresh cortical auto-implants. 3) Early and substantial vascular reaction of the bed which intensified after the fifteenth day, followed by early and deep vascular penetration of the implant, was found with fresh cancellous homo-implants. 4) Early and substantial vascular reaction of the bed which intensified after the fifteenth day, followed by delayed vascular penetration of the implant was found with fresh cortical homo-implants. 5) Early and very profuse vascular reaction of the bed without vascular invasion of the implant was found with all hetero-implants. 6) Moderate vascular reaction of the bed followed by substantial vascular penetration of the implant was found with frozen auto-implants and homo-implants. 7) The least vascular reaction of the bed, followed by poor or absent invasion of the implant, was found with all types of boiled implants.

This analysis suggests that the vascular reaction of the bed elicited by the implant is not the cause of the vascular penetration, for the reactive vascularity of the host may either be very great—heterogenous implants—or alternatively very poor—boiled implants—without any significant revascularisation occurring.

THE REVASCULARISATION OF THE IMPLANT

Guinea-pig. *Fresh cancellous auto-implants and cancellous homo-implants*—By the second day the implants were all adherent on the side facing the iris. The vessels progressively covered the surface of the implant which showed pyknotic osteocytes in distress and dying. Some fibroblast-like cells had appeared round the implant. At the fourth day vessels were seen covering, but not in, the implant. Young connective tissue cells were seen in the medullary spaces. By the sixth day no vascular penetration had occurred but on one occasion a vessel was perfused in a Haversian canal. Profuse vascularity and cellularity around the implant was seen. At the eighth day, in the cancellous implant, a sudden vascularisation of the whole of the implant had taken place both in auto-implants and homo-implants (Fig. 3). Osteogenesis as well as bone removal was taking place actively (Fig. 4). Very few osteoclasts were seen.

Cortical auto-implants and homo-implants—At the tenth day hardly any penetration of vessels

into the Haversian canal had begun but new bone was being laid down around the implant. By the fifteenth day the vascularisation in the Haversian canals was nearing completion, with osteogenesis and bone absorption round the vessels, but more so in the auto-implant than in the homo-implant. This was the first time that a different behaviour was observed between the two types of implant. From then on the intolerance of the homo-implant was seen to increase and by the eighteenth day, when the auto-implant was considered to be vascularised and incorporated, the homo-implant was suffering from both a severe vascular inhibition and lysis of the new bone (Fig. 5), accompanied by a profuse cellular reaction. By the twenty-eighth day the intertrabecular spaces of the homo-implants had lost most of their vessels but those few still remaining appeared dilated. A large cellular mass occupied all the marrow spaces. By the end of the experiment, at the forty-fifth day, a few scattered thin vessels were the only vasculature remaining in the implant.

Boiled implants—The least vascular reaction of the host was found in all the experiments and few vessels covered the surface of the implant. At the fifteenth day (Fig. 6) a vascular penetration could occasionally be shown but the implant was filled by young mesenchymal

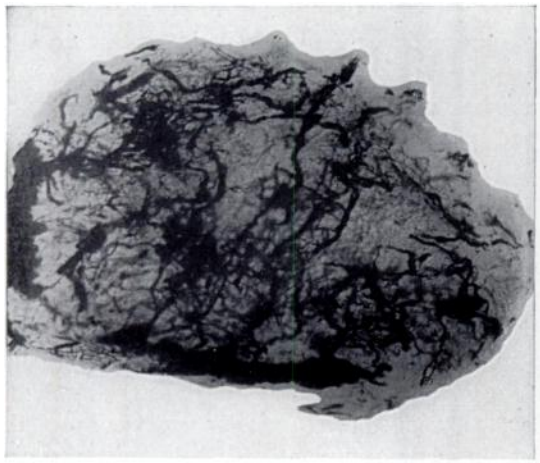

FIG. 3

FIG. 4

Figure 3—A Spalteholz preparation of an autogenous cancellous implant at the eighth day showing sudden and complete vascularisation. There is no difference at this stage between a homo-implant and an auto-implant.
Figure 4—A perfused vessel with its endothelium closely connected to active osteoblasts.

tissue of low cellularity. Those few vessels which were present were found away from the trabeculae and without any of the characteristics of being engaged in osteogenesis (Trueta 1963). Old osteocytes were found in the implant presumably having been fixed by heating. The Spalteholz preparation of these specimens gave evidence of the scarcity of the vascularisation (Fig. 7). Specimens at the forty-fifth day showed practically no changes. No differences were seen between boiled auto-implants and homo-implants, nor between implants boiled for three or ten minutes.

Frozen implants—In cancellous auto-implants as well as in homo-implants a moderate vessel ingrowth with a marked cellularity could be demonstrated by the eighth day. Progress continued for the subsequent fifteen days but the vessels remained thin, and even at the forty-fifth day some areas of the implants were still free from vascular invasion; but at the twenty-first day rows of osteoblasts along the old and new bone trabeculae were seen, always with a perfused vessel nearby (Fig. 8). There was no difference in the degree of vascularisation between the two types of implants.

In cortical implants hardly any vascular penetration was seen at the forty-fifth day.

Hetero-implants—Despite the extreme increase of vascularity around the implant it was not

FIG. 5

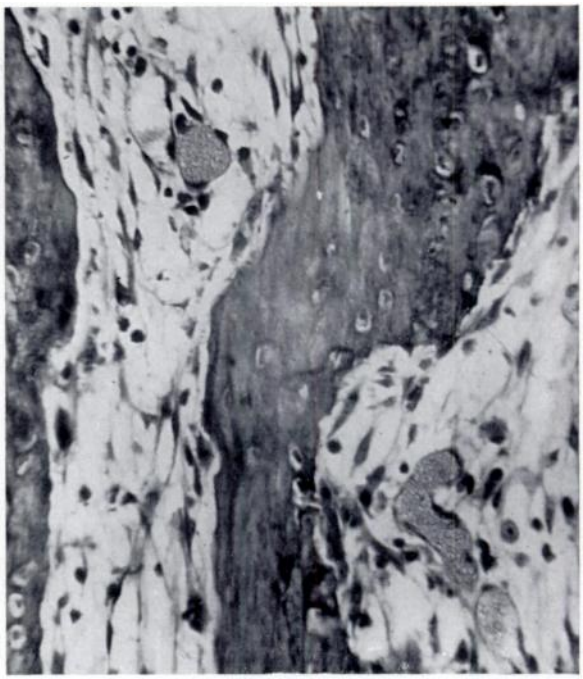

FIG. 6

Figure 5—A Spalteholz preparation of a homo-implant at the eighteenth day showing severe regression of vascularity probably caused by an antigenic reaction. Compare this with the implant at eight days in Figure 3. Figure 6—A boiled implant at fifteen days with very scanty vascular penetration. The few vessels found are away from the trabeculae and have none of the characteristics of being engaged in osteogenesis. Osteocytes, fixed by boiling, can be seen.

FIG. 7

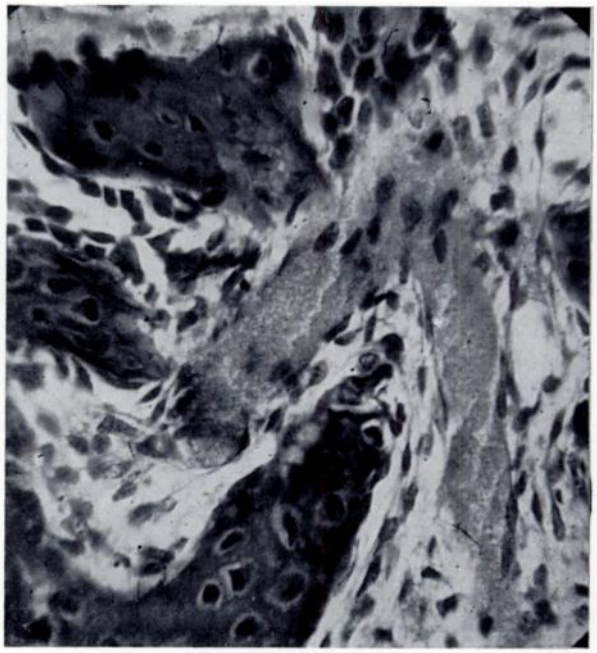

FIG. 8

Figure 7—A Spalteholz preparation of a boiled implant at fifteen days showing the paucity of the vascularisation. The few vessels visible are at the surface of the implant. Figure 8—A frozen cancellous auto-implant at twenty-one days showing active osteogenesis around the vessels.

penetrated and did not even become adherent to the bed. Residual haemorrhage was still present at the eighth day and all the bone lacunae appeared empty. By the fifteenth day there was an intensive polynuclear cellular reaction. In one experiment there was a complete resorption of the implant by the fifteenth day.

Rats. *Fresh auto-implants*—During the first two days the graft was surrounded by a variable amount of bleeding. There appeared to be vessels coming from the ciliary processes.

By the fourth day vessels were seen to be well perfused and running through the pre-existing medullary spaces of the implant. Rows of osteoblasts could already be seen close to the vessels and in line over the trabeculae of the implant. Round cells, exhibiting periodicity, were in the process of filling the medullary spaces. At the sixth day osteoblasts, always parallel to the wall of the vessels, were laying down new bone and this was also evident by the eighth day.

DISCUSSION

The complete covering of the surface of the bone implant by perfused vessels was seen to occur by the second day in rats and at the end of the fourth day in guinea-pigs, and complete vascularisation of a cancellous auto-implant appeared suddenly between the third and fourth days in rats and the sixth and eighth days in guinea-pigs. The tiny fragments of cancellous bone used in these experiments needed only two days in guinea-pigs and one day in rats to become completely vascularised once the vascularisation had started. This extreme rapidity of vascular invasion cannot adequately be explained other than by an end-to-end anastomosis between the vessels of the host and those of the medullary spaces of the implant. This implies that, during the time before the beginning of the blood flow from the host, the vascular endothelium of the implant remained totally—or mostly—alive, because otherwise it would have caused thrombosis, which was never found in the course of this study. The survival of the vascular endothelium also explains why such a vigorous osteoblastic activity occurred close to the vessel wall adjacent to the bone lamellae of the implant (Trueta 1957, 1961, 1963).

The smallness of the implants, which favour fluid diffusion and the very early re-establishment of the blood flow, may explain why osteocytes have been seen to survive in numbers not encountered in bone grafts of larger size (Barth 1908; Leriche and Policard 1928; Loeb 1930; Nishimura *et al.* 1962). Anderson (1961), on the other hand, found surviving osteocytes only in large cortical implants.

Some authors believe that the implant induces a metaplasia of the connective tissues (Ely 1924; Castiglioni and Venturini 1954; Bassett 1955; Ray and Sabet 1963). That the implant may exert a direct influence on the rate of vascular penetration and that this may condition the osteogenetic activity of the endothelial wall does not seem to have been fully considered. We believe that the evidence submitted here supports the view that these two actions exist.

Our findings may help to explain the observations of Cooley and Goss (1958) that a non-irradiated graft will fuse in an irradiated bed whereas an irradiated implant will not take in a non-irradiated bed. It seems that it is the nature and condition of the implant together with the vasculature of the host area that determines the final outcome of the implanted bone.

It was of interest that bone resorption in our experiments occurred despite the striking scarcity of osteoclasts. Marked osteolysis of the new bone took place following homo-implantation. Antigenic reactions causing delayed ischaemia and subsequent vascular reinvasion appeared responsible for the osteolysis.

That the vascular penetration was not necessarily dependent on the survival of the osteocytes of the implant was shown by the experiments with frozen bone which exhibited an active vascularisation despite the death of the osteocytes. Whatever the substance, enzyme or otherwise, which guided the vascular penetration, this was suppressed by boiling, for the boiled autogenous implant behaved like a fresh heterogenous one in refusing revascularisation.

The only difference between the two was found in the lack of vascular proliferation in the bed round the boiled implant, in great contrast to the exuberant vascularity which was elicited by a heterogenous bone graft.

In our findings it is important to emphasise once more the smallness of the bone implants used, because it is believed that this may have enhanced the chances of cell survival and thus have made vascular penetration easier. It would be improper to extend the implication of the present work to bone grafts in general, particularly those of large size. It seems permissible, however, to assume that the same relationship between vascular penetration, vascular osteogenesis and vascular survival encountered in the present experimental work may be found with grafts of larger size, although vascularisation would be much more difficult. The work of Stringa (1957) and others suggests this view.

The order of qualitative and quantitative success in the incorporation of small bone implants found in the present work on a decreasing scale was as follows: fresh autogenous cancellous, fresh autogenous cortical, fresh homogenous cancellous, frozen autogenous cancellous, fresh homogenous cortical, frozen homogenous cancellous and frozen homogenous cortical grafts. Boiled grafts of all kinds and fresh heterogenous grafts were rejected.

SUMMARY AND CONCLUSIONS

In this study the direct relationship between the type of bone implant used, the vascular reaction caused to the host and the revascularisation of the implant has been studied. It was found that the best graft was that which was the most rapidly and permanently vascularised. Not only was the biological affinity between the graft and the bed important, but the structural facilities offered by the implant for the " penetration " by the host vessels were also of paramount importance. Thus small, fresh, cancellous bone grafts offered the best chance of rapid incorporation provided they were not crushed to the point of making vascular progress difficult. The findings from this investigation so strongly suggest that the rapid revascularisation of the bone grafts was because of an end-to-end anastomosis of the vessels of the host with those in the implant that it seems justified to consider that the best bone graft is that which is richest in vessels. Apart from a recent short paper by Graf (1960), we have not found this assertion before. It is this which seems to make the fresh, autogenous, cancellous implant so superior to all others.

We believe that any new material for bone grafts should be tested by the technique described here. The material which one day may replace fresh, autogenous, cancellous implants will have to show the same readiness to vascular penetration, vascular osteogenesis and vascular permanency that at present is exhibited only by the cancellous autograft.

REFERENCES

ALGIRE, G. H., WEAVER, J. M., and PREHN, R. T. (1954): Growth of Cells *in Vivo* in Diffusion Chambers. I. Survival of Homografts in Immunized Mice. *Journal of the National Cancer Institute*, **15**, 493.

ANDERSON, K. J., SCHMIDT, J., and CLAWSON, D. K. (1959): The Vascularization and Cellular Response Induced by Homogenous Deproteinized Bone Transplants in the Anterior Chamber of the Rat Eye. *Transplantation Bulletin*, **6**, 97.

ANDERSON, K. J. (1961): The Behaviour of Autogenous and Homogenous Bone Transplants in the Anterior Chamber of the Rat's Eye. *Journal of Bone and Joint Surgery*, **43–A**, 980.

AXHAUSEN, W. (1908): Die histologischen und klinischen Gesetze der freien Osteoplastik auf Grund von Tierversuchen. *Archiv für Klinische Chirurgie*, **88**, 23.

BARTH, A. (1908): Ueber Osteoplastik. *Archiv für Klinische Chirurgie*, **86**, 859.

BASSETT, C. A. L. (1955): Discussion on Immune Reaction in Tissue Homotransplants. Part IV: The Immune Concept: its Relation to Bone Transplantation by M. Bonfiglio. *Annals of the New York Academy of Sciences*, **59**, 432.

BASSETT, C. A. L., CREIGHTON, D. K., and STINCHFIELD, F. E. (1961): Contribution of Endosteum, Cortex, and Soft Tissues to Osteogenesis. *Surgery, Gynecology and Obstetrics*, **112**, 145.

BERT, P. (1865): Sur la greffe animale. *Comptes Rendus des Séances de l'Académie de Sciences*, **61**, 587.

BILLINGHAM, R. E., and MEDAWAR, P. B. (1951): The Viability of Mammalian Skin after Freezing, Thawing and Freeze-drying. In *Freezing and Drying*. London: The Institute of Biology.

BILLINGHAM, R. E., BRENT, L., and MEDAWAR, P. B. (1956*a*): Quantitative Studies on Tissue Transplantation Immunity. III. Actively Acquired Tolerance. *Philosophical Transactions of the Royal Society of London*, Series B, **239**, 357.

BILLINGHAM, R. E., BRENT, L., and MEDAWAR, P. B. (1956*b*): The Antigenic Stimulus in Transplantation Immunity. *Nature*, **178**, 514.

BLOOM, W. (1937): Cellular Differentiation and Tissue Culture. *Physiological Reviews*, **17**, 589.

BLOOM, W., BLOOM, M. A., and MCLEAN, F. C. (1941): Calcification and Ossification: Medullary Bone Changes in the Reproductive Cycle of Female Pigeons. *Anatomical Record*, **81**, 443.

CARREL, A., and Baker, L. E. (1926): The Chemical Nature of Substances Required for Cell Multiplication. *Journal of Experimental Medicine*, **44**, 503.

CASTIGLIONI, G., and VENTURINI, G. (1954): Osservazioni comparative su diversi tipi di innesto osseo nella camera anteriore dell'occhio. *Archivio di Ortopedia*, **67**, 35.

CHALMERS, J. (1959): Transplantation Immunity in Bone Homografting. *Journal of Bone and Joint Surgery*, **41-B**, 160.

CLARK, E. R., and CLARK, E. L. (1939): Microscopic Observations on the Growth of Blood Capillaries in the Living Mammal. *American Journal of Anatomy*, **64**, 251.

CONVERSE, J. M., and RAPAPORT, F. T. (1956): The Vascularization of Skin Autografts and Homografts; an Experimental Study in Man. *Annals of Surgery*, **143**, 306.

COOLEY, L. M., and Goss, R. J. (1958): Effects of Transplantation and X-irradiation on the Repair of Fractured Bones. *American Journal of Anatomy*, **102**, 167.

DANIS, A. (1956): L'ostéogénine existe-t-elle? *Acta Orthopaedica Belgica*, **22**, 501.

DOBROWOLSKAJA, N. A. (1917): On the Regeneration of Bone in its Relation to the Cultivation of Bone Tissue. *British Journal of Surgery*, **4**, 332.

ELY, L. W. (1924): Bone Growth in Transplanted Bone; an Experimental Study. *Archives of Surgery*, **9**, 215.

GOLDHABER, P. (1958): Preliminary Observations on Bone Isografts within Diffusion Chambers. *Proceedings of Society for Experimental Biology*, **98**, 53.

GRAF, R. (1960): Blutversorgung und Ueberleben autoplastischer Spongiostransplantate. *Bulletin de la Société Internationale de Chirurgie*, **19**, 602.

HAMMACK, B. L., and ENNEKING, W. F. (1960): Comparative Vascularization of Autogenous and Homogenous-Bone Transplants. *Journal of Bone and Joint Surgery*, **42-A**, 811.

HANCOX, N. M. (1947): The Survival of Transplanted Embryo Bone Grafted to Chorioallantoic Membrane, and Subsequent Osteogenesis. *Journal of Physiology*, **106**, 279.

HESLOP, B. F., ZEISS, I. M., and NISBET, N. W. (1959): Relation Between Cell Survival and Osteogenesis in Fresh Autologous and Homologous Cortical Bone Implants in Rats. *Proceedings of the University of Otago Medical School*, **37-1**, 2.

HOLMSTRAND, K. (1957): Biophysical Investigations of Bone Transplants and Bone Implants. *Acta Orthopaedica Scandinavica*, Supplementum 26.

HURLEY, L. A., STINCHFIELD, F. E., BASSETT, C. A. L., and LYON, W. H. (1959): The Role of Soft Tissues in Osteogenesis. *Journal of Bone and Joint Surgery*, **41-A**, 1243.

KEITH, Sir A. (1927): Concerning the Origin and Nature of Osteoblasts. *Proceedings of the Royal Society of Medicine* (Section of Surgery), **21**, 301.

KIEHN, C. L., and GUTENTAG, J. (1955): The Uptake of Radioactive Isotopes by "*in vivo*" Bone Transplants in Diffusion Chambers. *Transplantation Bulletin*, **2**, 97.

LÄWEN, A. (1909): Zur Histologie des frei transplantirten periostgedeckten Knochens beim Menschen. *Archiv für Klinische Chirurgie*, **90**, 469.

LERICHE, R., and POLICARD, A. (1928): *The Normal and Pathological Physiology of Bone; its Problems*. St Louis: The C. V. Mosby Company.

LOEB, L. (1930): Transplantation and Individuality. *Physiological Reviews*, **10**, 547.

MAATZ, R., LENTZ, W., and GRAF, R. (1953): Experimentelle Grundlagen der Transplantation konservierter Knochen. *Archiv für Klinische Chirurgie*, **273**, 850.

MAATZ, R., LENTZ, W., and GRAF, R. (1954): Spongiosa Test of Bone Grafts for Transplantation. *Journal of Bone and Joint Surgery*, **36-A**, 721.

MACEWEN, W. (1912): *The Growth of Bone. Observations on Osteogenesis. An Experimental Inquiry into the Development and Reproduction of Diaphyseal Bone*. Glasgow: James Maclehose and Sons.

MARCHAND, F. (1901): *Der Process der Wundheilung mit Einschluss der Transplantation*. Stuttgart: F. Enke.

MAXIMOW, A. (1926): Über undifferenzierte Blutzellen und mesenchymale Keimlager im erwachsenen Organismus. *Klinische Wochenschrift*, **5**, 2193.

MORO, F. (1947): Potere condro ed osteogenetico dei diversi tessuti oculari (uvea). *Annali di Oftalmologia e Clinica Oculistica*, **73**, 541.

MOSIMAN, R. S. (1950): A Study of Bone Growth. *Surgical Forum*, 424.

MOWLEM, R. (1941): Bone and Cartilage Transplants: their Use and Behaviour. *British Journal of Surgery*, **29**, 182.

NISHIMURA, K. K., YAEGER, J. A., and SABET, T. Y. (1962): Fate of Osteocytes in Adult Mouse Whole Bone Isografts and Homografts. *Anatomical Record*, **144**, 85.

ODELL, R. T., MUELLER, C. B., and KEY, J. A. (1951): Effect on Bone Grafts of Radio-Active Isotopes of Phosphorus. *Journal of Bone and Joint Surgery*, **33–A**, 324.

PHEMISTER, D. B. (1930): Repair of Bone in the Presence of Aseptic Necrosis Resulting from Fractures, Transplantations, and Vascular Obstruction. *Journal of Bone and Joint Surgery*, **12**, 769.

PHEMISTER, D. B. (1947): Treatment of Ununited Fractures by Onlay Bone Grafts Without Screw or Tie Fixation and Without Breaking Down of the Fibrous Union. *Journal of Bone and Joint Surgery*, **29**, 946.

POLACCO, E. (1929): Ricerche comparative sulla neoformazione ossea negli innesti auto- ed omoplastici di periostio e di giovane osso. *Archivio per Ier Scienze Mediche*, **53**, 476.

RAY, R. D., DEGGE, J., GLOYD, P., and MOONEY, G. (1952): Bone Regeneration. *Journal of Bone and Joint Surgery*, **34–A**, 638.

RAY, R. D., and SABET, T. Y. (1963): Bone Grafts: Cellular Survival Versus Induction. *Journal of Bone and Joint Surgery*, **45–A**, 337.

SABET, T. Y., HIDVEGI, E. B., and RAY, R. D. (1961): Bone Immunology; II. Comparison of Embryonic Mouse Isografts and Homografts. *Journal of Bone and Joint Surgery*, **43–A**, 1007.

SIFFERT, R. S. (1955): Experimental Bone Transplants. *Journal of Bone and Joint Surgery*, **37–A**, 742.

STRINGA, G. (1957): Studies of the Vascularisation of Bone Brafts. *Journal of Bone and Joint Surgery*, **39–B**, 395.

TRUETA, J. (1957): The Normal Vascular Anatomy of the Human Femoral Head During Growth. *Journal of Bone and Joint Surgery*, **39–B**, 358.

TRUETA, J. (1958): La vascularisation des os et l'ostéogénèse. *Revue de Chirurgie Orthopédique*, **44**, 3.

TRUETA, J. (1960): Bone Grafting. *Nursing Mirror*, May 20, **110**, 5.

TRUETA, J. (1961): The Housing Problem of the Osteoblast. *Journal of Trauma*, **1**, 5.

TRUETA, J. (1963): The Role of the Vessels in Osteogenesis. *Journal of Bone and Joint Surgery*, **45–B**, 402.

URIST, M. R., and MCLEAN, F. C. (1952): Osteogenetic Potency and New-Bone Formation by Induction in Transplants to the Anterior Chamber of the Eye. *Journal of Bone and Joint Surgery*, **34–A**, 443.

WEISS, P. (1939): *Principles of Development*, *p.* 93. New York: Holt.

ZEISS, I. M., NISBET, N. W., and HESLOP, B. (1958): Vascularisation of Fresh Auto- and Homologous Implants of Cortical Bone. *Proceedings of the University of Otago Medical School*, **36–3**, 29.