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MUSCLE-PEDICLE BONE GRAFTS

An Experimental Study

By

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After grafting of fresh autogenous bony material most of the osteocytes and the included soft tissues will perish before revascularization has taken place.

It is generally accepted, however, that the most peripheral cells of the graft are able to survive by diffusion, if the recipient site is favourable. Apart from the action due to surviving cells, it is known that bone grafts exert an inductor function upon the surrounding soft tissues (2). Furthermore, the osteogenic tissue arising from the recipient site is assumed to have an affinity for the grafts, which thereby exercise a conductor function.

Nothing definite is known about the share of these individual functions in the final result, but the most common view is that the mechanical properties of the graft and its inductor function are of cardinal importance, whereas it is not believed to play any major role as an active participant in osteogenesis. In the case of an inferior recipient site, the induction may be deficient as well, and this may result in poor healing of the graft.

In order to obtain a more dependable osteogenetic action, therefore, it seemed natural to apply a bone graft which had preserved its blood supply and viability through a soft-tissue pedicle.

In the course of time, clinical attempts at employing muscle-pedicle bone grafts have in fact been made.

It may be mentioned, as a curiosity, that *Phelps* (21), in 1891, was the first to transplant a muscle-pedicle bone graft from a dog to an

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osteomyelitic defect in a boy. The boy and the dog were on excellent terms, but the graft was expelled, and all subsequent attempts have been carried out as autotransplantations.

The method has been used for various purposes, most often to cover bony defects. *Watson-Jones* (26), for instance, covered a large defect in the frontal bone with a pedicled graft consisting of the external lamina of the temporal bone, the temporal muscle serving as the pedicle.

Incidentally, the most common indication has been pseudarthrosis. *Payr* (20), in a case of mandibular pseudarthrosis, used a rib with skin and muscle as a pedicle, while *Cole* (4), for the same purpose, used a graft from the mandible itself with a pedicle of platysma. In pseudarhtrosis of the humerus, *Codivilla* (3) used the axillary border of the scapula pedicled by the teres major and minor. For large defects of the tibia he used a piece of the fibula with preserved muscle attachment. *Cuneo & Block* (5), in femoral pseudarthrosis, used a pedicled sliding graft with intact muscles, taken from the femur distal to the pseudarthrosis. In congenital tibial pseudarthrosis *Reichel* (23) and *Farmer* (7) raised pedicled grafts from the contralateral tibia in several stages.

Furthermore, the method has been used to some extent in arthrodesis procedures. For example, in arthrodesis of the hip *Hibbs* (12, 11) used the greater trochanter with the gluteus medius as a pedicle and in arthrodesis of the knee he used the patella.

As far as experimental investigations are concerned, *Hellstadius* (10) arrived at a rather negative result, while *Davis & Taylor* (6), *Hartley & Silver* (9), and *Woodhouse & Farouk* (27) have reported more positive results.

Recently pedicled bone grafts have been used in the treatment of medial fractures of the femoral neck by *Movin* (17, 18, 19) and *Judet* (14) and in the treatment of pseudarthrosis by *Movin*. This has given rise to a renewed interest in the procedure.

The present study was undertaken in order to ascertain whether a fundamental difference between pedicled and free bone grafts could be demonstrated experimentally.

TECHNIQUE AND MATERIAL

The experimental animals were 24 young, mature or nearly mature rabbits of mixed stock, weighing between 2 and 3.5 kg, and ranging in age between 6 and 12 months. These rabbits were divided into two groups.

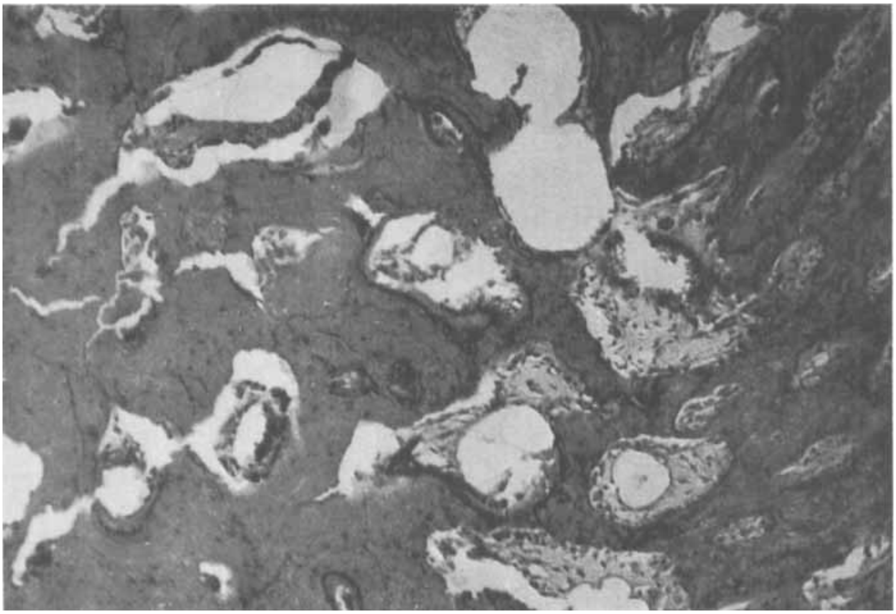

Fig. 1.
10th day. Histological preparation of a pedicled cortical graft.
Creeping substitution and periosteal callus. × 125.

Group 1 was used for comparing pedicled and free bone grafts, 5 rabbits with cancellous and 5 with cortical grafts. The right-sided grafts were pedicled and the left-sided ones free.

The rabbits were anaesthetized by injecting Nembutal into an ear vein, about 40 mg/kg body weight. The operative sites were shaved and disinfected with iodine. The bone grafts were cut with a rotating bone-saw, and the muscle pedicle was dissected to a length of 1 cm. The cut surface of the grafts was isolated from the surroundings by polyethylene foil, 0.05 mm thick.

The free grafts were dissected in exactly the same way, but the muscle attachment was stripped from the periosteum. The graft was fixed, so as to be in touch with the cut-off muscle, with a few sutures of non-traumatic silk.

The cancellous grafts were obtained from the greater trochanter. They measured approx. 10 × 10 × 5 mm and were furnished with a strong muscle pedicle consisting mainly of the gluteus medius and minimus.

The cortical grafts consisted of the dorso-radial part of the proximal end of the ulna. They measured approx. 20 × 5 × 2 mm and consisted of full-thickness compact bone, one side of which carried an endosteal surface corresponding to the medullary cavity of the ulna. The muscle pedicle covered more than half the periosteal surface and consisted mainly of the extensor carpi ulnaris muscle.

Penicillin, 300,000 units, was injected i.m. The rabbits tolerated the operation well. There was no mortality and no infection.

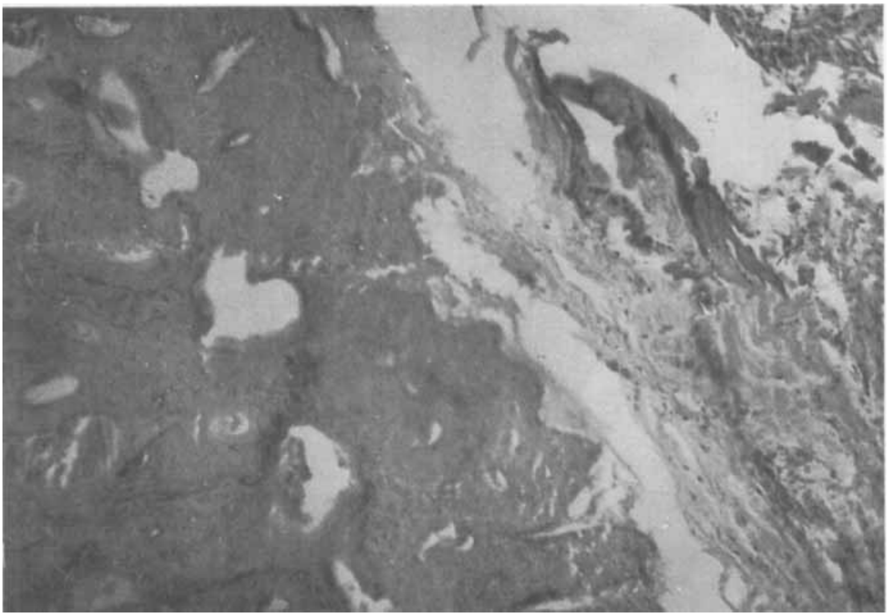

Fig. 2.
10th day. Free cortical graft. The graft is necrotic, and there is no callus. × 125.

The rabbits were killed 1, 3, 6, 10, and 13 days after the operation. Twenty-four hours previously they had been injected with ^{45}Ca, 50 μC/kg into an ear vein and, in the case of the cortical grafts, with tetracycline 50 mg/kg.

Under i.v. Nembutal anaesthesia the heparinized rabbit was bled through the aorta. The vascular bed was rinsed with 500 ml saline solution of body temperature to which had been added amyl nitrite, and 500 ml of a mixture of equal parts India ink and physiological saline was injected. The injection pressure was 100 mg Hg. In all cases there was an unobstructed flow out through the vena cava, and in 7/10 of the cases the injection was successful.

Half the specimen was fixed in formalin, decalcified in formic acid-sodium formate, embedded in paraffin, and cut into 7 μ sections, stained with haematoxylin-eosin and van Gieson's connective-tissue stain.

200–400 μ sections were made transparent by the Spalteholz method (24), and the course of the vessels was studied in a stereo-microscope.

The other half was used for preparing undecalcified, ground sections. The cortical grafts were embedded in methyl metacrylate according to a method described by *Løe* (16). After hardening, the preparations were sawn off and manually ground to a thickness of 50–75 μ. From the cancellous grafts ground sections were prepared without preceding embedding.

The undecalcified, ground sections were used for autoradiography by the contact method on Ilford Nuclear Research Plate G5, 50 μ. The films were exposed for 4 days and developed in an Ilford Rapid Dental Developer.

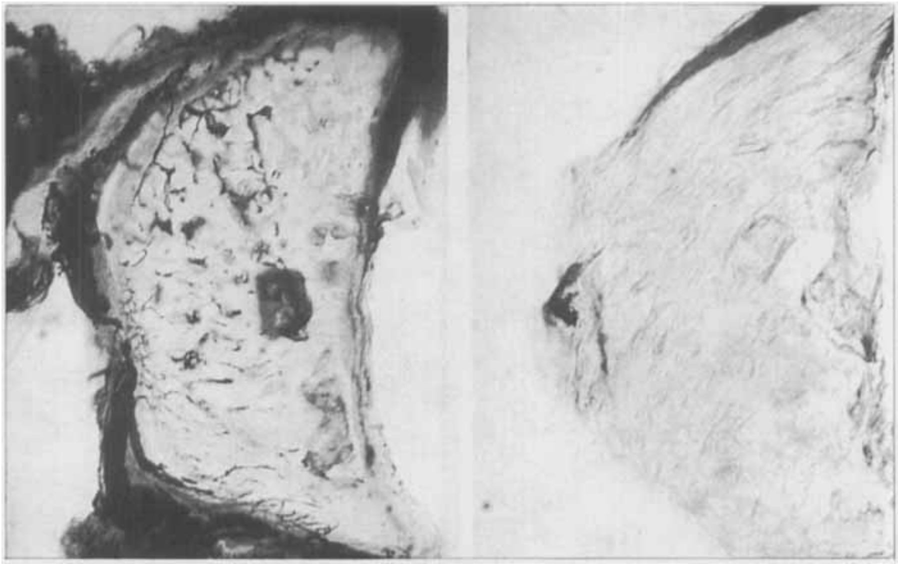

Fig. 3.
3rd day. Injection preparation of cortical bone. Numerous vessels in the periosteum and in the pedicled graft, none in the free graft. × 25.

The same sections of the cortical grafts were studied by fluorescence microscopy in ultraviolet light.

Group II, used for studying the histological changes during the further course, consisted of 7 rabbits with cancellous and 7 with cortical grafts. The operations were performed as described above, but with a pedicled graft on both sides. These rabbits were killed 1–7 weeks after the operation.

Lastly, one rabbit was used for investigation with ^{32}P. This rabbit had operations on all 4 limbs. On the 5th day ^{32}P, 50 μC/kg, was administered, and 90 min. later the rabbit was killed. The soft tissues were removed from the preparations, and the radiation activity was measured.

RESULTS

Group I.

Histological study of the pedicled cancellous preparations showed the osteocytes to be of normal appearance apart from a narrow rim of empty lacunae close to the cut edge. Only in one preparation was there a small, well-defined area, most remote from the pedicle, in which the lacunae were empty, and another preparation showed scattered pyknotic nuclei. The periosteum survived and showed, on the 3rd day, proliferation of osteoblasts. On the 6th day there was periosteal callus and callus formation between the trabeculae close to the

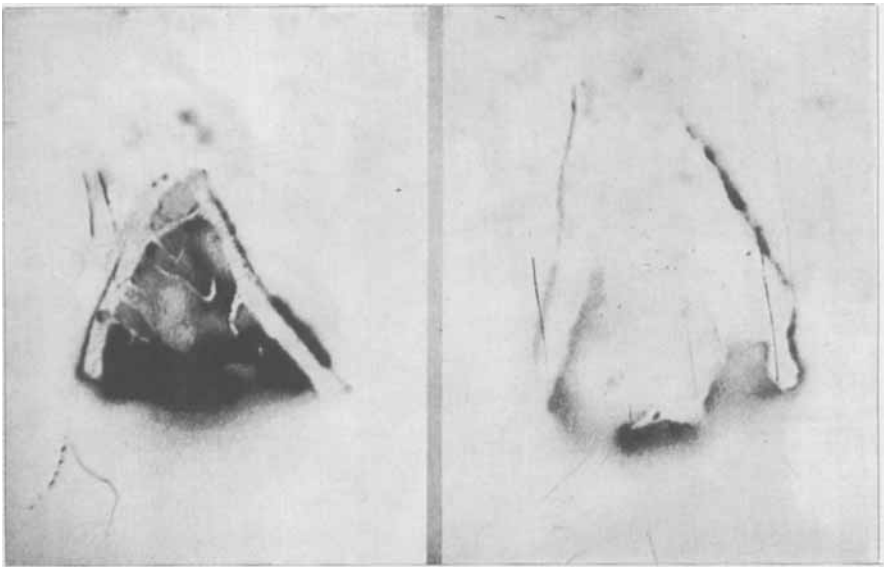

Fig. 4.
Autoradiography of cancellous grafts, the pedicled one on the left.
The marked blackening around the pedicled graft represents callus formation. × 5.

cut surface. On the 10th day the entire preparation was surrounded by callus. There were no signs of resorption or creeping substitution.

In the free cancellous preparations the lacunae were empty or contained pyknotic nuclei, and there were only a very few normal-looking cells peripherally in the graft. In the earliest preparations the periosteum was necrotic. On the 6th day there was incipient regeneration of periosteum, and on the 13th day periosteal callus.

The pedicled cortical preparations showed that despite a marked decrease in the number of normal osteocytes—to one-half in the oldest preparations—the periosteum of the pedicled graft survived, being from the 3rd day hyperplastic and showing proliferation of osteoblasts. On the 6th day these changes had become further accentuated, and there was callus formation as a layer on the periosteal surface. At the same time, regeneration of bone-marrow tissue and endosteal callus formation were observed. Internally, the corresponding changes occurred later, osteoblastic proliferation not occurring in the Haversian canals until the 6th day and creeping substitution from the 10th day.

In the free cortical grafts all the osteocytes disappeared. Not until the 10th day was there any osteoblastic proliferation and incipient

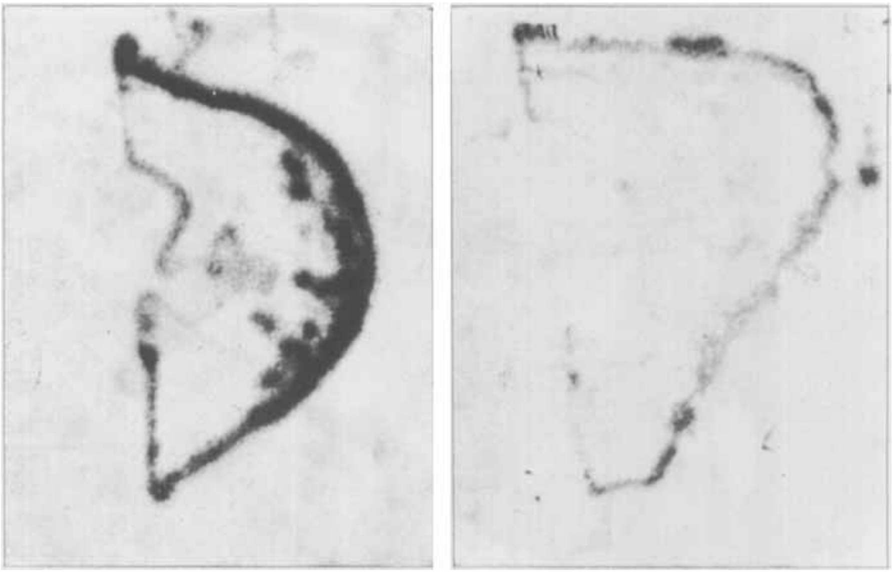

Fig. 5.
3rd day. Autoradiography of cortical grafts, the pedicled one on the left. A number of "hot spots" and pronounced marginal blackening around the pedicled graft; only faint marginal blackening around the free graft. × 20.

periosteal callus formation, and no signs of creeping substitution appeared within the observation period.

The injected preparations showed good agreement with the histological findings. The pedicled grafts showed on the first day numerous filled vessels extending from the hyperaemic periosteum deep into the graft, some vessels debouching on the endosteal surface. The older preparations gradually exhibited an increasing number of filled vessels, and the graft seemed to be fully vascularized. In contradistinction, the free grafts had, not until the 6th day, a single vessel, and in the older preparations still only a few vessels, peripherally in the graft.

Autoradiographically, the pedicled cancellous grafts resembled the appearances of a normal trochanter. At the site of callus formation, there was a more pronounced blackening, and in the small, well-defined necrotic areas there was no blackening. The free grafts were hardly visible until periosteal callus formation had set in, and the inside of the graft was empty.

Autoradiography of the pedicled cortical 1-day preparation presented itself as normal autoradiography with diffuse blackening of the inside, interspersed with numerous, rather faint, but yet distinctly

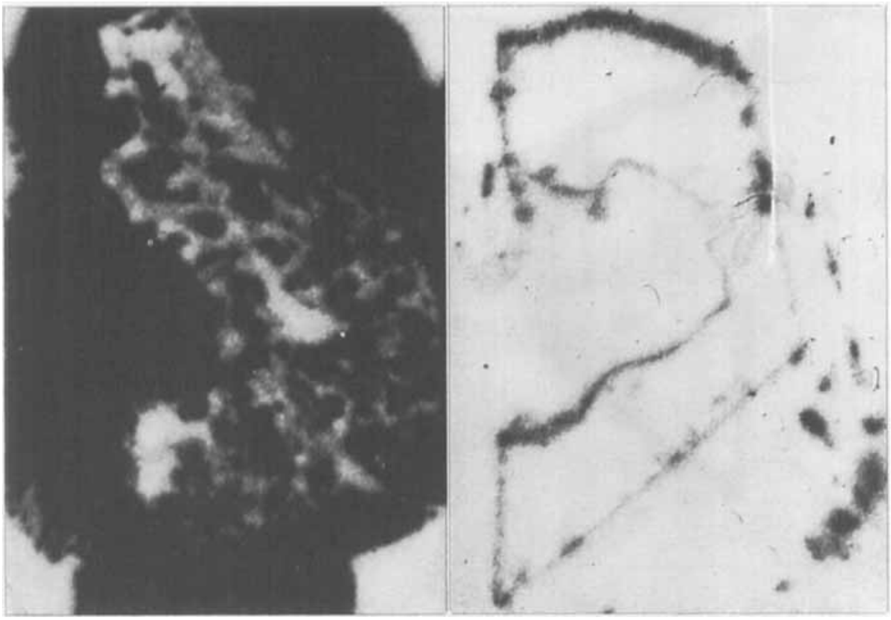

Fig. 6.
10th day. Autoradiography of cortical grafts, the pedicled one on the left. The violent blackening around the pedicled graft represents callus. × 20.

recognizable centres with a higher activity, the so-called "hot spots". Moreover, there was a more pronounced, blackened line corresponding to the entire surface of the graft. This surface phenomenon was less marked on the free graft whose inside was empty. On the 3rd day the marginal blackening on the pedicled graft was wider, and now there were about ten "hot spots". After this juncture, the activity of the pedicled graft greatly increased, in the periosteum as well as in the inside, and by far exceeded that of the corresponding free grafts which showed blackening only at the sites of the more scanty periosteal callus.

Fluorescence microscopy of the pedicled cortical grafts showed on the 1st day a thin, fluorescent streak around 3 vessels in the graft. On the 3rd day there was a more pronounced circle around several small vessels, filled with India ink, in the same numbers as the "hot spots" on the autoradiographs of the same section. Not until the 6th day could fluorescence be observed on the surface, at the site of the periosteal and endosteal callus, and from that time on the fluorescence accurately corresponded to the newformed bone.

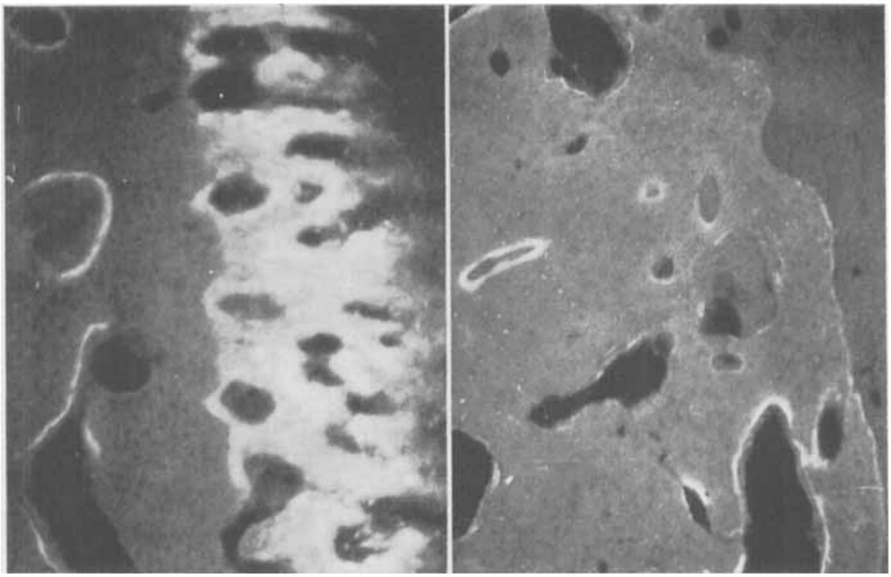

Fig. 7.
Fluorescence microscopy of pedicled cortical grafts, 6th day on the left 3rd day on the right. There was no fluorescence of the corresponding free grafts. × 125.

The free grafts showed no fluorescence until the 10th day, when it was apparent in one site of the periosteum, corresponding to incipient callus formation.

Group II.

Histological investigation of the cancellous grafts from the 1st–7th week revealed that on the whole the grafts had survived. Only 2 preparations exhibited necrosis which did not comprise more than one-third of the graft. Callus formation was satisfactory, but not equally intense in all the preparations, although in other respects they looked like normal, living bone. On the older preparations it was difficult to distinguish newformed bone from the original structure. However, the newformed bone was somewhat paler and contained larger osteocytes. There was normal bone marrow in all grafts.

In the cortical preparations there was an even development from the 1st to the 7th week. The osteocytes disappeared almost completely from the endosteal half of the graft, and gradually most of these cells also disappeared from the subperiosteal part, leaving only small areas of normal osteocytes. Pronounced osteoclastic absorption took place, so

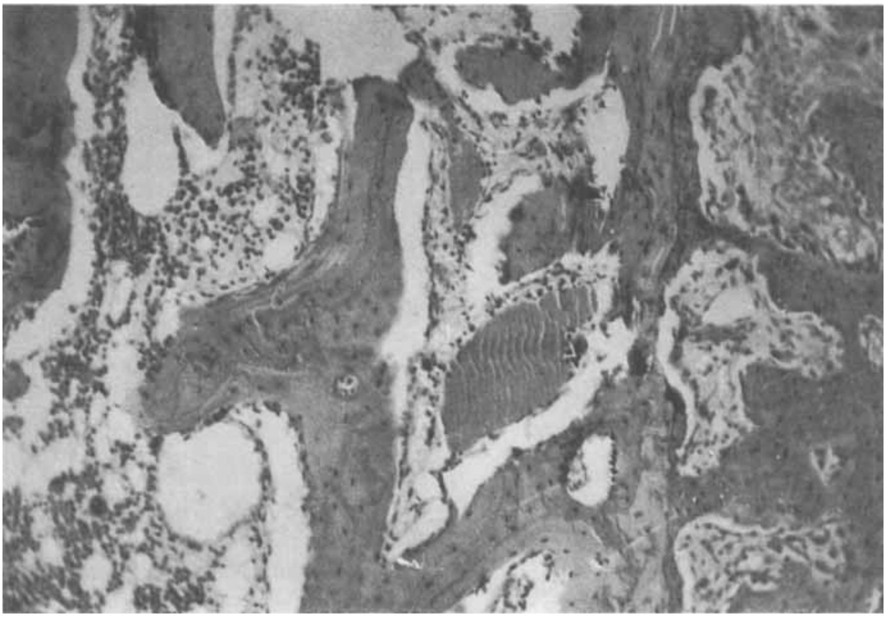

Fig. 8.
7th week. Histological preparation of pedicled cancellous graft. Normal osteocytes and bone marrow. Periosteal callus. × 125.

that towards the end of the experimental period the original graft showed a coarse-meshed, almost spongy structure. Concurrently, there was marked creeping substitution. Periosteal and endosteal callus formation was very pronounced, eventually surrounding the entire graft. In the most pronounced cases the preparation was transformed into a small ossicle, consisting of a thin layer of newformed compact bone lined with periosteum envelopping a cavity with ample medullary tissue, loose-structured callus, and remnants of the original, greatly substituted graft.

The results found in the last rabbits, used for ^{32}P experiments, are presented in the table.

TABLE
Counts / g / min.

Cancellous bone		Compact boee		Humerus	Blood
Pedicled	Free	Pedicled	Free		
1000	116	649	488	648	461

DISCUSSION

If a soft-tissue pedicle is to contribute to preserving the vitality of a bone graft, it is mandatory that a reasonable number of vessels enter the bone from the surrounding soft tisues.

The blood supply of the long bones has been thoroughly studied by *Lexer* (15) and *Johnson* (13) who have, consistently, described the following systems:

The nutrient arterial system. The nutrient artery divides into ascending and descending branches which supply the bone marrow and the inner half to two-thirds of the diaphyseal cortical bone.

The metaphyseal and epiphyseal system which consists of a large number of small vessels, penetrating the bone from muscular and ligamentous attachments, dividing profusely in the bone marrow which they supply together with the inner half of the metaphyseal cortical bone.

The periosteal system which surrounds the bone in the form of a dense, anastomosing network, supplying the outer one-third to one-half of the cortical bone.

There are fully sufficient anastomoses between the nutrient arterial system and the metaphyseal system which thus can compensate for each other. On the other hand, the anastomoses from the periosteal system to the other two systems are scanty. This was confirmed by *Trueta & Cavadias* (25) who in cases of medullary nailing found necrosis of the inner half to two-thirds of the cortical bone.

Our grafts, cancellous as well as cortical, were obtained from the metaphysis, and their blood supply therefore consists partly of metaphyseal and partly of periosteal vessels.

The thickness of the muscle pedicle decides the number of metaphyseal vessels which penetrate the graft and thereby the nutrition of its profound part, while the periosteal system can be supplied through a more slender pedicle. We have tried to preserve a pedicle as thick as possible. Thus, on the trochanter the pedicle covered the entire periosteal surface, while on the ulna it covered only half. When considering, moreover, that in the compact bone there are only a few anastomoses, and at that only of capillary size, between the individual Haversian systems, it is evident that the blood supply of the compact graft is more critical than that of the cancellous graft. This presumably affords the explanation of the difference observed between the osteocytes in the cancellous and in the cortical graft.

The histological assessment is disturbed by the fact that a long time may elapse until the perished osteocytes disappear from the lacunae. As a rule, however, they can be distinguished from normal osteocytes, as their nuclei generally show distinct pyknosis. They are small and stain intensely and uniformly. In the event of karyorrhexis doubt may arise, as a similar phenomenon may be observed as an artefact in normal bones. Thus, the histological evaluation carries some uncertainty. Therefore, the investigations were supplemented by injection of the vessels, autoradiography, and fluorescence microscopy.

The injection experiments afforded a convincing impression of the early and marked hyperaemia of the periosteum and the ample vascularization of the pedicled grafts, thus supplementing the histological findings.

In an effort to obtain a quantitative evaluation of the vascularization of the graft, we performed the above-mentioned ^{32}P experiment. Considering the short period between the administration of the isotope and the counting, we expected to obtain a result in which the vascularization was the decisive factor. The investigation showed a distinct difference between pedicled and free grafts, but did give an impression of considerable uncertainty. As a result, this method was not used in further case.

The results of autoradiography were largely consistent with the histological findings and the injection experiments, showing a distinctly higher activity of the pedicled grafts. It is well-known that a dead bone can also take up ^{45}Ca. Thus, using experimental conditions comparable to ours, *Ray et al.* (22), by autoradiography of dead bone, could obtain a picture which in respect to marginal blackening was like the corresponding living bone. However, the exposure period was 15 times longer. Although the uptake of ^{45}Ca cannot be taken directly to be a vital process, there are considerable quantitative differences between dead and living bone.

In one of our younger preparations there was a marginal blackening and an intensity of "hot spots" (Fig. 5) which did not only exceed that of the free graft, but also that of the corresponding normal preparation. This cannot be explained by callus formation, because at that time there was only incipient osteoblastic proliferation. However, there was only unmistakable hyperaemia of the periosteum (Fig. 3) which may perhaps have caused the phenomenon. In that event, it might be compared with *Bauer*'s (1) finding of the increased uptake of ^{47}Ca by bone tumours, sometimes observed long before the lesion is demonstrable by X-rays.

The fluorescence microscopic findings resembled the autoradiographic results. This was only to be expected, since—like ^{45}Ca—the tetracyclines label the not fully mineralized newformed bone and callus. However, the tetracycline labelling is more sharply defined and more specific. Thus, the above-mentioned marginal area showed no uptake of tetracycline (Fig. 7), which accurately followed the periosteal callus formation.

One factor is worthy of special mention. In all phases the pedicled cortical grafts exhibited a strikingly greater osteogenic activity than the cancellous grafts. A possible explanation is the above-mentioned, more critical blood supply of the cortical bone which involves more widespread necrotic changes and thereby exerts a more potent stimulation for hyperaemia and new-bone formation. Owing to the somewhat artificial experimental conditions—motivated by the wish to avoid actions from the surroundings structures—the surviving cancellous graft lacks a stimulus and therefore responds only by callus formation on the surface.

SUMMARY AND CONCLUSION

Comparative studies on pedicled and free bone grafts were carried out on rabbits.

On the basis of histological investigations, injection of vessels, autoradiography, and fluorescence microscopy, the authors conclude that free bone grafts undergo necrosis, while pedicled, cancellous grafts survive. In pedicled, compact bone grafts the majority of the osteocytes disappear, but the blood supply, and thereby the osteogenetic ability, persists.

RESUME ET CONCLUSION

Avec des lapins comme animaux d'essai, il a été procédé à des examens comparatifs de greffes osseuses pédonculées et libres.

Sur la base d'un examen histologique, d'injections vasculaires, d'autoradiographie et de microscopie fluorescente, il est conclu que les greffes osseuses libres se nécrosent alors que les greffes spongieuses pédonculées survivent. Dans le greffes osseuses pédonculées compactes, la plupart des ostéocytes disparaissent, mais l'approvisionnement des vaisseaux est maintenu et par là le pouvoir ostéogénétique.

ZUSAMMENFASSUNG UND SCHLUSSFOLGERUNG

An Kaninchen als Versuchstiere führte man vergleichende Untersuchungen von gestielten und freien Knochenspangen aus.

Auf Grundlage von histologischen Untersuchungen, Gefäsinjektionen, Autoradiographie und Fluoreszensmikroskopie schliesst man, dass freie Knochenspangen nekrotisieren, während gestielte spongiöse Spangen überleben. In gestielten kompakten Knochenspähnen verschwinden die meisten Osteozyten, aber die Gefässversorgung und damit die osteogenetische Potenz wird beibehalten.

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