Acta orthop. Scandinav. 42, 82–93, 1971

Institute for Experimental Research in Surgery, University of Copenhagen, Surgical Department A, Frederiksberg Hospital, and Department of Plastic Surgery, Rigshospitalet, University of Copenhagen, Denmark.

BONE GROWTH IN THE FEMORAL HEAD FOLLOWING PEDICLED BONE GRAFTING

An Experimental Study

SANDOR MEDGYESI

Accepted 13. x. 1970

The common occurrence of aseptic necrosis of bone in the head of the femur following intracapsular fracture of the femoral neck—not only in the presence of non-union, but also when the fracture has apparently healed—must be due to the special blood supply of the femoral head (Trueta & Harrison 1953). The supplying blood vessels are torn or thrombosed by the fracture, and the cells in the bone and bone marrow perish. The radiographic phenomena of increased density, a mottled appearance, and collapse are due partly to necrosis and resorption and partly to the new formation of bone. Reduction and osteosynthesis are followed by revascularization, leading to more or less intensive new formation of bone. However, this new formation has a tendency to become arrested. The explanation is that in the course of time the dead bony tissue in the femoral head loses its inductor capacity and that the increasing number of multipotential connective tissue cells are no longer converted into osteogenic cells.

Several surgeons insert an alloprosthesis in cases of intracapsular femoral neck fracture. Others try a more physiological solution, consisting in the insertion of a live bone graft, small or large, into the head across the fracture (Judet 1963, Movin 1966). Part of the dead bony tissue is thereby replaced by live tissue. Of course, there always remains no small amount of dead bony tissue to be revascularized and replaced by living tissue. It was the object of the present study to elucidate whether this live bone graft is able to contribute to this process.

In previous experiments on pedicled bone grafts, good survival of the grafts was obtained (Baadsgaard & Medgyesi 1965, Medgyesi 1968). The ability for healing to avascular sites has also been studied (Medgyesi 1965). In this latter investigation it was suggested that the live bone graft is able to induce "creeping substitution" in the deeper parts of the avascular bone.

METHOD

The experimental animals were mature or almost mature rabbits, i.e. in the latter the epiphyseal cartilage was still discernible. The animals were anaesthetized by Nembutal administered slowly by the intravenous route.

After cutting the ligamentum teres and dislocating the head of the femur, the femoral head on one side was sawn off by a rotating saw just distally to the articular cartilage in 28 cases. The pedicled graft

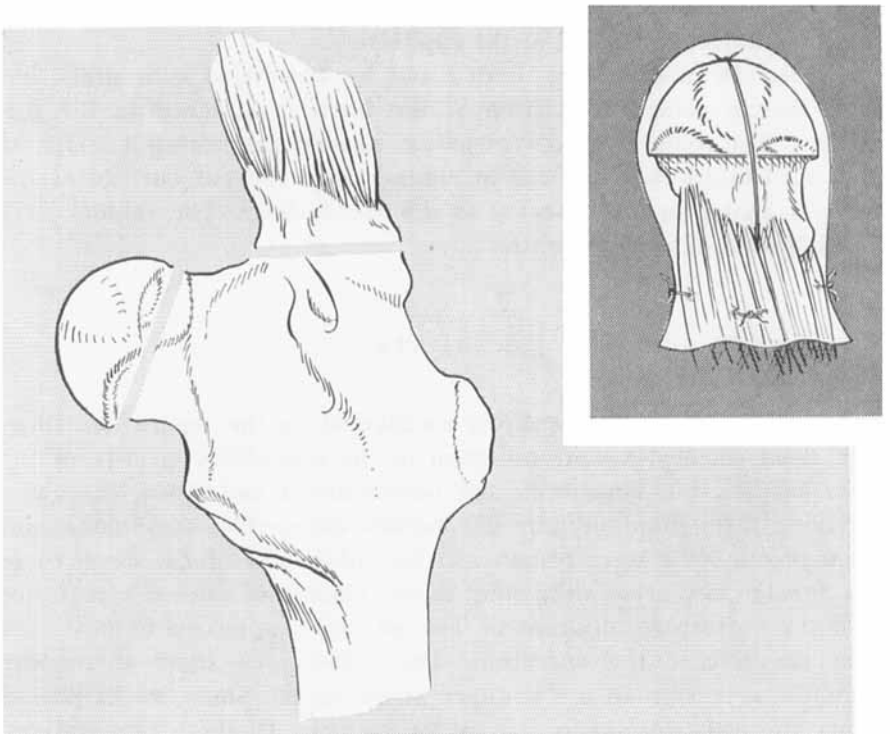

Figure 1. Graphic presentation of the operative method.

was sawn off the greater trochanter of which it made up the greater part. During the operation care was taken not to damage the periosteum, and all the muscles attached to the graft were preserved as a pedicle. The pedicle was dissected to a length of 1 cm. The entirely freed femoral head and the pedicled graft were kept together by a silk ligature, cancellous bone facing cancellous bone. In addition, the head and pedicled graft were isolated by a small hood of polyethylene foil. The foil was fixed by a few superficial silk sutures to the periosteum and muscle pedicle (Figure 1). It must be pointed out that presumably it was inevitable to compromise the blood supply of the graft to some extent by the sutures.

The rabbits were killed 4 days—14 months after the operation. Nine received 50 μC Ca 45/kg 24 hours before being killed. In these cases autoradiography was done by the aid of 200 μ thick, undecalcified ground sections, kept in contact with an Ilford Nuclear Research Plate G_5 50 μ. For histological study haematoxylin-eosin and van Gieson stainings were used. In addition, angiography using Micropaque filling of the blood vessels was attempted in a number of cases, but without obtaining sufficient filling of the capillaries.

Another 16 rabbits were treated, not by a pedicled bone graft, but by a muscle pedicle consisting of the muscles attached to the trochanter. The femoral head was placed against the muscle pedicle, so that the cancellous bone was in contact with the cut surface of the muscles. Isolation was effected as described above. The rabbits were killed from 4 days to 6 months later.

RESULTS

The Pedicled Grafts

Half the grafts proved entirely unaffected by the separation. However, dead osteocytes were observed in the immediate vicinity of the sawn surface, but bone cells and bone-marrow cells were otherwise surviving throughout (Figure 2). Normal osteocytes, osteoblasts, and haemopoietic cells were present. In the other half of the cases there was damage in places to the bony tissue, usually of limited extent, but sometimes affecting major areas. The osteocytes appeared to have been most sensitive to the operation. The well-known signs of nuclear pyknosis were seen in the younger preparations. Some weeks passed before the cells disappeared from the lacunae. In these preparations there was reduced haemopoietic activity, but the cells in the reticular

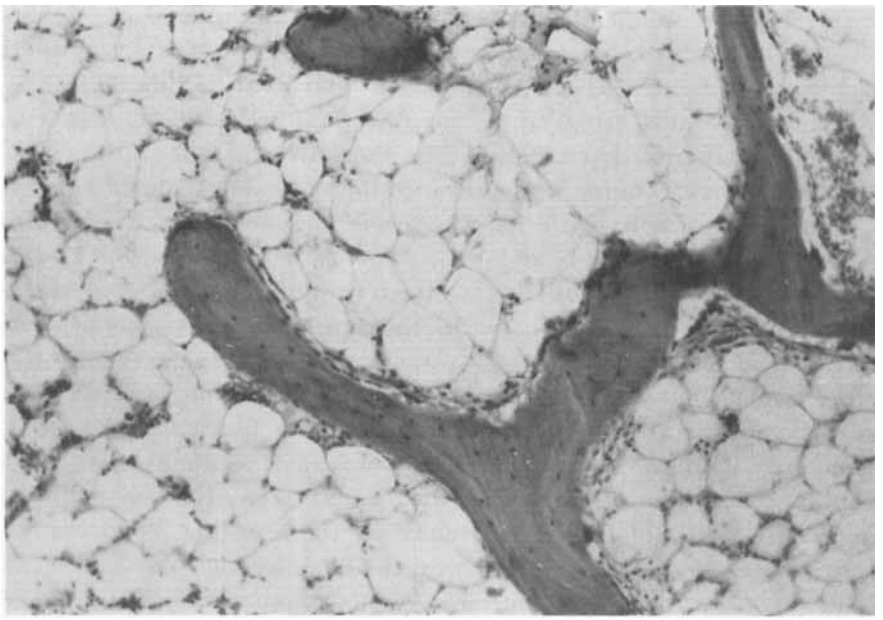

Figure 2. Live bony tissue from the inside of the graft. The bone marrow is fatty in this case. One-month preparation.

connective tissue, the osteoblasts, and the cellular elements of the sinusoids and capillaries looked unaffected. Total necrosis of the grafts was observed in two cases. These phenomena were also easily recognized in older preparations because there was little osteoclastic activity around the dead bony trabeculae, i.e. they were lying *in situ*. The newly formed bony trabeculae could be distinguished from the old ones because of their more irregular structure and often more intense basophilic staining. Quite rapidly, a certain new formation of bone took place in a number of the grafts, subperiosteally as well as endosteally, mainly in those grafts where some of the osteocytes had perished. In the older preparations the bony trabeculae were thinner than normal, presumably owing to inactivity.

There were no changes in the muscle pedicles apart from the accumulation of connective tissue cells and capillary proliferation in the vicinity of some grafts.

Femoral Head in Contact with the Pedicled Bone Grafts

In a one-week specimen there was already a large number of cells between the two pieces of bone. These cells originated partly in the

intertrabecular tissue of the pedicled bone graft and partly in its periosteum. At the outset, and especially where a small haematoma had been present, the cell population consisted of macrophages, lymphocytes of different size, a very few giant cells and plasma cells, and occasional leukocytes. In addition, there were already at that time a few cells corresponding in appearance to the histological descriptions of primitive mesenchymal cells. The term "mesenchymal cells" is also used in connection with bone induction (Urist et al. 1967), but in this context it seems more correct to use the term undifferentiated or multipotential connective tissue cells. In the course of approximately two weeks, this cell population had yielded to more regular connective tissue which continues the invasion into the femoral head together with the proliferating capillaries and gradually larger blood vessels. The entire femoral head had been permeated in the course of 2 months. Little osteoclastic activity was found. Only a few cases showed penetration of the old bony trabeculae by the invading blood vessels. It must be assumed that in most cases the blood vessels and connective tissue invaded the intertrabecular space, where the original blood vessels had been. As already mentioned, the epiphyseal cartilage was still present in the femoral head in some of the rabbits at the time of the operation. In these cases the tissue invasion stopped at the epiphyseal line. The epiphyseal cartilage appeared to act as a barrier to the tissue invasion. On the outside the connective tissue grew up along the articular cartilage and encapsulated the entire head in most cases. However, the articular cartilage acted in the same way as the epiphyseal cartilage. It was very seldom penetrated despite the fact that its cells had perished.

The invading connective tissue consisted of two types, in several cases co-existing in the same preparation (Figure 3). One, the less common, was dense connective tissue with a number of fibrils and numerous fibrocytes as well as a few other cells, such as macrophages and lymphocytes. This fibrous tissue was fairly avascular. The other type was a connective tissue with a smaller content of fibrils, but more vascular, reminiscent of areolar or reticular connective tissue. In this tissue there were a number of the above-mentioned multipotential connective tissue cells. In relation to the cytoplasm, these cells had a large vesicular nucleus which, however, was smaller and more rounded than the nucleus of the fibroblasts. The cytoplasm stained faintly. The greater part of the nuclear chromatin was often of a peripheral situation, and the nuclear membrane was distinct. This tissue also con-

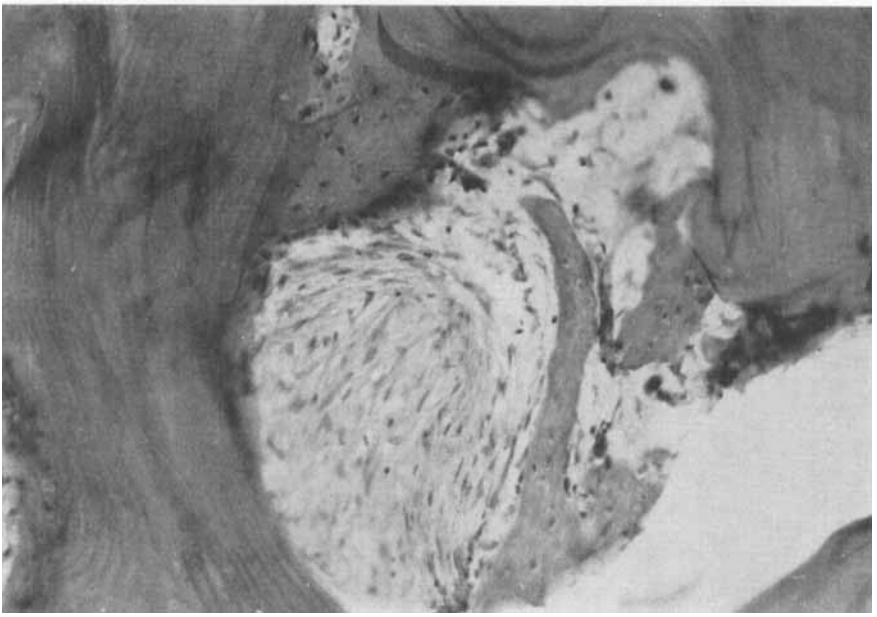

Figure 3. Loose connective tissue with newly formed bone as well as non-productive, fibrous connective tissue from the inside of the femoral head.

tained fibroblasts and transitional varieties between fibroblasts and the named cells; therefore, the differentiation was often difficult. In addition, there were typical macrophages and other connective tissue cells. The undifferentiated connective tissue cells were often seen in larger quantity in the vicinity of the proliferating small blood vessels. It was in this type of connective tissue that bone formation took place in most of the cases. Bone marrow formation followed only upon invasion of connective tissue of this type.

Callus formed between the two pieces of bone, at least before the lapse of two weeks. It consisted of irregularly arranged, densely placed cancellous bone. Incidentally, bone formation was delayed in relation to connective tissue invasion inside the preparation. In preparations removed a short time after the operation this delay was about two weeks, but in older preparations it was longer. As already mentioned, the connective tissue took two months to penetrate, but in the most distal part of the femoral head bone formation did not appear until 4 months had elapsed. Autoradiography gave an excellent survey of the appearance of bone formation (Figure 4). However, conclusions concerning the relative quantity of newly formed bone cannot be

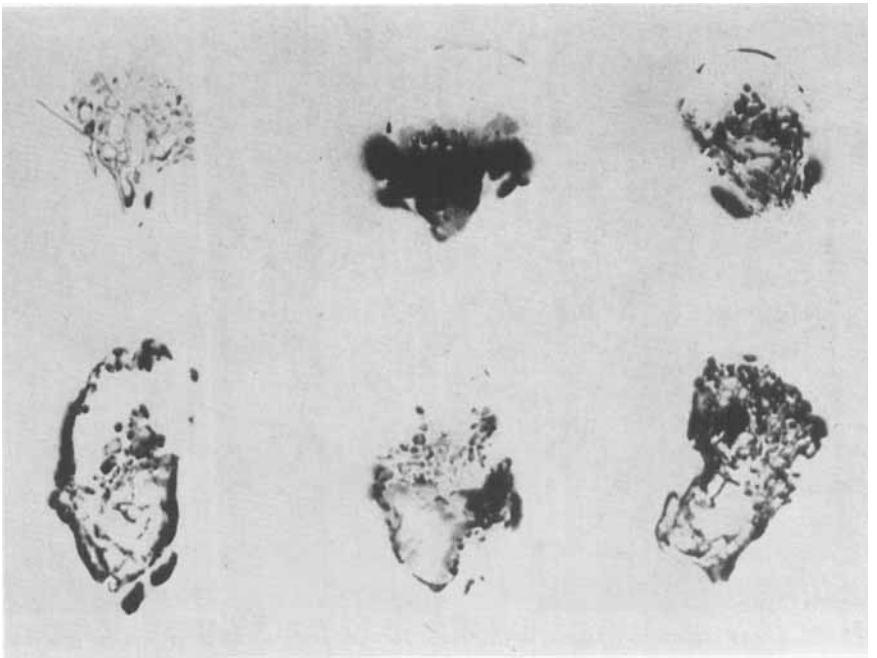

Figure 4. Autoradiography. Top, left: a normal femoral head. Thereafter, a 2-week, 1-month, 2-month, 3- and 5-month preparation. The pedicled grafts at the bottom.

drawn on the basis of the intense blackening. The autoradiographic exposures give the impression that the volume of new bones exceeds that of the old bony trabeculae in most places, but this was not so. In most cases the newly formed bone had settled on the old bony trabeculae, i.e. it was a case of "creeping apposition". These deposits were about 1/5 as thick as the original bony trabeculae (Figure 5). However, there were instances in which major islets of woven bone or entirely new trabeculae would form.

In the older preparations a return of the haemopoietic bone marrow might be seen, often parallel with new bone formation. This bone marrow was less cellular than normal, but otherwise showed the same characteristics as normal bone marrow in the rabbit.

Femoral Head in Contact with a Muscle Pedicle

No primary callus formed in these cases. Only one case exhibited minimal bone formation before the lapse of two weeks. In this case there was a suspicion that a few osteogenic cells had survived in the

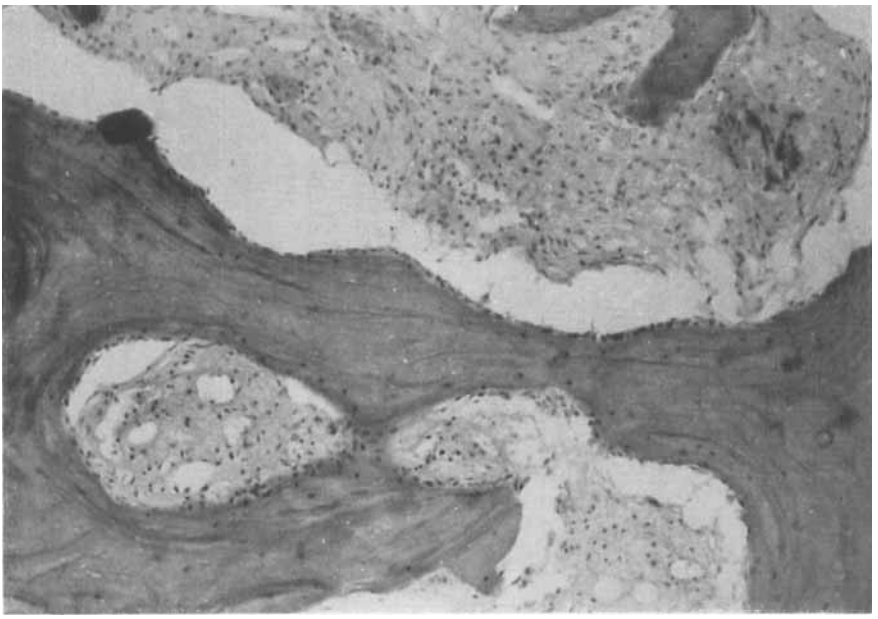

Figure 5. The old bony trabeculae with deposits of newly formed bone. At the top, an independent island of new bone. The photograph is from the distal part of the femoral head. 5-month preparation.

head itself. The fact is that a few normal-looking osteocytes were seen in the immediate neighbourhood of the newly formed bone, indicating that a small area of the head had survived the grafting and had formed an ossification centre. Apart from the fact that primary callus formation was usually absent, the entire process was surprisingly like that seen in the experiments using pedicled bone grafts. The nature of the invading connective tissue was the same as described above. Invasion of blood vessels and connective tissue was followed by new formation of bone, slightly delayed in early preparations and more delayed in older ones. Again, there were the same thin deposits of newly formed bone on the surface of the old trabeculae, but there were also a few apparently detached ossification centres, also like those observed previously (Figure 6).

DISCUSSION

The main object of the present study was to elucidate whether a pedicled bone graft is able to secure revascularization and new forma-

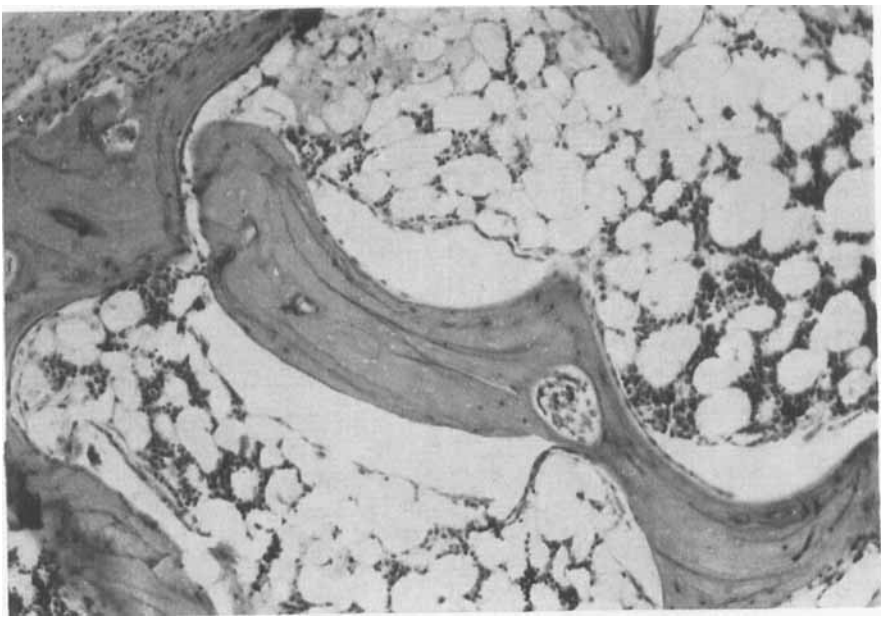

Figure 6. Newly formed bone as well as bone marrow in a femoral head which has been in contact with a muscle pedicle. 6-month preparation.

tion of bone in a femoral head which is cut off from its blood supply. In addition, it was investigated how long these processes take. The experiments using a muscle pedicle served as a control on the rate of revascularization and at the same time supported the induction theory.

Isolation with polyethylene foil ought to guarantee that invasion of tissue could not take place from any other site than from the pedicled graft. The tissue migration took place at the same rate centrally and peripherally. It must be assumed, therefore, that the endosteal blood vessels have contributed to the process at least as much as the periosteal vessels. Except in the two cases where the grafts were totally necrotic, the revascularization and reossification took place in the head, also in cases where major or minor quantities of dead osteocytes were seen in the grafts. Apparently, some of the osteocytes have been rather sensitive, even to minor disturbances of nutrition which otherwise had not influenced the other tissue components of the grafts and which had not interfered with their role as a source of revascularization. Tissue invasion went all through the femoral head except in the experiments where epiphyseal cartilage was still

present. The explanation of the latter phenomenon must be that osteoclastic activity has been relatively slight throughout the head. Newly formed bone was seen throughout, but not in particularly great quantities. This is in keeping with previous observations of bone formation in the human femoral head after intracapsular fractures of the femoral neck (Santos 1930). In this respect, muscular inactivity presumably plays an important role. The fact that the connective tissue took a couple of months to penetrate such a relatively small piece of bone may be explained by the relatively thick and densely arranged bony trabeculae in the head of the femur. In man, it has been reported that up to 2 years may elapse before the head has been completely revascularized (Phemister 1939, Scherman & Phemister 1947). To decide whether the possibly reduced resources of the pedicled grafts contribute to the revascularization and reossification being slower than would be expected, the head was brought into contact with a muscle pedicled in some of the cases. It was assumed that the well-vascularized muscle pedicle was able to secure revascularization and connective tissue invasion of the head as rapidly as at all possible. The invasion of connective tissue also involves a possibility of bone formation.* That the rate of invasion was the same in both events indicates that it is actually dependent upon the structure of the femoral head and a possible physiological stimulus, whereas the nature of the pedicled tissue means less, provided that the pedicle is well vascularized. Apart from the primary callus formation, the histological appearances were exactly identical and the changes took place in the same order in both types of experiment. This indicates an induction process following upon the invasion of connective tissue into the femoral head. At any rate, it is evident that bone cannot invade from a muscle pedicle. This is also indicated by the latent period between the invasion of connective tissue and of bony tissue. This latent period was longer in the older preparations than at the outset of tissue

* This has been elucidated in the studies of Leriche & Policard (1926) and of Levander (1938). The multipotential cells (primitive mesenchymal cells) of the connective tissue may be induced by bony tissue to form new bone. Some authors believe that the inducing substance is liberated by the dying osteocytes (Trueta 1963). At least, lyophilized, decalcified matrix contains this substance (Urist et al. 1957). Bertelsen (1944), in a comprehensive study, reviewed previous results on this subject and reported his own result. Since that time several admirable experiments have confirmed the induction theory (Urist & McLean 1952, Goldhaber 1961, Urist et al. 1967). However, there is not complete agreement as to which cells are induced (Trueta 1963), nor has the inducing substance been isolated biochemically.

invasion. From clinical observations it is also known that new formation of bone may become completely arrested, nothing but connective tissue being found in the deeper parts of the head. The dead bony tissue gradually loses its inductor powers.

CONCLUSIONS AND SUMMARY

In rabbits a pedicled bone graft is able to revascularize the isolated femoral head. Primary callus formation is seen within 2 weeks, followed by slow connective tissue invasion and bone formation, in the form of "creeping apposition", completed in about 4 months. A muscle pedicle exerts an identical effect, except for the primary callus formation. The slow rate at which the connective tissue invades the intratrabecular spaces must be assumed to be due to the bony structure of the femoral head as well as the lacking stimulus by muscular activity, independently of whether the source of revascularization is a pedicled bone graft or a muscle pedicle. New formation of bone is observed in preparations removed a few weeks after the connective tissue invasion. The bone formation shows a greater delay in the deeper part of the femoral head, indicating that the induction capacity decreases as time goes by.

REFERENCES

Baadsgaard, K. & Medgyesi, S. (1965) Muscle-pedicle bone grafts. *Acta orthop. scand.* **35**, 279–293.

Bertelsen, A. (1944) Experimental investigation into post-foetal osteogenesis. *Acta orthop. scand.* **15**, 139–181.

Goldhaber, P. (1961) Osteogenic induction across millipore filters *in vivo*. *Science* **133**, 2065–2067.

Judet, R. (1963) Fractures du col du femur. *Actualités chir. orthop. Hôpital Raymond-Poincaré. II*, 78–104.

Leriche, R. & Policard, A. (1926) *Les problemes de la physiologie de l'os*. Mason et Cie, Paris.

Levander, G. (1938) A study of bone regeneration. *Surg. Gynec. Obstet* **67**, 705–717.

Medgyesi, S. (1965) Healing of muscle-pedicle bone grafts. *Acta orthop. scand.* **35**, 294–299.

Medgyesi, S. (1968) Investigation into the carrying ability of pedicle-bone grafts during transplantation. *Acta orthop. scand.* **39**, 1–7.

Movin, R. (1966) Revascularisation de la tête fémoral (Discussion). Dixième Congrès International Orthopédique et de Traumatologie, Paris, pp. 98–100. *Acta medica Belgica*, Bruxelles.

Phemister, D. B. (1939) Pathology of un-united fractures of the femur with special reference to the head. *J. Bone Jt Surg.* **21**, 769–792.

Santos, J. V. (1930) Changes in the head of the femur after complete intracapsular fracture of the neck. *Arch. Surg.* **21**, 470–530.

Scherman, M. & Phemister, D. B. (1947) The pathology of un-united fractures of the neck of the femur. *J. Bone Jt Surg.* **29**, 19–37.

Trueta, J. & Harrison, H. M. H. (1953) The normal vascular anatomy of the femoral head in adult man. *J. Bone Jt Surg.* 35 *B*-**3**, 442–461.

Trueta, J. (1963) The role of the vessels in osteogenesis. *J. Bone Jt Surg.* **45** *B*-**2**, 402–418.

Urist, M. R. & McLean, F. L. (1952) Osteogenetic potency and new bone formation by induction in transplants to the anterior chamber of the eye. *J. Bone Jt Surg.* **34**-A, 443–470.

Urist, M. R. et al. (1967) The bone induction principle. *Clin. Orthop.* **53**, 243–283.