Acta orthop. scand. *50*, 491–499, 1979

THE OSTEOGENIC CAPACITY OF FREE PERIOSTEAL AND OSTEOPERIOSTEAL GRAFTS

A Comparative Study in Growing Rabbits

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The behaviour of free periosteal and 200 micron thick osteoperiosteal grafts was studied histologically in 40 six-week-old rabbits. The grafts were taken from the tibia and fixed on either side of the same lumbar vertebra between the spinous and mamillary processes. The free stripped periosteum had better osteogenic activity than the 200 micron thick osteoperiosteum. The new bone was formed by the osteogenic cells of the cambium layer in both types of graft.

Key words: osteogenesis; periosteal grafts; osteoperiosteal grafts

Accepted 1.ii.79

The cortical growth of long bones is brought about mainly by the diaphyseal periosteum. This fact has been widely accepted since the pioneer investigations of Duhamel (1739). However, some opposing concepts have been presented. For instance, MacEwen (1912) stated that the periosteum is merely a limiting membrane preventing the osteoblasts from being scattered from the surface of the bone into the soft tissues, where their presence could be harmful. MacEwen considered the osteoblasts of the osseous tissue responsible for osteogenesis. In some respects he was right. The question then arises as to where the boundary between bone and periosteum definitely lies. The periosteum comprises the outer fibrous layer and the inner proliferative "cambium layer". When studying the osteogenic capacity of free periosteal transplants, the results are influenced by many variables including age, species, bone type, site from which the bone is derived (Fang et al. 1934) and the bed to which it is grafted (Cohen & Lacroix 1955, Burwell 1969, Melcher & Accursi 1971, Alhopuro 1978). In addition, the method of detaching the periosteum from the underlying bone may also be of importance. For instance, in an adult dog the stripping of periosteum from a long bone leaves the cambium layer attached to the shaft (Phemister 1914). All these factors contribute to the controversial views concerning the osteogenic capacity of the periosteum and especially of free periosteal grafts.

The constantly postitive results achieved in our laboratory in investigations using young rabbits (Ritsilä et al. 1972a, Ritsilä & Alhopuro 1972a, b, 1973a, b, 1975, Alhopuro et al. 1973a, Alhopuro 1978) have led to some clinical applications, mainly spinal fusions of idiopathic scoliosis (Snellman et al. 1977). In these operations some technical difficulties were encountered. A free periosteal graft stripped from the tibia of the patient has a tendency to shrink to about one third of its original length. In addition it also has a tendency to curl. These difficulties prompted us to experiment with

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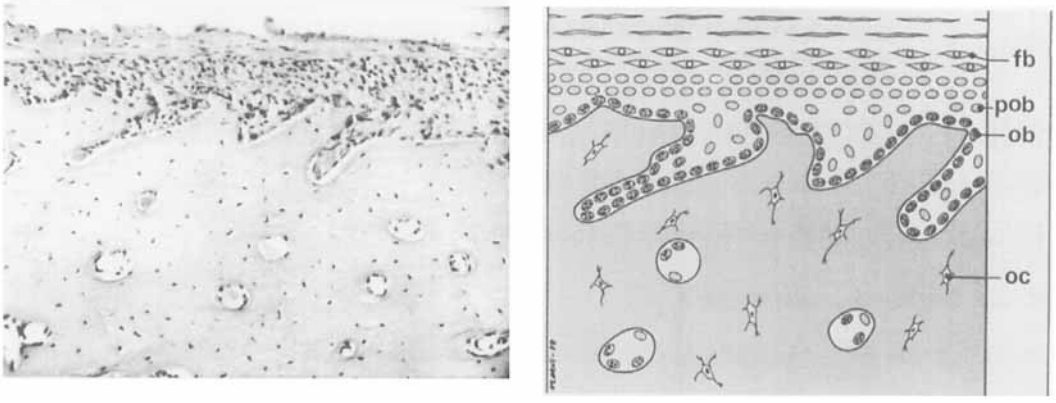


Figure 1. Photomicrograph from the surface of the proximal part of the tibia of a 6-week-old rabbit (H-E, × 250). On the right a schematic presentation (fb, fibroblast; pob, preosteoblast; ob, osteoblast; oc, osteocyte).

grafts comprising both the periosteum and a thin shaving of underlying bone, as such grafts would be easier to handle. Nevertheless, taking into consideration the weak osteogenic capacity of cortical bone, the effect of the underlying bone on the osteogenic capacity of the periosteum had to be investigated.

Microscopy of the surface of the cortical bone in 6-week-old rabbits (Figure 1) revealed a folded boundary between the cortex and the periosteum. The periosteum sends osteogenically active buds of cambium layer into the cortex. When the periosteum is stripped off, it is possible that part of the cambium layer, especially the buds, remain on the bone surface. This can be avoided by taking part of the underlying cortex with the graft. To test the hypothesis a comparative study was made in which the osteogenic capacity of free stripped periosteum was compared with periosteum attached to 200 micron thick cortical bone.

MATERIAL AND METHODS

Forty 6-week-old rabbits of both sexes were used in this series, 20 of them belonging to the pilot series. The animals were housed in wire mesh cages and received food pellets (Hankkija, Finland) and water *ad libitum*. The operations were performed under anaesthesia with Hypnorm® (Philips, Duphar) 0.5 ml/kg i.m. and local infiltration with 0.5 per cent Lidocain® without Exadrine. The periosteal and the 200 micron thick osteoperiosteal grafts were taken from the proximal third of the medial facet of the tibia (Figure 2). The area was first delineated with a scalpel and the periosteal graft was taken by stripping. The osteoperiosteal graft was detached as a whole with a circular saw. The cortex was made thinner by reaming the excess bone from the medullary side of the graft. When reaming, a very slow velocity was used and the graft was kept moist and prevented from overheating by using Ringer's solution during the procedure. The thickness (200 micron) was checked with an operating microscope. In some grafts the extra bone was removed with a chisel. No differences were noted in the behaviour of the grafts after these two methods of thinning. Thereafter, these two grafts were fixed to a lumbar vertebra (Figure 3). Through a midline incision the spinous and mamillary processes were exposed. Sutures were sewn with 6–0 nylon.

The rabbits were killed 3, 7, 11, 14, 21, 28, 42 and 84 days postoperatively. The whole lumbar vertebra was separated with its surrounding muscles and fixed in 10 per cent phosphate buffered neutral formaldehyde for 1 to 2 weeks, decalcified in formic acid, dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin. Transverse sections of 5–7 micron thickness were cut and stained with haematoxylin eosin and van Gieson.

RESULTS

The results are expressed as histological findings at various periods after the grafting

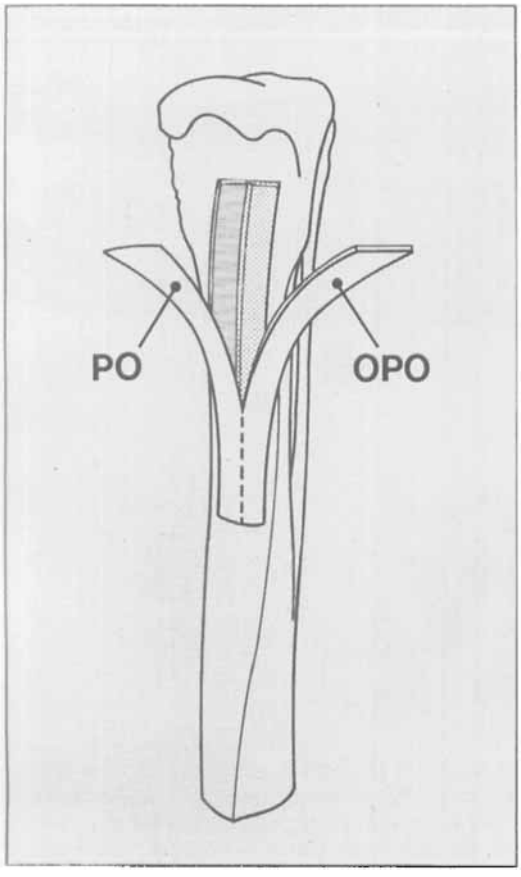


Figure 2. Schematic presentation of the way of detaching the grafts from the proximal part of the tibia (PO, periosteum; OPO, osteoperiosteum).

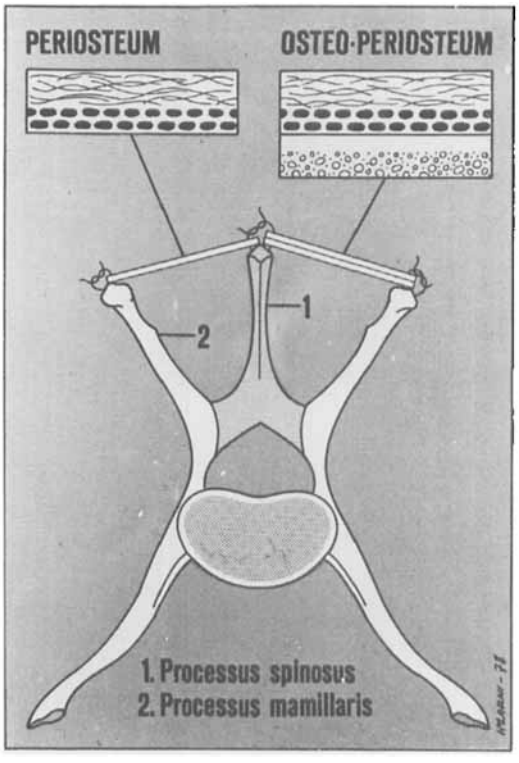


Figure 3. Schematic presentation of the method of fixing the grafts between the spinous and mamillary processes of the lumbar vertebra.

operation. Comparison is thus made between the two grafts in the same exposition.

Observation period, 3 days

On the side of the free stripped periosteum there was marked proliferation of the cells of the cambium layer (Figure 4). Mitoses were also seen in the cambium layer. The fibrotic layer, on the other hand, seemed very inactive.

In the 200 micron thick osteoperiosteum the two layers of the periosteum were clearly seen as well as the buds from the cambium layer into the cortical bone. No active proliferation of the cambium was noted. In the part of the cortical bone facing the muscle there was a thin layer of bone with empty lacunae. On the other hand, beneath the periosteum the osteocytes seemed intact and the nuclei were preserved.

Observation period, 7 days

In most preparations the free stripped periosteum had formed an ossicle through the activity of the cambium cells. It looked as if the cambium cells, through proliferation, first produced cartilage which then ossified and formed woven bone (Figure 5). In the last stage the cartilage cells seemed to hypertrophy and become replaced by woven bone. The ossicle was frequently situated in the middle part of the periosteal graft.

On the side of the 200 micron thick osteoperiosteum no marked activity was seen. Obviously the cortical part of the graft was dying, with an inflammatory reaction in the

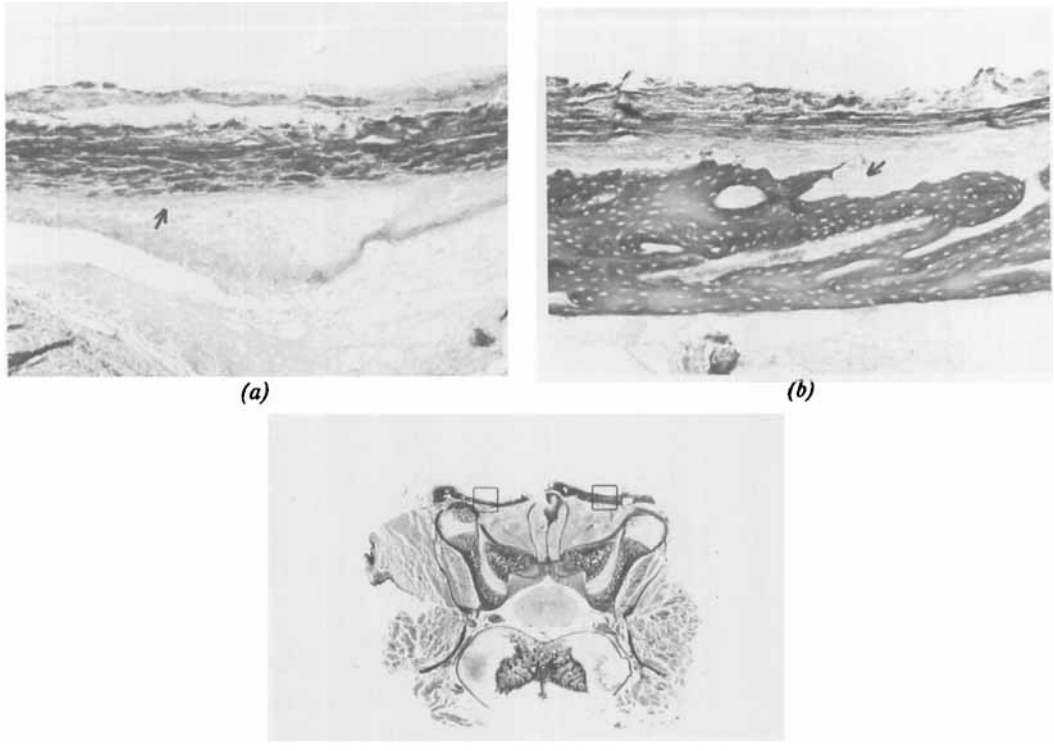


Figure 4. Low power photomicrograph (× 5) from the grafts in the lumber vertebra 3 days postoperatively. The cambium layer of the periosteum has proliferated. This is clearly seen in the higher magnification (× 125) of the area marked on the left (a). In the osteoperiosteum (× 125) (marked area on the right) the folded boundary between the periosteum and the underlying cortex is seen (b) (van Gieson).

muscle beneath the graft. A gap between the muscle and the cortex was frequently seen.

Observation period, 14 days

The free stripped periosteum had formed a uniform bone-cartilage bridge between the spinous and the mamillary processes (Figure 6). The bone here resembled young woven bone, abundant osteoblasts lining its trabeculae. A new thick proliferating periosteum surrounded this newly formed bone-cartilage bridge.

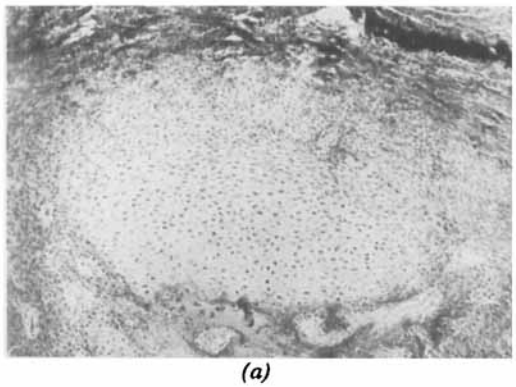
On the side of the 200 micron thick osteoperiosteum the cambium-layer cells formed cartilage in the space between the periosteum and the cortex. In some preparations there were signs of incipient bone formation in the cartilage. The cortical bone was obviously dead and there was a gap between the muscle and the cortex.

Observation period, 21 days

A uniform bone bridge had been formed by the periosteum (Figure 7). Also the osteoperiosteum had formed bone, but there still existed some cartilage between the cortex and the muscle. The cortical bone in the osteoperiosteum was being resorbed. The histological structure of the bone was trabecular, abundant osteoblasts lining its trabeculae. The cartilage here was so-called "secondary cartilage", i.e. it was being replaced by woven bone as maturation progressed.

Observation period, 28 days

A bone bridge was seen on both sides (Figure 8). On the periosteal side there was haematopoietic marrow in the middle of the bridge. On the osteoperiosteal side the grafted



(a)

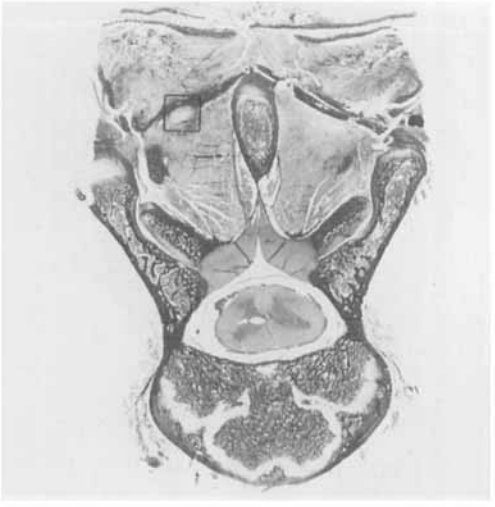


Figure 5. Low power photomicrograph (× 5) from the grafts 7 days postoperatively. The free periosteum (left side) has formed an ossicle, which at a higher magnification (× 250) (a) is seen to consist of cartilage and woven bone. No activity in the osteoperiosteum (van Gieson).

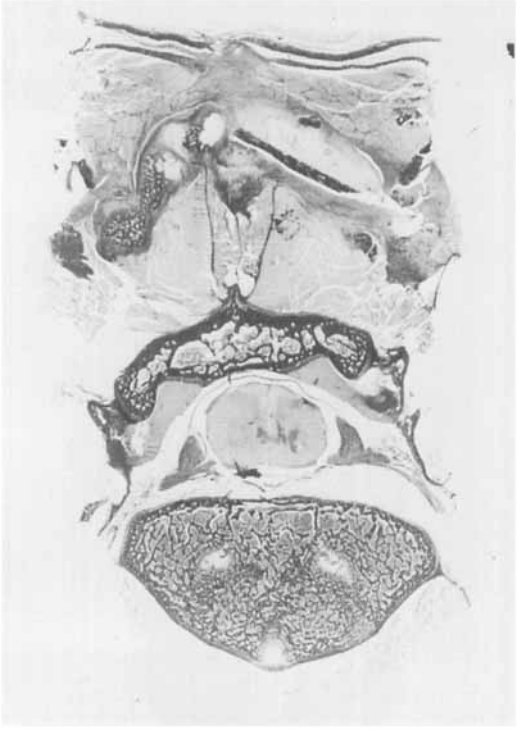


Figure 6. Low power photomicrograph (× 6) from the grafts 14 days postoperatively. The periosteum has formed a uniform bone-cartilage bridge. In the osteoperiosteum cartilage has been formed between the cortex and periosteum (van Gieson).

cortex was still clearly seen and surrounded by proliferating new bone.

Observation period, 42 days

A bone bridge with visible bone marrows had been formed on both sides (Figure 9). No difference could be observed in the stage of osteogenesis. The bone tissue formed began to resemble the structure of the neighbouring bones, i.e. that of the processus spinosus and mamillaris.

Observation period, 84 days

Remodelling of the previously formed bone was the main feature that separated this stage from the former one (Figure 10). The bone formed was structurally compact and resembled that of the adjacent processes.

DISCUSSION

The known prerequisites for bone formation are proper osteogenic cells, proper environment, and proper stimulus. Yet, the basic

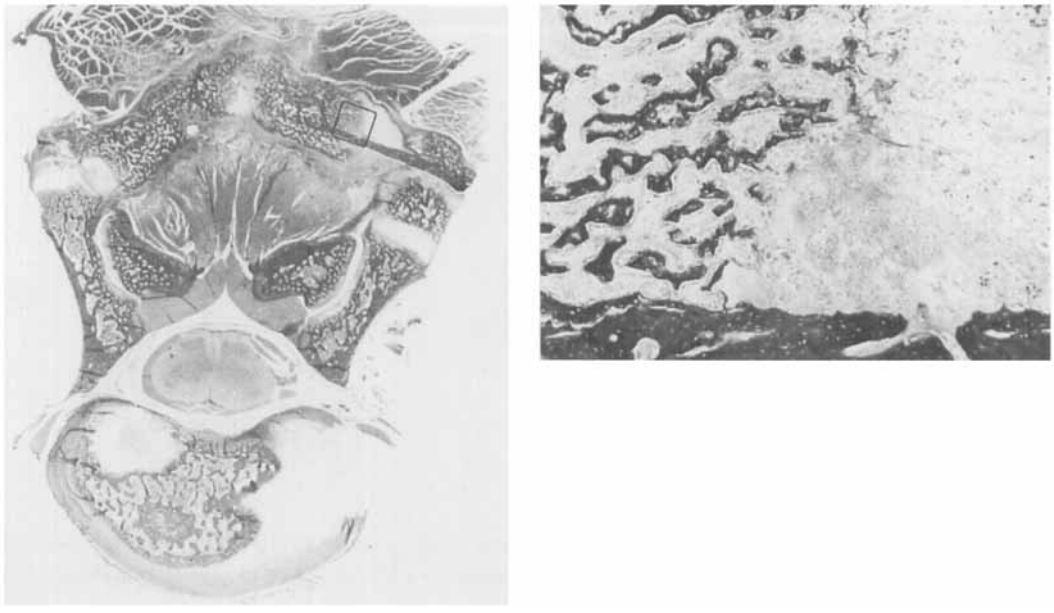


Figure 7. Low power photomicrograph (× 6) from the grafts 21 days after the grafting procedure. The periosteum on the left has formed a bone bridge between the spinous and mamillary processes. On the right in the osteoperiosteum the secondary cartilage is being replaced by trabecular bone. The hypertrophic cartilage cells are clearly seen in the higher magnification on the right (× 125, van Gieson).

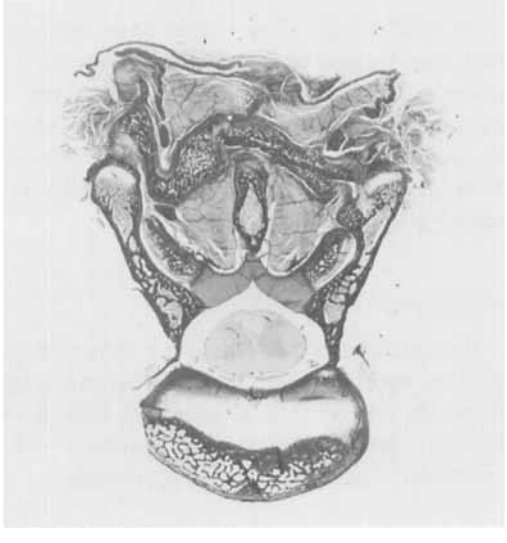


Figure 8. Low power photomicrograph (× 5) from the grafts 28 days postoperatively. On the left of the figure, bone formed by the periosteal graft, haematopoetic marrow in the middle. On the right, the cortex still visible, surrounded by new bone (van Gieson).

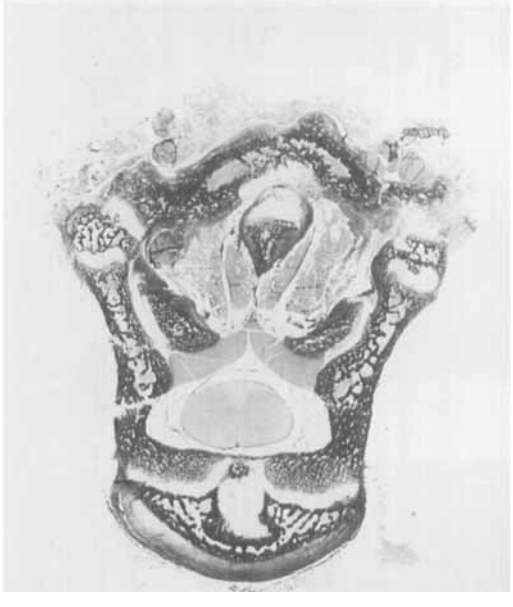


Figure 9. Symmetrical bone bridges 42 days post-operatively. Medullary cavity visible on both sides (van Gieson, × 5).

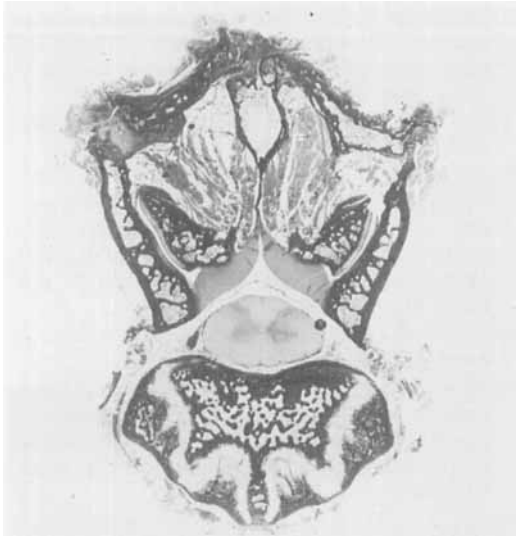


Figure 10. Remodelling of the bone bridges has occurred 84 days postoperatively (van Gieson, × 5).

mechanisms of osteogenesis are obscure (Basset 1962). It is now widely accepted that in bone transplantation part of the cells of the graft retain their vitality and after the transplantation begin to proliferate and form new bone (Heslop et al. 1960, Elves & Pratt 1975, Tervo 1975). In the inner layer of the periosteum of young animals there are a lot of osteogenically active cells. The present studies were performed with growing rabbits. On the other hand, in earlier studies free periosteal grafts from old rabbits also formed bone (Ritsilä et al. 1972a). Earlier studies have pointed out that with free periosteal grafts the bone formation mechanism is direct proliferation of the cambium cells (Tonna & Cronkite 1962, 1963, Ritsilä et al. 1972a, b, Ritsilä & Alhopuro 1973a, b). This quite a rapid process, as it was also in this investigation. Cortical bone, on the other hand, is known to be osteogenically weak (Gallie 1931, Burwell 1969), and the mechanism of osteogenesis is different. It occurs mainly through resorption of the graft and the new bone is formed through a mechanism of "creeping substitution" and remodelling.

There are some observations in the literature concerning the size of the graft. Andersson (1961) studied the size of cortical chips. Smaller, 0.3–7 mm, chips underwent complete necrosis and in addition incited a strong inflammatory reaction. So-called cortical shavings without periosteum are said to be of no value as grafting material (Keith 1934, Siffert 1955). A too massive bone graft, on the other hand, becomes necrotic because of difficulties in revascularization (Zeiss et al. 1960). The ideal size of a bone graft is one of the many unsolved problems of the grafting procedure (Burwell 1969).

The aim of this investigation was to study the effect of the cortical bone in the osteoperiosteal graft. In theory, several factors could have an influence. Firstly, removal of a piece of underlying bone with the periosteum means that all the osteogenic cells and thus the maximal amount of osteogenic material is included in the graft. Secondly, death of the cortical bone could stimulate the periosteum and in this way enhance osteogenesis in the graft (Richany et al. 1965, Gage et al. 1966). Thirdly, as resorption of the cortical bone takes place first a delay in bone formation is to be expected.

The results of this study showed that periosteum alone had a more potent bone forming capacity than the 200 micron thick osteoperiosteum. In the osteoperiosteal grafts the resorption of the cortical bone perhaps took place first and osteogenesis could begin first thereafter. Also the development of circulation which is known to occur simultaneously with resorption can be delayed in the osteoperiosteum when compared with free periosteum.

The process of bone formation seemed consistently to go through a cartilage phase. These cartilage cells showed great similarity to the cartilage cells seen in the zone of proliferation in the growth columns of epiphyseal lines. Although the arrangement was not as orderly there was often a suggestion of palissading of cartilage cells as they underwent enlargement and swelling. This cartilage can be called secondary cartilage because through the ingrowth of vessels and osteoblasts it was replaced by bony

structures. Here the periosteum was taken from the tibia, a bone of endochondral origin, but the same phenomenon has also been observed with periosteum from the calvarium and the scapula (Alhopuro 1978). The resulting bone resembled that of the spinous and mamillary processes and not that of the tibia. This effect of the surrounding tissues was also observed in earlier studies (Ritsilä & Alhopuro 1973a, b, Alhopuro 1978).

Further studies are envisaged to examine the effect of the thickness of the cortical bone, especially the effect of thinner grafts, as then the resorption process could be shortened or could perhaps be totally avoided.

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