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Effects on bone of vascular interruption

Turnover and morphology in isotope-prelabelled rats*

The effects of bone devascularization were evaluated histologically and metabolically in rats prelabelled with ^{45}Ca, ^{3}H-tetracycline and ^{3}H-proline by quantifying cortical bone resorption and formation. The interruption of blood supply to bone without invading its integrity resulted in a marked increase in bone turnover (resorption and formation) during the first and second months. The stimulated increase in bone resorption and formation did not affect the resultant mass of collagen and calcium. Thus, the increase in bone resorption was compensated by an equivalent increase in bone formation.

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Previous experimental studies (Ficat & Arlet 1980) evaluating the role of vascularity to bone utilized models which interrupted the major nutrient blood supply to segments of bone. These studies demonstrated pathological events which cause widespread bony necrosis and ultimate slow revascularization and creeping substitution. There have been a few studies recently (Ficat & Arlet 1980, Whiteside et al. 1978, Silberman et al. 1967) describing the histological and vascular response of bones to the interruption of the periosteal blood supply to a bony segment.

In order to evaluate the surgical interruption of the periosteum blood supply, the soft tissue cuff was subperiosteally dissected from the knee region of mature rats. This model did not interrupt the major endosteal blood supply to the bony segment. The prelabelling method (Klein & Jackman 1976) was employed to study the effects of devascularity on cortical bone resorption and formation in mature rats.

Materials and methods

Fourteen weanling male Lewis rats obtained from the vendor were utilized in this study. They were maintained in caged environments and fed a standard diet.

Eight male Lewis rats were labelled twice weekly for 6 weeks with increasing doses of ^{45}calcium, tritiated tetracycline and tritiated proline (Klein 1980) from 4 to 10 weeks of age. Total isotope per rat was 3 μCi (1 Ci = 37 GBq) of ^{45}calcium, 50 μCi of ^{3}H-tetracycline, and 120 μCi of ^{3}H-proline. Four weeks after the end of labelling, the periosteal blood supply to the left hind limb was interrupted for a 3 cm osteochondral segment, which included the knee joint. The subperiosteal dissection of the soft tissue cuff included the entire extensor mechanism and the hamstring, gastrocnemius and anterior tibial muscles, from 1.8 cm proximal to the knee joint to 1.2 cm distal to the joint (Figure 1). Thus, the periosteal blood supply to this osteochondral segment was compromised. The soft tissue cuff was repaired in standard fashion, and the subcutaneous layer and skin were closed. The rats were allowed to ambulate freely in their cages.

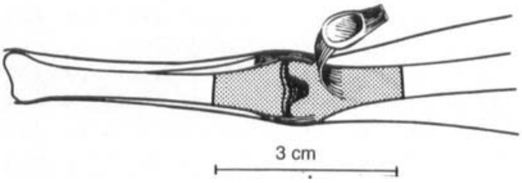

Figure 1. Diagrammatic scheme of the operation for vascular interruption by periosteal stripping.

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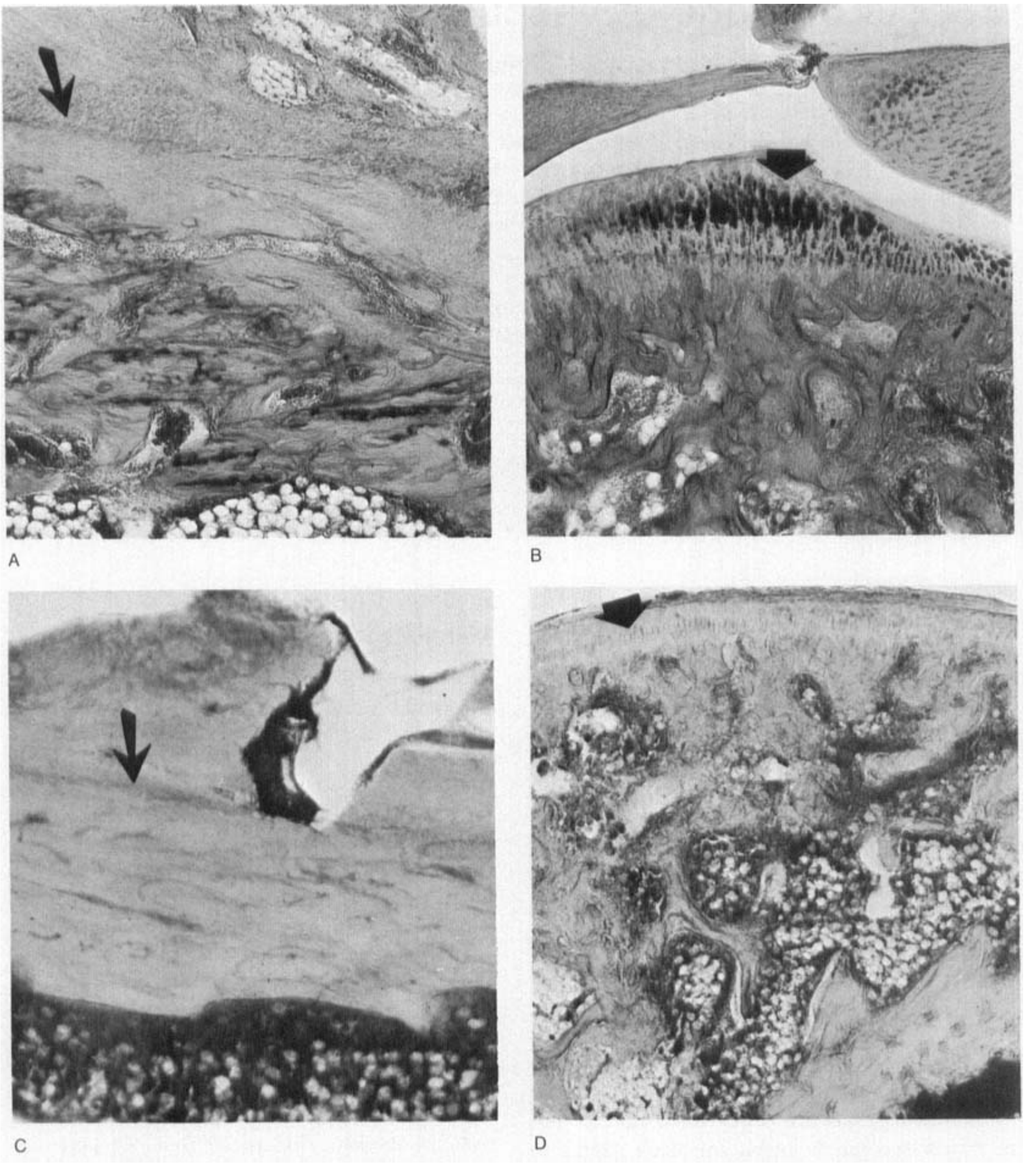

Figure 2. Sagittal section of a rat knee at 1 month following vascular interruption: (A) Periosteal proliferation is seen, and (B) the articular cartilage appeared viable (x 40). Sagittal section of a rat knee at 2 months following vascular interruption: (C) New bone formation is seen in the periosteum, and (D) the articular cartilage remained intact and viable (x 40). The original location of the periosteum and the vital cartilage are indicated by an arrow in each figure.

Six 14-week-old rats were operated on as described above and used for histological studies.

At each of 1 and 2 months after the operation, four rats were killed for biochemical and isotope studies, while three rats were killed for histological evaluation. A 3-cm osteochondral segment, as described, was removed as an intact structure from the right control and left experimental hind limbs.

The following measurements were made on each rat for biochemical and isotopic analysis (Klein 1980, Klein et al. 1983): dry defatted weight, calcium and collagen mass per bone, and total radioactivity of ^{45}calcium, tritiated tetracycline and tritiated collagen per whole specimen of bone (femur, tibia). The experimental data are presented as a percentage of the control bone (Klein et al. 1983). All results were analyzed for statistical significance by Student's *t*-test.

The osteochondral segment utilized for histological evaluation was embedded (Heiple et al. 1963) using standard decalcification methods. Sagittal celloidin sections at 25 μm were taken and stained with hematoxylin and eosin for histological evaluation. A qualitative evaluation of the histologic characteristics of the articular cartilage, growth plate, metaphyseal and diaphyseal bone, as well as the marrow, was performed.

Results

Histological results

At 1 month after the operation, there was marked thickening of the periosteal envelope about the entire segment (Figure 2A) and fibrosis of the muscular cuff surrounding the knee joint. The joint itself was abnormally stiff and more difficult to mobilize. Similar findings were present at 2 months, but there appeared to be increased thickening of the periosteal envelope.

Subperiosteal necrosis was noted throughout the diaphyseal portion of the segment; however, there was also a widespread thickening of the bony envelope. The periosteal cellular activity was marked and there was definite widespread evidence of repair and bony accretion. There was little attempt at reorganization of the new woven bone at 1 month. The endosteal bony surface was quiescent, but appeared viable. There appeared to be more metaphyseal resorption with visible osteoclastic activity than was seen in the diaphyseal bony segment.

The growth plate appeared viable, but somewhat disorganized. At 1 month, the articular cartilage appeared viable with the matrix staining in an appropriate fashion (Figure 2B). There appeared to be little attempt at any cellular proliferation or hypertrophy. There was, however, a definite increase in articular fibrosis about the meniscus. Areas of subchondral necrosis of cancellous bone were visible, more evident in the lamellar subperiosteal area. The bone marrow was viable, with active areas of subchondral irregularity, apparently both osteoblastic and osteoclastic in character.

At 2 months, the findings were similar with regard to the bony segment (Figure 2C). An increase of periosteal new bone was seen throughout the diaphysis with marked thickening of the shaft. There did not appear to be any increase in necrosis. Rather, there appeared to be increasing appositional new bone subperiosteally throughout the osteochondral segment. These results at 2 months were qualitatively similar to the earlier time period, but appeared to be more advanced as far as organization of the newly woven bone toward a more mature pattern of lamellar bone. The articular cartilage and growth plate remained intact, but there appeared to be less cellular viability than seen at 1 month (Figure 2D). There was also some disorganization of each component. The marrow remained active and appeared to be viable.

Table 1. Compositional and isotopic changes of eight devascularized rat knee joints

Components	Control Left/Right	Experimental Femur	 Tibia
^{45}Calcium	99(1)	80(4)	75(4)
^{3}H-Tetracycline	101(2)	58(6)	53(5)
^{3}H-Collagen	99(2)	66(5)	59(6)
Dry weight	99(3)	109(3)	102(3)
Collagen mass	98(2)	115(7)	105(6)
Calcium	99(1)	103(2)	97(4)

Values given are mean [Left/Right] × 100 (SE) and mean [expt/control] × 100 (SE).

Chemical results

Biochemically, comparison of the devascularized hind limb with the control contralateral limb demonstrated a marked loss of all three isotopes from the devascularized bone (Table 1). A comparison of the chemical and isotope measurements showed an equivalence between left and right bones from control rats (Table 1).

Since there was no statistical difference between the results of the two groups, the data for the 1- and 2-months groups were pooled. The operation produced a marked increase in bone resorption, as seen by a similar loss of tritiated tetracycline (53–58 per cent of control) and tritiated collagen (59–66 percent of control) from either the femur or the tibia. No net loss occurred in dry weight, collagen mass, or calcium mass, indicating that the resorbed bone (both collagen and calcium) was replaced with an equivalent amount of new non-radioactive bone. The difference between the larger loss of tritiated tetracycline (53–58 per cent of control) and a smaller loss of ^{45}calcium (75–80 per cent of control) demonstrates that approximately one-half of the resorbed calcium was conserved (Hevesy 1955, Klein et al. 1983) during bone formation by reutilization of the resorbed ^{45}calcium.

Discussion

In order to evaluate the role of revascularization in bone graft incorporation, it is important to distinguish between the initial effects of trauma on bone resorption at any graft site and the ultimate biological healing of the graft. Our study was an attempt to define the role of trauma and devascularization on a defined osteochondral segment in prelabelled rats. The results suggest that the compromise of the periosteal blood supply to bone without invading its overall structural integrity has a major effect on bone turnover during the first month. The devascularized bone was not completely resorbed over a 2-month period. The large increase in bone resorption and formation did not significantly affect the mass of collagen and calcium. This would indicate that the increase in bone resorption was compensated by an equivalent increase in bone formation. These results are in contrast to the significant loss of bone mass which was observed following immobilization; an increase in bone resorption occurred in rats and dogs (Klein et al. 1982, 1983), with only a partial compensatory increase in bone formation in rats and little, if any, increase in bone formation in dogs. In comparison to the maintenance of bone mass in devascularized bones, bone grafts at orthotopic sites definitely lose mass (Dollinger et al. 1984), and at heterotopic sites they show very large losses of mass (Burwell 1969). It is apparent that the ability to maintain bone mass varies greatly according to the experimental conditions.

The histological observations confirmed the marked new bone formation (Foster et al. 1951, Beneke & Deutschle 1968), which was evident mostly in the periosteal surface. The remaining cortical compartment appeared to be viable, with little change. This is consistent with previous histologic evidence, which suggests that only the outer one-third of the diaphysis is supplied by periosteal vessels, while the inner two-thirds are supplied by the major nutrient artery via the medullary vessels (Trueta & Cavadias 1963). It is well known that cartilage derives its nutrition from extravascular sources, and no loss of viability was seen. The increased fibrosis and scarring might well have been a result of the trauma and inactivity induced by the operation. Previous studies (Evans et al. 1960, Thaxter et al. 1965) have shown that reduced activity of diarthrodial joints induces a fibrotic response. However, the overall function of the median hind limb of the rat was maintained throughout the period of observation.

Delayed revascularization and the concomitant impaired anabolism were major factors which influenced the poorer outcome of bone allografts when compared with autografts (Hammack & Enneking 1960, Goldberg & Lance 1972, Goldberg et al. 1980). In addition, when the immune response was suppressed, revascularization was rapid and mineralization proceeded quickly (Goldberg & Lance 1972). It would appear that vascularity and bone formation proceed hand in hand, and it is important to distinguish between the effect of trauma on the vascularity as compared with inherent biological effects.

Our histologic and isotopic data indicate that periosteal stripping results in a major stimulation of bone resorption and formation in the subperiosteal region and the outer one-half of the cortex without a loss of bone mass. The maintenance of bone mass confirmed that disruption of periosteum does not prevent healing of bony segments or grafts.

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