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HEALING IN DENERVATED BONES

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In children suffering from myelomeningocele who needed orthopaedic or surgical correction of the lower extremities we found an increased number of fractures of the femur and tibia and an anomalous type of repair (fast and with abundant callus production on many occasions) (Navarro, in preparation).

These fractures are found not only in children with myelomeningocele, but also in people with paraplegia due to other causes, e.g. subdural haematoma (Caffey 1946, Alliaume 1950), spinal fractures associated with cord lesion (Katz 1953, Robin 1965), lumbosacral root avulsion, transverse myelitis (Jeannopoulos 1954) and cord tumours (Robin 1965).

We could not obtain clear conclusions due to the influence of many factors: the delay in recognizing the fractures (due to lack of sensation), the different levels of the fractures, and the variable ages of the patients. However, it was evident that the atrophic bone with a supposed low "metabolic flux" or turnover repaired well and quickly.

In an attempt to clarify these factors we made an experimental model choosing the rat because of the facility of obtaining animals of the same sex, age, weight and geniticity, and because it had been already used to study the repair of fractures in denervated limbs (Smith & Dunsford 1955, Hulth & Olerud 1965) and also because it had been shown that in bone atrophy following denervation, the turnover was variable with different periods of immobilization (Landry & Fleisch 1964). We studied histologically the fracture and bone defects produced for the same periods of denervation as those used by Landry & Fleisch (1964).

MATERIAL AND METHODS

Animals

The animals used were young male Wistar rats, weighing between 80 and 110 g. Eighty rats, divided into two main groups, were used.

I) Complete fractures (64 rats).
   a) Femoral series.
      Fracture of the denervated femur (16 rats).
      Fracture of the normal femur (16 rats).
   b) Metatarsal series.
      Fracture of the third denervated metatarsal (16 rats).
      Fracture of the third normal metatarsal (16 rats).

II) Bone defects (16 rats).

The bone defects were created in both femora and tibiae of the same animal.

Technique

All the animals had a denervation of the right hind leg. Under ether anaesthesia and through a latero-posterior approach the primary rami L-3, L-4, L-5, L-6 and S-1 were found between the insertion fibres of the psoas muscle. Five millimeters of each primary ramus were resected.

The fractures were all produced through a small incision, cutting the bone through with small scissors, at the level of the junction of lower and middle third of the femur and the proximal third of the third metatarsal; the bone defects were made with small bone biting forceps, in the femoral metaphysis and the tibial shaft; in every case the bone marrow was able to be seen through the defect.

Timing of the fracture and bone defects. These were produced immediately, two, four and seven weeks after the denervation.

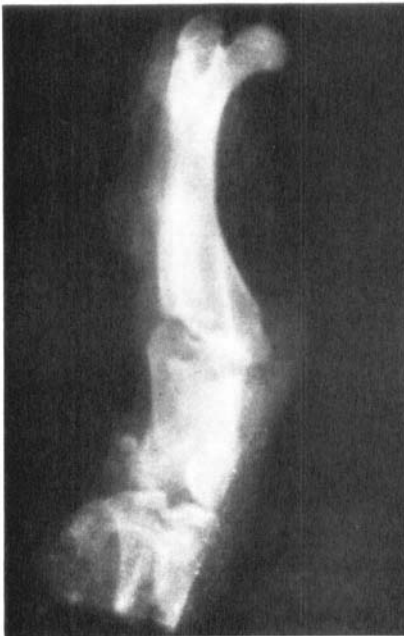

Figure 1. X-ray appearance (at seven weeks) of a femoral fracture produced four weeks after denervation.

Study of the repair

The repair was studied at one, two, four and seven weeks after the fracture, both radiographically and histologically (Figure 1).

The first fracture series served for the study of the fracture repair in bone without immobilization. The second fracture series served for the study of the fracture repair in conditions of immobilization (splintage of the second and fourth metatarsal bones).

The bone defects were made in both legs of the same animal, because the animal could manage well, in spite of the defects.

Histological technique. Ten per cent formol solution was used as a fixative. Decalcification with EDTA. Wax-paraffin inclusion. Six micron thick slices and haematoxylin-eosin (H-E) staining.

Prior to the fixation the specimens were radiographically studied.

Difficulties

Many more rats than the ones we used were operated on. A few were not studied due to operative infection, but most rats were not used because of trophic ulcers in the feet due to lack of sensation, and in many cases this was followed by autophagic phenomena. This forced us to change the animal cages for ones in which the walls and floors were completely smooth.

RESULTS

Femoral Fractures

First group: Fractures produced at the same time as the denervation.

Week 1: The marrow reaction is the same in both femora. There is no subperiosteal reaction with bone or cartilage formation. The newly-formed tissue is less differentiated on the denervated side.

Week 2: The periosteal reaction is quite similar on both sides, but on the normal side the cartilage formation is more noticeable. Much new vessel formation is observed in the callus.

Week 4: On both sides the fracture is united by bone at the periphery of the callus. Again the central part of the callus shows a very rich blood supply on the denervated side and this is not present on the normal side.

Week 7: Advanced union* is observed from the periosteal bone. Ossification is equal on both sides. Bone response is equal on both sides.

Summary: The bone response is much slower on the denervated side up to the second week, but from here onwards it increases and from the fourth week both processes look quite similar. The atrophic

* By bony union we mean lack of mobility of the fragments and a histological presence of a bridge of bone between the fragments.

side has *less* cartilage formation and a *much richer* blood supply to the callus.

Second group: Fracture two weeks after denervation.

Week 1: Marked bone formation in the marrow cavity as on the normal side and similar to the first group. Good periosteal bone formation, greater than on the normal side and in the first group. The bone formation is more abundant in the proximal fragment.

Between the fragments a large fibro-cartilaginous metaplasia is observed.

Week 2: The repair is much more advanced on the atrophic side. The periosteal bone formation is abundant with bony bridges and areas with ossification fronts and osseous-cartilaginous metaplasia. The osteoclasts are more abundant on the atrophic side.

Week 4: Bony union is seen. The periosteal bone is completely remodelled with a new cortex formation.

Week 7: Complete bony union is observed on the atrophic side but not on the normal side.

Summary: The repair process is much more advanced in the denervated femur.

Third group: Fracture four weeks after denervation.

Week 1: A large amount of bone formation on the atrophic side but not so abundant as in the second group.

Week 2: Similar to the normal side, but with less cartilage formation, and a greater number of osteoclasts.

Week 4: Complete bony union from the new periosteal bone. More advanced process than on the normal side. Excellent turnover judging from the richness in osteoclasts and osteoblasts (Figures 2 and 3).

Week 7: More solid union than on the normal side, but presenting slight mobility (manual).

Summary: The bone formation and the remodelling are very good and greater than on the normal side.

Fourth group: Fracture seven weeks after denervation.

Week 1: There is no bone formation. The fracture gap is entirely cartilaginous.

Week 2: Lamellar bone is already formed and a very marked number of osteoclasts. All this indicates a very good remodelling process, more active than on the normal side.

Week 4: Cartilaginous union with ossification fronts on both sides.

Week 7: Cartilaginous union with some areas of non-union on the normal side.

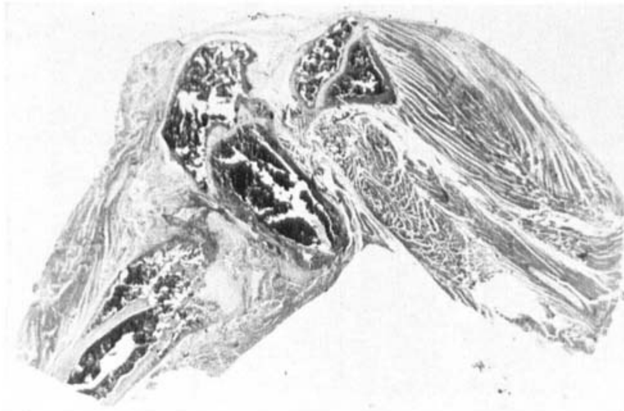

Figure 2.

Figure 3.

Figures 2 and 3. Magnified views of a slide showing the normal and denervated sides of a femoral fracture at four weeks.

Summary: On the atrophic side there are some bony bridges mixed up with the cartilaginous callus.

The process is quite similar to the normal side up to the second week, but after the fourth week on the atrophic side the union is complete.

Summary of the Femoral Series

None of the groups had less reaction on the atrophic side, but in the first group there was a lazy reaction during the first week.

In all the groups there was a smaller amount of cartilage formation on the atrophic side. The remodelling was good in every group, with a good lamellar bone formation.

The healing was faster and stronger on the atrophic side of the second, third and fourth groups.

Fracture of the Third Metatarsal Bone

First group: Fracture produced at the same time as the denervation.

Week 1: There are no morphological differences between the two sides.

Week 2: Periosteal bone formation. Cartilage formation changing to bone between the two fragments. The process of repair is more advanced in the denervated side.

Week 4: The fracture of the denervated limb is completely united. There is lamellar bone formation; the bone of periostic formation has been completely remodelled.

On the normal side the fracture is not yet united and there is a good deal of cartilage formation.

Week 7: Both fractures are united.

Summary: There is a greater bone reaction and a faster healing process on the atrophic side than on the normal one.

Second group: Fracture two weeks after denervation.

Week 1: There is no difference in the two sides. A large amount of periosteal reaction.

Week 2: Quite similar processes in both normal and denervated sides.

Week 4: Process is much more advanced on the atrophic side than on the normal one (Figure 4).

Week 7: Bony end separated by a cartilage bridge which is very thin on the denervated side and much thicker on the normal side.

Summary: The repair process is more advanced on the atrophic side, but bony union is not seen on either side.

Third group: Fracture four weeks after denervation.

Week 1: Bony metaplasia from granulation tissue in the fracture gap is observed on the atrophic side. This is not seen in any other group.

Week 2: Periosteal bone formation more abundant than in the former groups.

Week 4: Quite similar to the second group. The periostic reaction has subsided and there is a central bony bridge.

Figure 4. Shows the thin gap filled with cartilage and the new bone formation in the medullary canal (× 78.75).

Figure 5. Shows a bony bridge and a central gap filled with cartilage (× 31.25).

The difference on the normal side is that the periostic bone still persists showing that the union is perhaps not so advanced.

Week 7: Peripheral bony bridges are seen on the atrophic side, showing an advanced healing process (Figure 5).

Summary: Greater reaction during the first two weeks on the atrophic side. More rapid union on the atrophic side.

Fourth group: Fracture produced seven weeks after denervation.

Week 1: Periostic reaction with bone formation in both cortices, on the atrophic side. This was not observed in any other group.

Week 2: The callus formation is quite similar to that observed at four weeks in the second and third groups on the atrophic side.

Much more advanced process than on the healthy side.

Week 4: Not much difference from the former stage.

Week 7: There is no bony union yet, but there is a cartilaginous bridge. Lamellar bone formation.

Summary: A faster healing process than in other groups, but without union. Bigger fracture gap?

Summary of the Metatarsal Series

In groups one and two, the reaction was equal on both sides, during the first week. After that, the atrophic side reacted with a quicker union that ended with a bony union in the first group and a thin cartilaginous bridge in the second group.

In groups three and four, the response was stronger on the atrophic side during the first two weeks: in the following weeks the process of union was more rapid on the atrophic side, but not so complete as in the first group. Is this because of a greater fracture gap or a greater comminution of the fracture? The remodelling was good with an intense osteoclasia.

The greatest response was between four and nine weeks after denervation.

The amount of cartilage was quite similar on both sides and much less than in the femoral series.

Study of Bone Defects in Cortico-Cancellous Bone (Femur)

First group: Bone defect produced immediately after denervation.

The healing process is less marked up to the second week on the atrophic side, equalizing later on with the normal side. There is no cartilage formation.

Second group: Bone defect produced two weeks after denervation.

On the atrophic side a more advanced healing process is observed with a difference of at least one week, but there is less bone formation.

There is no cartilage formation unlike the normal side, and on both sides there is a marked periosteal bone-forming reaction of similar intensity.

Third group: Bone defect produced four weeks after denervation.

The bone formation is more rapid on the atrophic side up to the second week, but from then to the seventh week the reactions tend to equalize.

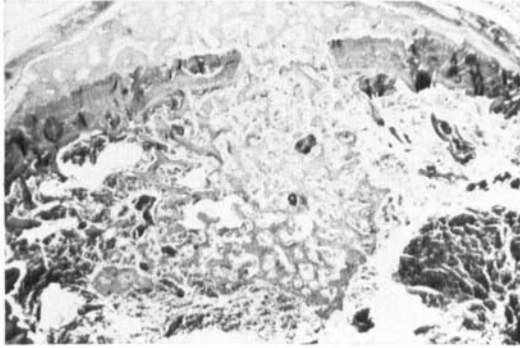

Figure 6. Seven weeks after denervation. Atrophic femur. Callus at two weeks. Observe the new bone in the medullary cavity communicating through the gap with the outer periostic bone. No cartilage (× 20).

On the normal side a marked periosteal reaction and cartilage formation is observed, but not on the atrophic side.

Fourth group: Bone defect produced seven weeks after denervation. The reaction is quite similar up to the second week but between the second and the seventh week the bone defect is completely repaired on the atrophic side, while it is still remodelling on the normal side (Figure 6).

The quantity of bone and cartilage formation is similar on both sides.

Overall Assessment

Cartilage: On the atrophic side cartilage is seen only in the fourth group. On the normal side there is minimal cartilage formation, except in the third group where it is abundant.

New bone formation: Very good formation in the second and fourth groups, but much less in the third group, taking the normal side as control.

Quickness of repair: At all stages the atrophic bone shows repair equal or superior to that of the normal bone. The periods in which the repair is superior vary according to the time of atrophy. In the second group the repair is quicker in every instance with a more advanced remodelling. In the third group it is greater up to the second week, equalizing later. In the fourth group it is equal up to the second week being greater later on (Figure 7).

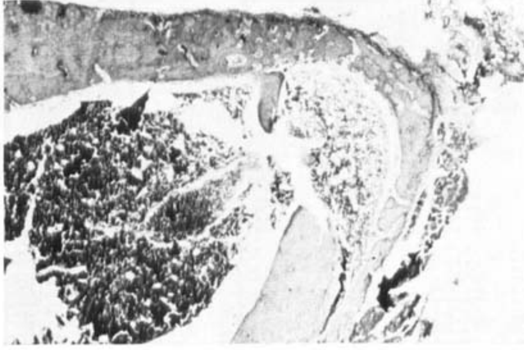

Figure 7. Two weeks after denervation. Four weeks after the defect was created. Normal femur. The defect is covered with new bone (× 20).

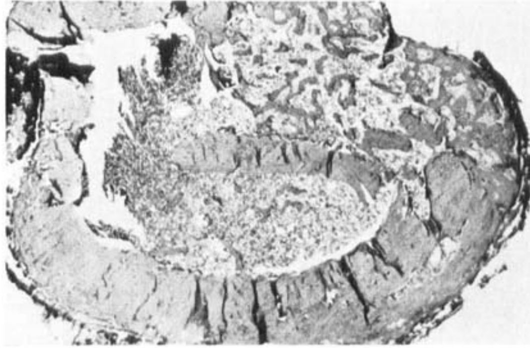

Figure 8. Four weeks after denervation. Atrophic tibia. Callus formation at two weeks. Shows no cartilage formation and abundant new bone (× 20).

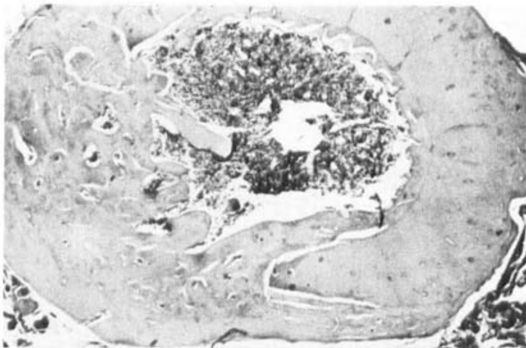

Figure 9. The same group as Figure 8. Denervated tibia. After seven weeks of repair. Observe the great quantity of bone plugging the gap (× 20).

Study of the Bone Defects in Cortical Bone (Tibia)

First group: Bone defect produced immediately after denervation. Equal repair during the first week, but from here onwards the atrophic side shows a faster and more complete repair.

Second group: Bone defect produced two weeks after denervation. The stage of repair looks the same. There seems to be less new bone on the atrophic side. There is no cartilage formation (Figures 8 and 9).

Third group: Bone defect produced four weeks after denervation. The normal side has a similar repair to the denervated one up to the second week, but from here onwards the defect is repaired more quickly and with more bone on the atrophic side. The cartilage formation is minimal on both sides.

Fourth group: Bone defect produced seven weeks after denervation. The repair process is much faster on the atrophic side. There is no cartilage formation, as there is on the normal side; however, this produces more new bone. A double cortex is observed on the atrophic side.

Overall Assessment

Cartilage: The atrophic side produces practically no cartilage to repair the bone defect. The normal side produces a large amount of cartilage in the fourth group.

New bone formation: The atrophic side has a richer new bone formation than the normal side in the third group. In the other groups there is less on the atrophic side (Figure 9).

Time of repair: The time taken for repair on the atrophic side is never longer than on the normal side, being equal in the second group; shorter in the fourth group; and equal up to the second week, and shorter from the second to the seventh week in the third group, as happens in the fourth group of the defects in cortico-cancellous bone.

In general, similar phenomena are observed in both the cortical bone and the cortico-cancellous bone, but at different periods of the bone atrophy.

DISCUSSION

Our results compared with the ones obtained by Smith & Dunsford (1955), Hulth & Olerud (1965) and Reyes-Cunningham et al. (1971) (The present work was finished before this last paper was published.)

Smith & Dunsford (1955) arrived at the conclusion that there was

delayed healing in fractures of the tibia produced thirty-five days after the denervation of the limb, with a sciatic nerve avulsion, and that this delayed healing was due to the bone atrophy. We shall discuss this later.

Amongst the other factors put forward by the same authors we can list:

– Smaller calibre of the vessels in the denervated bone. This is not so as shown by Kemp et al. (1947), Imig et al. (1953), Geiser & Trueta (1958), Hulth & Olerud (1960, 1961), Baungartl et al. (1958), Ferguson & Akahoshi (1960).

– Less soft tissue, especially muscle.

– The marked displacement of the fracture of the control limb compared with the denervated one (this argument would be acceptable if the authors were defending the opposite viewpoint).

These authors studied fracture healing only radiographically.

Hulth & Olerud (1965) also studied fracture of the tibia in rats following a motor-sensory mixed and peripherial denervation. They found exuberant callus formation at two weeks in all the series. The fracture was produced one week after denervation. Their results are in agreement with ours.

In the group in which the denervation is sensory or mixed, a greater quantity of cartilage is found. We found less cartilage in the denervated side; perhaps in cases with sensory denervation, there is a greater amount of cartilage due to the use of the insensitive limb, but in our cases with a mixed denervation the limb is completely useless, and in the bone defect series, in which there is no mobility, there is less cartilage formation in the denervated side; this favours the argument that the mobility is the cause of the cartilage formation.

Reyes-Cunningham et al. (1971) found delayed callus formation and scanty mineralization after radiological and histological studies. They studied callus formation of fractures produced at the time of denervation, and used an intramedullary rod which very probably alters the vascular pattern of the bone.

As is the case with fractures, the results in denervated skin are contradictory: Muren (1953) says that denervated skin heals normally, but Sandberg & Zederfeldt (1961) do not agree with these findings and say it shows a diminished tensional resistance.

Our work is more complete than the above-mentioned studies, because we studied bone healing after different periods of bone atrophy.

Our Findings

In fracture of the femur:

- Less cartilage formation than on the normal side.
- More adequate blood supply to the fracture gap tissue.
- Quicker and more complete union in every group. All the denervated groups showed union at between four and seven weeks; however, in the normal group there was no union in the second, third and fourth groups.
- The bone resorption is greater on the atrophic side judging by the number of osteoclasts.
- Bone remodelling is as good as on the normal side, with cortical and lamellar bone formation.

In the fracture of metatarsal bone:

- The amount of cartilage is similar on both sides, but much less than in the femoral group.
- Quicker union, with greater bone formation and bone absorption, on the denervated side.

In the femoral bone defect:

- There is less cartilage on the atrophic than on the normal side.
- There is more new bone formation on some of the atrophic sides, but not on all.
- The repair is always quicker on the atrophic side.

In the tibial bone defect:

- The findings are similar to the femoral bone defect but at different periods of bone atrophy.

Our results compared with the ones obtained by Landry & Fleisch (1964)

Landry & Fleisch (1964) found that bone atrophy following denervation can be divided into three periods:

a) During the first two weeks, bone formation is diminished.
b) From two to seven weeks bone formation and resorption increase; in other words the turnover or "metabolic flux" is increased.
c) At five months bone formation is again below normal, showing the lowest values in the whole study.

We agree with these findings, but we could not clearly separate the same periods due to the intermingling of the atrophy and fracture. We

observed, however, that between the ninth and fourteenth weeks after denervation bone formation was not as good as in former weeks.

Consideration of the diminished cartilage formation in the femoral series of the denervated limb

We can explain the diminished cartilage formation in the denervated femora in two ways:

1) The lesser mobility of the fragments.

2) The greater blood supply, due to new blood vessel formation and more rapid blood flux in the atrophic bone (Kemp et al. 1947, Imig et al. 1953, Geiser & Trueta 1958, Ferguson & Akahoshi 1960).

It seems that the movement of the fracture fragments does not permit the advancement of the new blood vessels and so hinders healing. This lack of blood supply would create a low oxygen tension and so enhancement of cartilage formation, a fact demonstrated by Bassett (1963) who showed that cells with bone forming capacity are very sensitive to variations in oxygen level.

This fact explains clearly why there is less cartilage formation in the atrophic femur, but not in the metatarsal bone. Perhaps the vascular changes are not so marked in the foot bones, which are surrounded by much less connective tissue.

It is more logical to explain the differences between femur and metatarsal bone, using the argument that excessive movement is the principal cause of cartilage formation. The denervated limb is useless and dragged behind by the animal, and so the femoral fragments have very little movement. The healthy limb has to be used in spite of pain during the first few days.

In the series of the bone defects, there is also less cartilage on the atrophic side, which is in favour of the mobility argument.

Study of the bone remodelling process

We have observed a good formation of primitive bone, lamellar bone and new cortical bone. These findings support the histological observation of Catto (1967) in one of our patients with myelomeningocele (Navarro, in preparation), in whom we found normal bone formation. Therefore we cannot agree with Eichenholtz (1963): "It seems that (the callus) never progresses to compact mature bone, but it seems to remain in limbo between fibre primitive bone and the adult cortical one".

CONCLUSIONS

1) In fracture of a denervated femur, we found less cartilage formation, a greater blood supply, a quicker and more complete union, and greater bone resorption.
2) Bone remodelling is normal.
3) In the fracture of the metatarsal bone, we found the same amount of cartilage formation on both normal and denervated sides; however, the amount was much less than in the femur.
4) The study on bone defects confirms the cyclic variations of bone turnover in atrophic conditions.
5) The differences in turnover depend on the type of bone (cancellous, cortico-cancellous, cortical) and the time of atrophy.
6) These findings may explain the differing results found in the literature.

SUMMARY

A study of the healing of complete fractures and bone defects was made in the rat with a denervated limb. The femoral and metatarsal bones were used to compare the healing of mobile and immobilized fractures. The metaphysis of the femur and the shaft of the tibia were used to study differences in healing of bone defects.

Various periods of bone atrophy were used to study how the behaviour of repair is altered by the degree of atrophy.

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