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The Relative Importance of Periosteum and Endosteum in Bone Healing and the Relationship of Vitamin C to Their Activities

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INTRODUCTION

SINCE the time of John Hunter (1835) there have been a multiplicity of hypotheses as to the relative functions of the various units of bone structure in bone regeneration which include that of Cohn and Mann (1915) who claimed that the bone cells themselves (the osteocytes) were responsible for regeneration. Leriche and Policard (1928) mainly as a result of speculation attributed the whole of the function of bone regeneration in fracture healing to the formation of bone from the connective tissue of the callus, Keith (1928), Girdlestone (1932), and Harris (1933) have stressed the importance of the osteoblast in bone formation. Hertz (1936) has noted that multiplication of cells and hyperæmia followed by the formation of bony trabeculæ occurred between the fibrous layer of the periosteum and the surface of the bone, after injury; but he noted that this reaction was greatest some distance from the fracture and that it diminished the closer the line of fracture was approached. This reaction he described as the "distant periosteal reaction". He found a multiplication of cells and hyperæmia close to the fracture line in the endosteum, but there was no "distant endosteal reaction". Some of the callus then ossified directly and the remainder became cartilaginous and subsequently underwent endochondral ossification. Urist and McLean (1941) have made the following rather interesting statement concerning the healing of a fracture: "Our preparations demonstrate invasion of the fibro-cartilaginous callus by new bone, which calcifies as it advances into the callus mass, and affords striking evidence that the bone originates outside of this mass. This process is analogous to the intra-cartilaginous growth of bone, in that the remnants of the invaded tissue are made use of and converted into bone matrix by the invading osteogenetic cells. . . . According to our observations, new intra-membranous bone is formed, chiefly from the deep or cambial layer of the periosteum and from the endosteum, including the osteogenetic cells within the Haversian canals."

EXPERIMENTAL

The stimulus to repair after a fracture is very great and rapid and since it is impossible to obtain a series of identical fractures (although the 1936 technique of Hertz approaches this ideal fairly closely) it is impossible by killing fractured animals at different times and examining sections of the fracture to be sure that one is observing the correct sequence of events in healing—since the process will proceed at different rates in different types of fracture. For the purposes of this work, therefore, small 1 mm. holes have been bored (with the aid of dentist's twist drill, using an aseptic technique; *see* Bourne, 1942*a*, for details) in the femora and in the parietal region of the skull of guinea-pigs and rats. By this means it is possible to obtain a small standardized area of regeneration and by killing animals at different intervals one can be reasonably sure of obtaining a continuous sequence of stages in the healing process.

Very little difference has been found in the healing processes in holes bored in the femora of rats and guinea-pigs. The process of healing is also fundamentally the same in the healing of holes in both the skull and the femora of guinea-pigs although it is slower in the former and in the latter small amounts of cartilage are sometimes formed.

Hertz (1936) found that in the healing of fractures of the fibula in rat and guinea-pig there were two processes of ossification: (1) Direct and (2) Endochondral. In the rat he found the second process to play a more important part than the first. Since in the repair of the holes described in this paper, there is little cartilage found in any case, this difference in healing between the two species was therefore eliminated; the one description will serve to show the healing processes in both. Animals were killed at twenty-four hours, three days, five days, one week and two weeks after boring the holes. The bone bearing the hole was removed at autopsy and fixed, in some cases in 5% formol saline and in others in acetic acid silver nitrate. The object of the latter fixative was to demonstrate vitamin C histologically in bone repair, but there were so many factors complicating the reaction in healing bone that it was not possible to obtain reliable results. After fixation for twenty-four hours, the bones were decalcified for a further twenty-four hours in trichloracetic acid. They were then washed, dehydrated, embedded in wax, sectioned, stained with hæmatoxylin and van Gieson and mounted in balsam.

In the specimens used for the section on the relation of vitamin C to the periosteum and endosteum, groups of animals were placed on a scorbutic diet (the same as that described by Bourne, 1942*b*) for two weeks and various animals were given different doses of vitamin C.

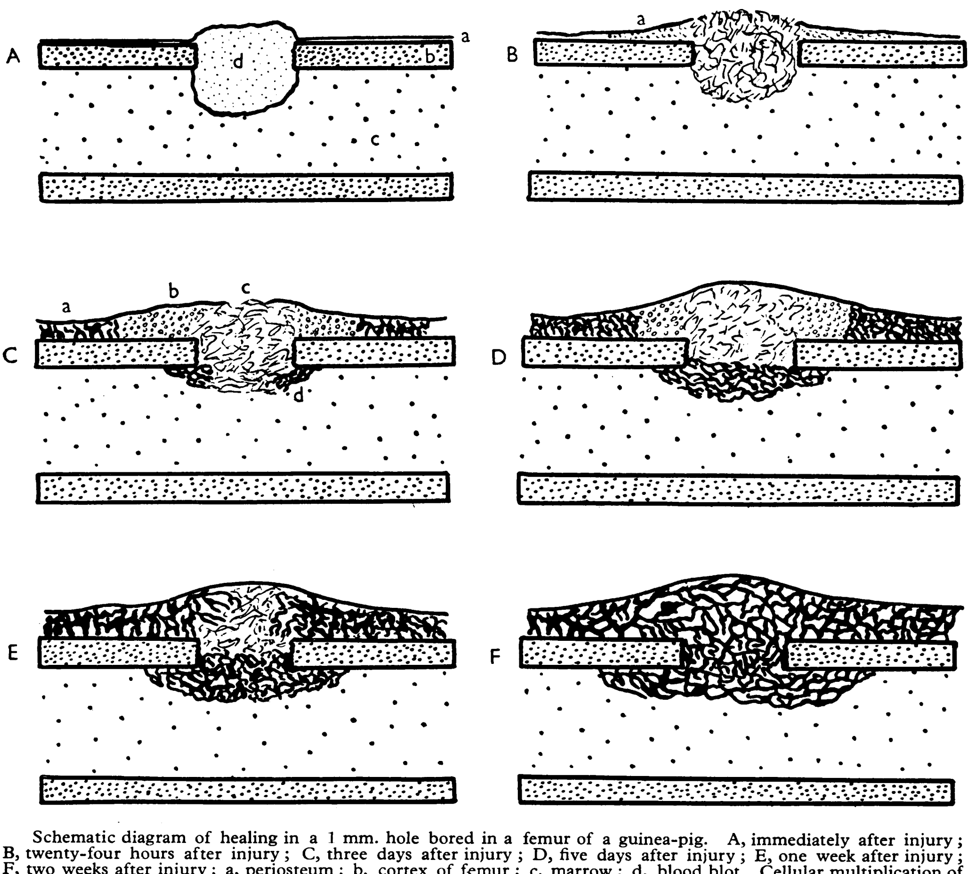
No. of Animals	*Amount of Vitamin C*
5	2 mg.
5	1 mg.
5	0·25 mg.
5	no supplement given

At the end of the first week a hole was bored in each femur of each animal. At the end of the second week, all animals were killed and their femora treated as described above.

Immediately the hole was bored there was a hæmorrhage from the medulla. The blood soon clotted and within twenty-four hours of the operation the clot became invaded by cells which had the appearance of fibroblasts and which were probably osteoblasts. They appeared to come from the periosteum and the marrow. By twenty-four hours signs of fibrous organization were apparent. The cells gave the appearance of having moved through the clot spinning fibres as they went. Danielli (1942) has pointed out that strands of protein can be spun from the surface of protein monolayers which have the same molecular orientation as collagen fibres and he suggests that these fibroblasts "spin" such fibres from a layer of protein absorbed on their cell membranes. These first fibres which were produced in the clot were very fine, and since they did not retain van Gieson stain (which gives a bright red colour with mature collagen) they were probably in the nature of reticular "pre-collagen" (Hunt, 1941) fibres. This process of fibre production continued for the next two days. By this time (three days after the operation) most of the blood corpuscles of the clot had been removed and their place taken by a close network of mixed collagen and "pre-collagen" fibres. In the case of holes bored in the guinea-pig's skull, however, there was often a considerable amount of the original blood clot still present at the end of a week. In such cases, the clot had frequently caused the formation of a deep depression in the brain substance.

By three days after the operation a marked reaction could be seen in the cambial (inner cellular) layer of the periosteum. The cells in this region had proliferated and lifted the fibrous layer of the periosteum away from the bone. This process spread outwards from the hole for some distance (a centimetre or more). By the end of three days the fibrous layer of the periosteum appeared to be growing over the fibrous callus which had formed. The periosteal fibres engaged in this migratory activity behaved like true collagen because they retained van Gieson stain. Small splinters of bone which were present in the hole in some cases as a result of the boring were in the process of being broken down and reabsorbed. Those which had a small piece of periosteum attached to them, however, showed activity of the periosteum. At three days bony trabeculæ appeared to be forming in the region of the endosteum and apparently in association with it. This process appeared to take place by the progressive metamorphosis of the adjacent pre-collagen fibres and their arrangement into thick trabecular strands. The first formed trabeculæ were always associated with the endosteum and some endosteal trabeculæ were always present before bone was formed by the periosteum.

At four days a striking feature of the healing process was the formation of numerous osteoid trabeculæ (which stain intensely with van Gieson after decalcification) in the proliferated cambial layer of the periosteum. In some cases, by this time, the reaction had travelled completely round the femur. In some specimens the fibrous layer of the periosteum had by this time penetrated into the callus from the periosteum. It seems that the behaviour of the periosteum is one of the factors which influence the rate and orderliness of the healing of bone. The main function of the fibrous layer of the periosteum appeared to be to grow across and seal off the repair tissue from the tissues outside the bone.



Schematic diagram of healing in a 1 mm. hole bored in a femur of a guinea-pig. A, immediately after injury; B, twenty-four hours after injury; C, three days after injury; D, five days after injury; E, one week after injury; F, two weeks after injury; a, periosteum; b, cortex of femur; c, marrow; d, blood blot. Cellular multiplication of the cambial layer of the periosteum, between the fibrous layer of the periosteum and the bone can be seen in B and C. Periosteal and endothelial trabeculæ can be seen developing in C, D, E and F.

A tendency which the fibrous layer sometimes exhibited to dive into the callus would appear abnormal. In some specimens, where it had not penetrated into the callus it had actually grown completely across the hole by four days.

Some specimens at four days showed the presence of masses of what appeared to be collagenous material apparently derived from the periosteum, which were losing their ability to stain with van Gieson and which appeared to be forming hyaline ground substance. The enclosed cells were altering their appearance and the whole mass of tissue was apparently in an early stage in the formation of cartilage.

By seven days, in every specimen, collagenous strands from the periosteum had passed completely across the fibrous mass plugging the hole. In some cases, on either side of the hole some of the periosteal trabeculæ had a cartilaginous appearance. The hole was filled in most specimens (with the exception of the holes in the skull) with solid-looking trabeculæ which anastomosed with each other in typical fashion. In other words, the hole was now plugged with membrane bone which acted as a very serviceable repair tissue. In some rat femora, for no obvious reason, the whole medulla became filled with trabeculæ. The maximum number of trabeculæ in the rat appeared to be formed by seven days, but in the guinea-pig it took longer for this to happen (ten to fourteen days). In many skull holes in guinea-pigs there was only a small number of trabeculæ present, even at fourteen days. The bones of the skull, therefore, do not seem able to repair damage as rapidly as those of the limbs.

In the rat by eleven days there were some signs of the formation of cartilage from the osteoid trabeculæ. By the end of two weeks there was a certain amount of cartilage derived from the periosteum and some appeared to have formed from the trabeculæ. There was a thick coat of collagen fibres which was derived from the periosteum. There was no evidence in the guinea-pig that there was a metamorphosis of any osteoid trabeculæ into cartilage.

Relation of Vitamin C to Periosteal and Endosteal Activity

It has been shown by Bourne (1942*b*) that in guinea-pigs receiving less than 2 mg. of vitamin C a day there is a deficient production of trabeculæ in the hole. Since the production of trabeculæ appears to be initiated by the endosteum, this suggests that the endosteal activity can only take place if adequate vitamin C is provided in the diet.

The endosteum is normally composed of a layer of osteogenetic cells closely pressed against the inside of the bone. Also there are endosteal cells lining the Haversian canals. Embryologically they are derived from the cambial layer of the periosteum. When a bone is injured, as for example in the boring of holes described in this paper, there is an enlargement, multiplication and migration of the cells near the injury. The migrating cells appear to form collagen fibres and these are eventually aggregated together into osteoid trabeculæ. In animals receiving no vitamin C there is still some enlargement and multiplication of endosteal cells near the injury and in some cases some fibres are formed. More rarely just the beginnings of osteoid trabeculæ are apparent. Normally the endosteal cells which are situated some distance from the injury, are closely pressed against the bone and are difficult to see. In completely scorbutic animals these endosteal cells are much more apparent. They appear swollen (particularly the nuclei) and in most cases have a pale-staining œdematous appearance. In animals receiving an amount of vitamin C in excess of 0·25 mg. a day, this œdematous condition of the endosteal cells does not appear to be present.

A definite relationship can be observed between the periosteal activitv and the amount of vitamin C. (Some particulars of this have been given by Bourne, 1942*b*.)

In an animal receiving no vitamin C there is often, at the end of a week after injury to the bone, no trace of a reaction by the cambial layer of the periosteum. In some cases the cells of this layer undergo a few divisions so that there is a slight thickening, but all other processes are inhibited. The outer fibrous layer of the periosteum does not grow over the tissue filling the hole in the bone in the usual way and the attachment of this layer to the bone is loosened. As a result, it often comes away during the process of sectioning.

In animals receiving 0·25 mg. of vitamin C per day, there is at the end of one week a multiplication of the cambial layer of the periosteum but no formation of collagen fibres by the cells. There is still no sign of the growth of the fibrous layer of the periosteum over the hole in the bone.

In animals receiving 1 mg. of vitamin C per day, the cells of the cambial layer of the periosteum have after a week multiplied to such an extent that this layer is six times as wide as it was in the preceding group. In addition, there are the beginnings of trabeculæ formation. There is some growth of the fibrous tissue of the periosteum over the hole.

In animals receiving 2 mg. of vitamin C the cambial layer of the periosteum at the end of a week has formed solid bony trabeculæ continuous with the bone of the femora and the fibrous layer of the periosteum has grown, in most cases, across the hole, which by now contains an appreciable number of bony trabeculæ.

SUMMARY

This work suggests that both endosteum and periosteum play an important part in the regeneration of an injured bone and that the first reaction and production of repair

bone in the region of the injury is by the endosteum. Only some days after the original sealing off of the injury (in this work, a hole bored through the bone) by the endosteum does the periosteum cover the injured area with a layer of bone. Any cartilage present appears to be formed in the first instance in association with the periosteum, but some cartilage appears to be formed by conversion of osteoid trabeculæ. The activity of both periosteum and endosteum is impaired by a deficiency of vitamin C.

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Ossifying Chondroma Replacing the Infrapatellar Pad of Fat

By Paul Bernard Roth, F.R.C.S.

This condition appears to be one of extreme rarity. I have only been able to find two published cases, one by Cassou in 1936, and one by Robillard in 1941. Cassou's case occurred in the left knee of a man aged 43: no operation was done, Cassou contenting himself with remarking that the ossification caused the owner but little discomfort. Robillard's case concerned the left knee of a woman aged 35, who was admitted to hospital on June 6, 1939.

"Two years before she had injured her knee in a fall. The knee was not X-rayed at the time. The patient had since complained of pain in the knee, and was treated for osteoarthritis. In March 1939 she fell again, and pain, tenderness, swelling, and immobility of the left knee resulted, persisting until the date of her admission to hospital. Examination revealed a swelling of the left knee, reaching a maximum between the patella and the tubercle of the tibia, with diffuse tenderness and marked restriction of motion. X-rays revealed a large calcified mass, apparently in the anterior chamber of the knee-joint. A utility incision was employed and the patella displaced laterally. The joint cavity was opened and explored. The mass was exposed, dissected free, and excised. It measured 5½ by 4 by 2 cm. . . . The post-operative course was uneventful. Motion at the knee-joint improved until 60 degrees of flexion were present and the patient was free of pain.

Robillard's case is illustrated by lateral radiographs before and after operation, by a photograph of the osseous mass, and also by a photomicrograph showing bone trabeculæ.

My case was as follows:

H. P. S., a busy solicitor, aged 69, was sent to me by Dr. Barnet Stross on January 7, 1943, with the history that for twenty years he had had increasing stiffness in the right knee. For the last three or four years he had noticed an enlargement in front. The knee was gradually beginning to ache more and more, especially when near a fire, and he could only go downstairs a step at a time.

On examination.—The knee was seen to be markedly enlarged in front, below the patella: there was a prominence which gave the appearance of a second patella below the normal one. This was of bony hardness, and could be moved by me slightly to either side, but not up or down. The knee could only bend to 90 degrees, and extend to 150 degrees; the movement was accompanied by gross creaking. Any attempt to force the knee beyond these limits at once caused pain and was objected to. X-ray (fig. 1) showed that practically the whole of the space normally occupied by the infrapatellar pad of fat was filled with a dense opaque mass, which gave a much darker shadow than the adjacent bones. I diagnosed calcification of the pad, and advised its removal.

On June 12, under spinal anæsthesia administered by Miss E. M. P. Law, M.B., I split the ligamentum patellæ and patella, exposing the mass, which had the appearance of a large mulberry, being deep purplish red in colour. It was freed easily above and on either side, by blunt dissection with a periosteal detacher; below, a few light touches with a scalpel were needed to free it from the front of the tibia. The two halves of the patella and its ligament were then brought together and secured in position with a continuous catgut suture passed through the overlying fibres of the quadriceps tendon. The skin was closed with Michel's clips.