EXPERIMENTAL PRODUCTION OF CARTILAGE DURING THE REPAIR OF FRACTURES OF THE SKULL VAULT IN RATS

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Ham (1930) noted that the cartilaginous areas of fracture callus were poorly supplied with blood vessels, in contrast with the richly vascular new bone. He also pointed out that both bone and cartilage apparently developed from similar periosteal cells, and that the two tissues graded into one another. He therefore suggested that the osteogenic cells of the periosteum, during fracture repair, have dual potentiality, forming bone in places where the blood supply is adequate and cartilage where it is not. Urist and Johnson (1943), on the other hand, stated that the avascularity of callus cartilage is secondary, and is caused by the blood vessels' being pushed aside as the cartilage forms. Other workers (among them Enneking 1948) believe that the cartilage develops from different cells from those which give rise to bone. There is also to be considered the widely held opinion (Urist and McLean 1941) that cartilage formation in the course of bone repair is a reaction to mechanical stressing, such as that resulting from imperfect immobilisation of the fragments. It was felt nevertheless that ischaemia might have some part to play in the genesis of cartilaginous callus, and so it was decided to study bone repair in the cranial vault under conditions in which the blood supply to the fracture site was greatly reduced. The cranial vault was chosen because cartilage almost never appears there during fracture repair (Pritchard 1946), and because the rigidity of the skull minimises the mechanical factor.

The method chosen was to make a number of incisions right through the pericranium, skull and dura mater, in order to restrict the blood supply to the area, and, if necessary, to scrape the periosteum from the bone as well. Very young rats were used to take advantage of the more rapid and vigorous cellular responses of immature animals, for it was believed that the presence or absence of cartilage might well turn on the outcome of the race between cellular proliferation and revascularisation, as Ham (1930) originally suggested. The results were to be studied histologically with and without prior injection of the blood vessels with indian ink.

MATERIAL AND METHODS

A total of 102 rats between three and thirty-four days old were operated upon, of which eighty-six survived, and of these the skulls of sixty-seven were sectioned. Under ether anaesthesia a parasagittal incision was made through the scalp, and the skull vault was then incised with a scalpel. To begin with (Group A), two longitudinal cuts and a connecting transverse incision were made through the left parietal bone and underlying dura mater. In a later series (Group B), the periosteum over the left parietal bone was scraped away before similar incisions were made. Finally, and this applied to most of the animals operated upon (Group C), the periosteum was scraped as before and then four incisions were made, two running longitudinally in the left parietal, one longitudinally in the right parietal, and then one transversely connecting the three previous incisions (Fig. 1).

Most of the animals were killed three to thirty-six days after the operation, and their skulls fixed in Bouin's fluid and sectioned in paraffin. Twenty-four of the animals, however, at the time of killing were deeply anaesthetised with ether, and while the heart was still contracting, a fine glass cannula was introduced into the left ventricle and a dilute solution of indian ink (1:3 in distilled water) slowly run in. After some hours the whole animal was fixed in 10 per cent formalin. The head was then removed and cleared as a whole mount or

FIG. 1

Diagram showing incisions and area of scraped periosteum in Groups A, B and C.

else embedded in celloidin and cut at 80–120 μ . Sections were usually lightly stained with haematoxylin and eosin or Weigert's haematoxylin and van Gieson, and counterstained with toluidine blue to show up any cartilage that might have formed.

TABLE I

FREQUENCY OF CARTILAGE FORMATION IN GROUP C.
Animals less than seven days old at the time of operation

Days after operation	Cartilage found	Number of animals
3	0	3
4–6	1	9
7–9	7	15
10–12	9	14
13–15	1	7
16–18	1	2
	19	50

RESULTS

General—None of the five animals which were recovered and sectioned from group A showed any cartilage at the fracture sites when examined histologically ten to twenty days after operation, but one of the four animals recovered from group B did show some cartilage seven days after fracture.

None of the eight animals in group C which were operated on after the first week of life showed any cartilage; but nineteen of the fifty animals studied which were operated on during the first week did show cartilage. The relative frequency of cartilage production in this last mentioned group, according to the time elapsed since operation, is shown in Table I.

It will be seen that cartilage was found with any frequency only between seven and twelve days after fracture. This may well be because, before seven days, callus differentiation is not sufficiently advanced to show cartilage formation, whereas after twelve days any cartilage formed earlier may have been replaced by endochondral bone. This is supported by the time-course of cartilage formation and replacement in fracture repair of rat long bones (Pritchard and Ruzicka 1950).

To sum up: about half of the animals operated upon in the first week of life, and in which the interference with the blood supply was most drastic, showed cartilage formation when examined seven to twelve days later. Less severe restriction of the blood supply, and operations on older animals, did not usually lead to cartilage formation.

Histology—The repair of single linear incised fractures of the rat's skull vault has been studied by Pritchard (1946). By twelve days osteoblast-covered trabeculae of new bone were to be

FIG. 2

Ischaemic fracture eleven days after operation. Note cartilage cells capping bony callus. (Weigert's haematoxylin, van Gieson and toluidine blue, $\times 200$.)

seen surrounding the old bone at the fracture site, but, with a single exception, no cartilage was ever found.

In the present series, however, twenty examples of cartilage formation at one or other of the fracture sites (usually the central one of the three longitudinal incisions) were observed. In most instances the cartilage projected into the fracture gap from the bone edges, and in some cases extended right across the fracture line to give cartilaginous union. In a few cases the cartilage was present a little way distal to the fracture, and in two cases bony union had occurred and there was an island of cartilage cells in the midst of the bridging bone.

Figure 2 shows the most usual appearances. New bone, arising beneath the fibrous periosteum and projecting towards or into the fracture gap, is capped with a mass of cartilage cells. The pleomorphic, hypertrophic character of the cartilage cells is clearly shown in Figure 3. The same figure also shows the way in which the more centrally placed, and largest, cartilage cells grade on the one hand into smaller cartilage cells and then into osteocytes and

FIG. 3

Ischaemic fracture twelve days after operation. Note gradation of cartilage cells into bone cells on the right and into fibroblasts above. (Weigert's haematoxylin and van Gieson, $\times 420$.)

FIG. 4

FIG. 5

Figure 4—Ischaemic fracture eleven days after operation. Note nest of cartilage cells in the midst of the bony callus. (Weigert's haematoxylin, van Gieson and toluidine blue, $\times 200$.) Figure 5—Ischaemic fracture twelve days after operation. Note gradation of cartilage cells into bone cells. (Weigert's haematoxylin, van Gieson and toluidine blue, $\times 420$.)

osteoblasts, while on the other hand they grade into pro-cartilage cells and then into fibroblasts.

Figures 4 and 5 show a mass of cartilage cells a little way from the fracture line, the former showing cartilage cells completely surrounded by young bone, the latter a gradation of cartilage cells into bone cells. Figure 6, on the other hand, shows cartilage cells in the midst of granulation

FIG. 6

Ischaemic fracture twelve days after operation. Note cartilage and bone in the granulation tissue. (Weigert's haematoxylin, van Gieson and toluidine blue, $\times 200$.)

FIG. 7

Ischaemic fracture seven days after operation. Note cartilage on right, pro-cartilage on left, indicating impending cartilaginous union. (Weigert's haematoxylin, van Gieson and toluidine blue, $\times 100$.)

tissue and not in contact with the periosteal new bone applied to the surface of the old bone. Here again gradations of cartilage into bone and young connective tissue are clearly seen. Impending cartilaginous union is shown in Figures 7 and 8, the latter section being stained with thionin to show the basophilic nature of the cartilage matrix.

FIG. 8
Ischaemic fracture ten days after operation. Note cartilage masses about to unite. (Thionin, $\times 225$.)

FIG. 9
Ischaemic fracture nine days after operation. Note cartilaginous union across fracture gap. (Toluidine blue, $\times 150$.)

Actual cartilage union is illustrated in Figure 9, and bony union, with an island of cartilage, is shown in Figure 10.

It should not be supposed that every section from a given specimen showed cartilage. In most cases the cartilage was in the form of isolated nodules, and sections taken between these showed only bony callus.

Indian ink injections—The injected and cleared specimens of the skull vault, one or two days after operation, showed that relatively little ink reached the scraped and damaged area as compared with the rest of the skull. Ten days after operation, however, the vessels of the

FIG. 10

Ischaemic fracture fourteen days after operation. Note bony union on pericranial side, and cartilage on the right. (Haematoxylin and eosin, $\times 100$.)

FIG. 11

Ischaemic fracture twelve days after operation. Vessels injected with Indian ink. Note avascular island of cartilage. (Haematoxylin, eosin and toluidine blue, $\times 100$.)

damaged area were well filled, indicating extensive revascularisation. Sections from injected material nevertheless brought out the fact that areas of cartilage were conspicuously free from injected vessels as compared with the new bone in the neighbourhood (Fig. 11).

CONCLUSIONS AND DISCUSSION

These experiments have shown that it is indeed possible to induce cartilage formation in the course of bone repair in the skull vault provided the animal is very young, the damage is extensive and the fracture site is examined between seven and twelve days after injury. That ischaemia is the causal factor in such cartilage production is probable from the injection studies, from the fact that single incised fractures of the skull vault very seldom show cartilage, and from the rigidity of the skull, which eliminates mechanical determinants such as shearing stresses. The fact that cartilage was produced only in very young animals, and then only when the damage was extensive, supports the view that the rapidity of cell proliferation in relation to the speed of revascularisation is a decisive factor. Restriction of cartilage to the second week after fracture is easily explained by the normal lag in matrix deposition in bone repair, and the inevitable bony replacement of any cartilage formed. The fact that the cartilage was of the secondary, hypertrophic type, and was always closely associated with new bone, without a clear line of demarcation between the cells or matrices of the two tissues, confirms Ham's view that both tissues have a common cellular origin, a hypothesis that is supported by tissue-culture studies (Fell 1933). Ham's view that ischaemia in fracture repair induces rapidly proliferating osteogenic cells to form cartilage instead of bone is thus fully supported by these experimental findings.

However, it must not be inferred from this that mechanical factors play no part in the genesis of cartilage in bone repair, for the hypothesis that pressure, and particularly shearing, stresses stimulate cartilage formation is well attested. All that is claimed here is that ischaemia is also a factor. In fact it seems probable that the two factors are closely related, for mechanical stresses may well disrupt existing blood vessels, or prevent the ingrowth of new vessels, and so produce and maintain a stage of ischaemia.

Such considerations may also explain secondary cartilage formation in the course of the normal development of some membrane bones, particularly at sutures (Pritchard, Scott and Girgis 1956), and at the extremities of bony projections (Dixon 1953). The tissues there may well be exposed to pressure stresses, and so be subject to periods of temporary ischaemia, the osteogenic cells being induced thereby to produce cartilage instead of bone.

SUMMARY

Cartilage formation was provoked in the skull vault of the young rat by making multiple incisions, and scraping the periosteum to reduce the blood supply to the injured area. The hypothesis that ischaemia induces osteogenic cells to produce cartilage in the course of fracture repair thus receives experimental support.

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