

PROTECTION OF THE BLOOD CLOT IN HEALING CIRCUMSCRIBED BONE DEFECTS

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Murray, Holden and Roschlau (1957) showed that, if a portion of the cortex of the ilium of a dog is removed and the resulting blood clot is protected with a plastic cage, the interior of the cage becomes filled with new bone which is raised above the normal contour of the ilium. The normal healing process in a defect which communicated with the medullary cavity of the femur of the albino rat was described by Melcher (1960), who also showed that homogenous organic bone implanted into these defects readily undergoes resorption. The present investigation was undertaken in order to determine what effects protection of the blood clot with plastic and organic bone shields has on the healing of a penetrating defect in the rat's femur.

MATERIALS AND METHODS

Saucer-shaped shields were made from cellulose-acetate* and organic bone. The latter were prepared by immersing homogenous femurs in 0.5 M versene (ethylenediamine tetra-acetic acid pH 7.4) at room temperature for two weeks, the versene being changed every two days. The bone was then washed in tap water for twenty-four hours and stored in water at 4 degrees Centigrade.

Under ether anaesthesia the lateral aspects of both femurs of twenty-five adult rats of the Wistar strain weighing approximately 250 grammes were exposed, and circumscribed defects about three millimetres in diameter were cut through the cortex with a slow-running water-cooled drill. In fifteen of the animals the defect in the right femur was covered with a cellulose-acetate shield, while the defect in the left femur was not protected and was allowed to heal as a control. In the remaining ten rats the defects in both femurs were protected with homogenous organic bone shields. The shields were sufficiently rigid to hold the adjacent muscle away from the defect. The overlying soft tissues were repaired with catgut sutures.

The animals were killed at varying periods up to eighteen months after operation. The thighs were removed and fixed in formol-Zenker fluid. After decalcification the specimens were embedded in paraffin wax and transverse serial sections were cut and stained with haematoxylin and eosin.

Difficulty was encountered in sectioning the specimens containing the cellulose-acetate shields. During the process many of the shields were displaced, resulting in varying degrees of damage to the sections.

OBSERVATIONS

Control defects—The changes that took place in the control defects were the same as those which had been previously described (Melcher 1960).

Healing of defects covered by shields well adapted to the outer surface of the femur—Most of the shields in this group were made of homogenous organic bone, the periphery of which was often, but not always, fused to the healing callus. The bone from the healing defect proliferated into the concavity provided by the shield and eventually occupied almost the whole of the protected area. A layer of connective tissue separated the inner surface of the shield from the bony protuberance. Very occasionally, however, a trabeculum of bone was found to extend between the two (Fig. 1). The original contour of the femur, which was easily visible, was changed by the proliferating bone. During the process of resorption and

* Supplied by G. J. Wevell (Pty.) Ltd., Johannesburg.



FIG. 1



FIG. 2

Figure 1—Two months after operation the homogenous bone shield covering the defect can be readily recognised. The protuberant callus follows the inner contour of the shield closely and the two are separated by a layer of connective tissue. At one side the edge of the shield appears fused to the adjacent bone while the other is closely adapted. At the apex of the callus the ends of two bone trabeculae are fused with the shield. Pericanalicular resorption of the organic bone is evident. (Haematoxylin and eosin, $\times 25$.) Figure 2—In this specimen two months after operation the bone shield is fused to the femur on one side but poorly adapted on the other. Despite the distortion in the section the callus can be seen to follow the inner contour of the shield more closely on the fused than on the unattached side, where the connective tissue between the two is thick and continuous with that outside the shield. The shield is undergoing resorption particularly in the portion which is unattached. The medullary cavity of the protuberant callus is separated from that of the femur by a shelf of bone. (Haematoxylin and eosin, $\times 25$.)

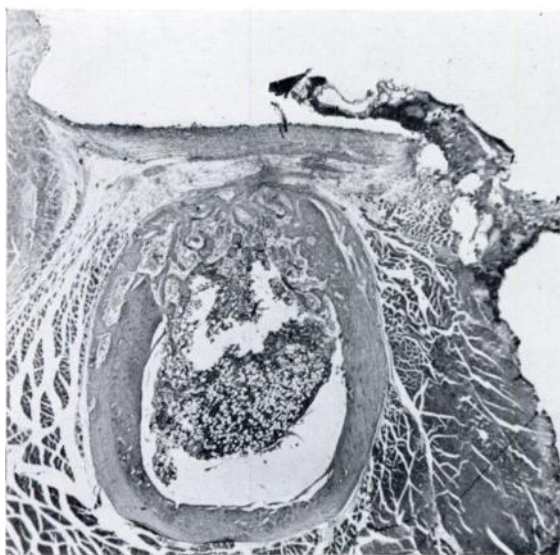


FIG. 3



FIG. 4

Figure 3—The defect was covered by a cellulose-acetate shield which was displaced. Although the shield was lost during preparation its position can be readily recognised. There is no protuberant callus three weeks after operation. (Haematoxylin and eosin, $\times 10$.) Figure 4—The organic bone shield has been displaced from the defect and, two weeks after operation, covers an area adjacent to the defect which is partly occupied by a well developed proliferation of subperiosteal callus. No protuberance is present over the defect, which shows the part played by endosteal callus in healing this type of defect. (Haematoxylin and eosin, $\times 10$.)

remodelling of the callus a separate, or nearly separate, medullary cavity was often formed in the protruding bone. This medullary cavity was separated from that of the femur by a shelf of bone which joined the endosteal aspect of the margins of the defect (Fig. 2). Sometimes the medullary cavity in the bony protuberance was loculated.

Healing of defects covered by shields partly adapted to the outer surface of the femur—There were shields of both types of material in this group. In some of the organic bone shields fusion had occurred to the bony callus at one side, but usually there was only close approximation; this applied to all the cellulose-acetate shields. The callus from the healing defect proliferated into the concavity provided by the shield but did not fill the space to the same degree as in the group described above, but was more closely related to the well adapted side of the shield than to the other side. The connective tissue between the shield and the

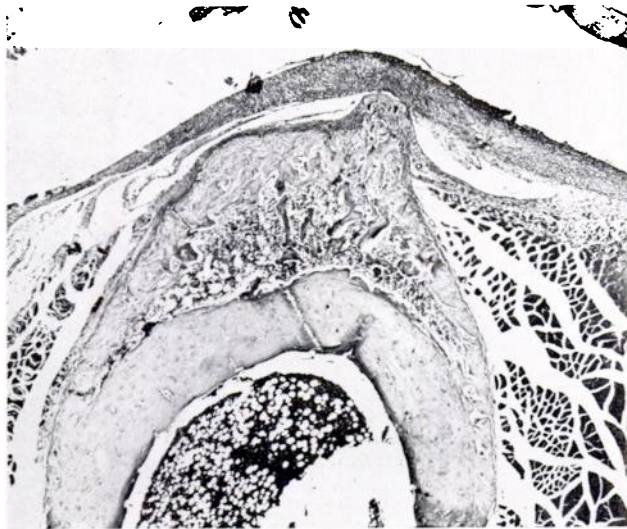


FIG. 5

In this specimen, three weeks after operation, the cellulose-acetate shield has been displaced outwards from the defect and also slightly upwards on the femoral shaft. The protuberance is papillary-shaped and is undergoing active resorption. (Haematoxylin and eosin, $\times 25$.)

callus was thicker, and was continuous with that outside the protected area on the side where the shield was more remote from the femur (Fig. 2).

Healing of defects from which the shields were badly displaced—Although the shield was always placed over the defect, sometimes it was displaced later. When there was considerable movement away from the bone surface, but with the shield still overlying the defect, the space between the healing bone and the shield was occupied by connective tissue and muscle, and the protuberance did not develop (Fig. 3). The shield was displaced once so that the edge of the defect and a portion of the adjacent periosteum was covered; no protuberance developed over the defect but the concavity of the shield was partly

occupied by a small amount of subperiosteal callus (Fig. 4). In another case in which the displacement was both outward from the defect, and slightly up the femoral shaft, a papillary-shaped protuberance developed under the shield (Fig. 5).

Reaction of host tissues to the shields—Apparently no attempts were made to resorb the cellulose-acetate shields. On the other hand active resorption of the organic bone took place and multinucleated giant cells were associated with this. Although resorption was most active in the walls of the canalicular system it also took place from the outer surfaces of the shield. Removal of the shields appeared to be most extensive near the exposed cut edges. The rate of resorption of the organic bone shields varied considerably in different animals and even between that of the right and left legs of the same animal.

Induction of new bone by the implant material—There were no examples of induction of new bone by the cellulose-acetate shields. In one case, however, there were signs of induction of new bone by a homogenous organic bone shield (Figs. 6 to 9).

Stability of the bony protuberance—Bony protuberances protected by cellulose-acetate shields have been recognised up to eighteen months after operation. There does not appear to be any evidence of active resorption of the exostosis in these cases (Fig. 10). There are indications, however, that marked resorption of the organic shield is accompanied by removal



FIG. 6



FIG. 7



FIG. 8

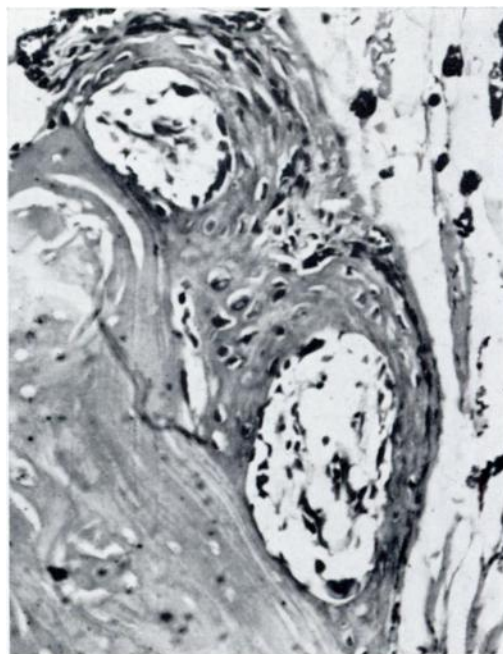


FIG. 9

These serial sections, four months after operation, demonstrate the apparent induction of new bone by a homogenous organic bone shield. Figure 6—This shows a bony protuberance that has developed beneath an organic bone shield. On the less well adapted side there is early evidence of new bone formation. (Haematoxylin and eosin, $\times 25$.) Figure 7—A higher magnification of the area marked in Figure 6 shows two areas of osteogenic activity adjacent to the organic bone shield. In one, osteoblasts are differentiating, and in the other new bone has already been laid down. The space between the new bone and the shield is an artefact. (Haematoxylin and eosin, $\times 250$.) Figures 8 and 9 show further development of the induced bone. (Haematoxylin and eosin, $\times 250$.)

of the protuberance (Fig. 11) and in one instance the outgrowth beneath a partially displaced cellulose-acetate shield also appeared to be undergoing resorption (Fig. 5).

Subperiosteal reaction on the surface of the femur opposite the defect—In all except one of the specimens examined a subperiosteal bony reaction occurred on the surface of the femur opposite the defect. This may take place directly opposite the defect, but usually to one or other side of this point. A striking feature of the reaction is the marked proliferation of the associated fibrous layer of the periosteum which very often appears as a dense feltwork of fibrous connective tissue.

DISCUSSION

Subperiosteal reaction on the surface of the femur opposite the defect—This reaction is not confined to femora in which a protuberance is being developed. We have also recognised it in relation to grafted defects and untreated control defects. It is possibly stimulated by the alteration of the direction of the forces which are normally transmitted through that part of the bone.

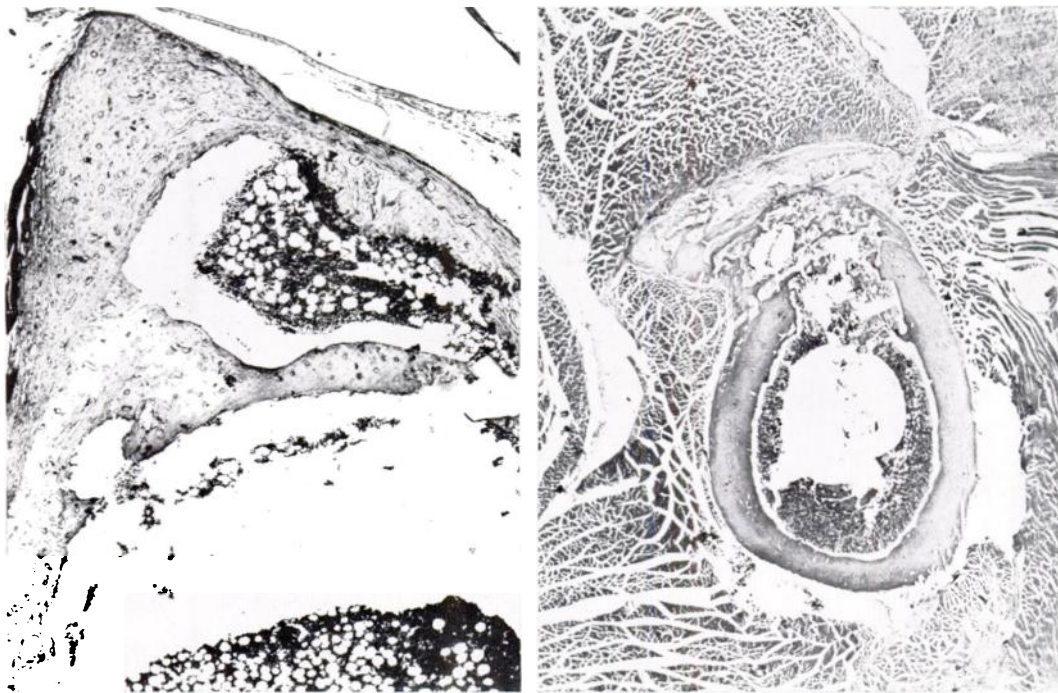


FIG. 10

FIG. 11

Figure 10—The cellulose-acetate shield has been lost during histological preparation but its position can be readily recognised eighteen months after operation. The protuberance is still present and the medullary cavity in it is almost wholly separated from that of the femur. There appears to be little cellular activity associated with the bone of the protuberance. (Haematoxylin and eosin, $\times 40$.) Figure 11—Four weeks after operation there is considerable resorption of the organic bone shield and the protuberance. (Haematoxylin and eosin, $\times 10$.)

Induction of new bone by organic bone—It is not known what conditions were responsible for the predisposition towards the induction of new bone by the implanted organic bone. The results of this experiment and also those obtained from the implantation of homogenous organic bone into bone defects (Ray and Holloway 1957, Melcher 1960) suggest that organic bone does not usually act as an inductor substance and that this one example is an unusual occurrence.

Removal of organic bone—The presence of multinucleated giant cells, morphologically identical with osteoclasts, in sites where organic bone is being removed has been described

previously (Melcher 1960). The most active removal of organic bone takes place from the walls of the canals which traverse it and resorption is generally most advanced in relation to the cut ends of the implant. This may be because the invading host connective tissue can more readily gain access to the canals exposed at a cut surface. An analogous situation has been described by Siffert (1952) who noticed that Howship's lacunae are more commonly found on the cut surfaces of cancellous bone grafts than on the intact borders.

Role of the shield in the production of the bony protuberance—The development of a bony protuberance under a protective shield may be ascribed to two main local factors. Firstly, the shield may permit the extension of osteogenesis into an area from which muscular pressure on the haematoma has been removed. Secondly, it may confine the colonisation of the haematoma to connective tissue elements which have an osteogenic potential.

Pressure on a haematoma can change its configuration and influence the morphology of the healed bone. We have observed that if a piece of muscle is inadvertently pushed into a bone defect it will displace the haematoma and will eventually cause deformity of the healing bone. It does not necessarily follow, however, that when absence of adjacent tissue pressure allows the formation of an excessively large haematoma a comparably large overgrowth of bone will occur. Marked upward displacement of the shield from the defect showed that, in spite of partial protection of the haematoma, repair of the defect was not accompanied by an outgrowth of bone.

The osteogenic potential of the young differentiating connective tissue is the other important local factor. The views of Pritchard (1956), although not wholly in accord with those of other workers (Urist and McLean 1953, Collins and Curran 1959), were that "the typical osteoblast is a temporary modification or modulation of a series of mutually transformable connective tissue cells resident in and around bone within the confines of the fibrous periosteum." On this assumption it might be expected that colonisation of the haematoma by granulation tissue elements, originating within the area defined by Pritchard, will give rise to new bone formation. Conversely, if the haematoma is colonised by non-osteogenic connective tissue elements it is more likely that mature connective tissue will be developed in preference to bone.

The work of Linghorne (1960) lends weight to this hypothesis. He showed that if a portion of the fibula of a dog was removed and the fragments were connected by a piece of polyethylene tubing, which was allowed to fill with blood, restoration of the lost bony tissue took place. If, under the same conditions, the fragments were not joined by a tube the gap was not bridged by new bone but by fibrous connective tissue. The protection of the blood clot and the prevention of its invasion by non-osteogenic connective tissue appeared to be of prime importance in this experiment. Similar conclusions may be drawn from the present investigation. In the defects where the shield has not only protected the haematoma from the pressure of the overlying soft tissue but also has excluded the non-osteogenic connective tissue, the new bone formation is of similar configuration to that of the haematoma. Where the shield has only partially excluded the non-osteogenic connective tissue, the new bone formation does not conform to the configuration of the haematoma in the areas where non-osteogenic connective tissue has apparently been able to proliferate beneath the shield. In the case where the protuberance of subperiosteal callus is shaped like a papilla the non-osteogenic tissue has proliferated beneath the shield from around its whole circumference. This suggests that the osteogenic connective tissue from the bone adjacent to the defect has proliferated upwards towards the shield, but that lateral proliferation has been restricted by the ingrowth of non-osteogenic tissue confining it to a papillary-shaped area. When the shield was displaced so far from the defect that it no longer protected the blood clot adequately, repair proceeded in a manner similar to that seen in unprotected defects.

It is therefore concluded that the exclusion of the proliferating extra-skeletal connective tissue from the haematoma is perhaps the main function of the shield in the formation of

the protuberance. The dimensions of the protuberance are, however, affected by the size and shape of the shield and its ability to protect the haematoma from the pressure exerted by the overlying soft tissues. The careful adaption of the shield to the surface of the femur is important, but it is thought that even if the shield is not in contact with the bone it will fulfil its function, provided that the proliferating subperiosteal callus is able to make contact with it before the ingrowth of non-osteogenic connective tissue.

When the shield was displaced away from the defect to cover the area from which the subperiosteal callus proliferated a similar type of protuberance developed. This is in keeping with the findings of Murray *et al.* (1957).

Maintenance of the protuberance—This investigation confirms the results obtained by Murray *et al.* (1957) and serves to show that if a shield remains in place a bony protuberance will be formed and maintained. There are indications that the papillary type of protuberance developed in relation to a wholly displaced shield, beneath which there has been ingrowth of non-osteogenic tissue, may not be stable. Resorption of the shield, such as eventually occurred in those made of organic bone, is apparently followed by removal of the protuberance. It is thought that the protuberance will only persist for as long as it is protected.

SUMMARY AND CONCLUSIONS

1. Penetrating defects were cut in the femora of twenty-five albino rats. In fifteen of the animals the defects in the right legs were protected with cellulose-acetate shields while those in the left legs were unprotected and allowed to heal as controls. In the remaining ten animals the defects in both legs were protected with shields made of homogenous organic bone.
2. New bone was found to proliferate into the concavity of the shields in most of the animals and this protruded beyond the contour of the femur. The development of the protuberance appeared to depend upon the degree to which the shield was adapted to the femoral surface.
3. The cellulose-acetate shield was not removed by the host, but the homogenous organic bone was actively resorbed; multinucleated giant cells were associated with this process.
4. There are indications that the maintenance of the protuberance is dependent upon the continued presence of the shield. Exostoses protected by intact cellulose-acetate shields have been recognised up to eighteen months after operation.
5. The function of the shield in the formation of the bony protuberance is thought to be two-fold, in that it protects the haematoma from invasion by non-osteogenic extra-skeletal connective tissue, and that it governs the size of the haematoma and prevents its distortion by the pressure of the overlying soft tissue.

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