

https://dspace.org/

The influence of alkaline phosphatase, calcium glycerophosphate and alginate on the repair of bone

Dreyer, C. J.; Melcher, A. H.

1962

http://hdl.handle.net/10539/20198

Dreyer, C. J., Melcher, A. H. 1962. The influence of alkaline phosphatase, calcium glycerophosphate and alginate on the repair of bone. Journal of the dental association of South Africa;17(2):34-38

Downloaded from a DSpace repository. https://dspace.org/

DIE TYDSKRIF VAN DIE T.V.S.A.

THE Journal OF THE D.A.S.A.

DIE Tydskrif VAN DIE T.V.S.A.

FEBRUARY, 1962.
Vol. 17. No. 2.

THE INFLUENCE OF ALKALINE PHOSPHATASE, CALCIUM GLYCEROPHOSPHATE AND AND ALGINATE ON THE REPAIR OF BONE

C. J. DREYER and A. H. MELCHER

Joint Dental Research Unit of the C.S.I.R. and the University of the Witwatersrand, Johannesburg

BLUM (1944) has concluded that alkaline phosphatase and calcium glycerophosphate in an anchoring medium accelerate the repair of experimentally prepared bone defects in rabbits. In one of his series of experiments three to five mm. defects were made in the shaft of the radius of adult rabbits. The defects were filled at the time of the operation with alkaline phosphatase and calcium glycerophosphate in an anchoring medium. The anchoring medium was formed by adding 2 per cent calcium chloride to a 5 per cent alginate solution.

The process by which femoral defects in Wistar Institute strain rats heal, as well as the effect of implanting various substances into these defects, has been previously described (Melcher 1960, Melcher and Dreyer). In view of the possible clinical application, part of Blum's investigation has been repeated to assess the effects of the implant materials used by him on the healing process in femoral defects in albino rats.

Materials and Methods

(*a*) *Materials*

The calcium chloride, calcium glycerophosphate, and sodium alginate were produced by B.D.H. while the alkaline phosphatase was supplied by Sigma Chemical Company.

Table 1

Number of Femora	*Implant Material*
10	Calcium glycerophosphate and alkaline phosphatase in sodium alginate gelled with calcium chloride.
20	Calcium glycerophosphate in sodium alginate gelled with calcium chloride.
10	Calcium glycerophosphate in sodium alginate.
10	Sodium alginate gelled with calcium chloride.

(*b*) *Method*

Mid-shaft defects of 2—3 mms. in diameter were made in both femora of 25, 5—6 week old Wistar Institute strain rats. The defects in the femora were filled with the material to be investigated. This material consisted of alginate and various combinations of the substances used by Blum. The number of defects implanted with each type of material are listed in Table 1.

Where applicable, the calcium glycerophosphate and alkaline phosphatase were mixed with the alginate immediately before the addition of the calcium chloride. At first, an attempt was made to confine the implant material to the bony defect, but the graft was constantly displaced by haemorrhage. This problem was overcome by packing the material into the medullary cavity and allowing the excess to protrude through the defect. The animals were sacrificed at varying periods from four days to 35 days post-operatively. Serial sections of the femora were stained with haematoxylin and eosin and also with toluidine blue. Histological material obtained from rats of the same strain and age group which were used in previous experiments on the healing of a bone defect (Melcher, 1960) were utilized for comparison.

RESULTS

In a pilot study, sections containing alginate were treated with various stains, since haematoxylin and eosin did not stain the alginate differentially. The most successful of these was toluidine blue which stains the alginate metachromatically. Thin films and the edges of heavy concentrations of alginate appear bright red while thick portions are black. The alginate undergoes a marked degree of shrinkage during histological processing.

Many of the features previously described in the healing process of grafted defects (Melcher, 1960) also occurred in this series. Where the implanted material occupied the defect, it impeded the bridging of the gap by acting as a physical barrier; but as the material in the defect was gradually resorbed it was replaced by bone (Fig. 1).

There were however variations from the usual pattern of healing. The most striking of these were an increase in the formative response and a resorptive reaction in the femoral cortex.

The Formative Response

An increased osteoblastic response involved either the subperiosteal or endosteal callus. An exaggerated subperiosteal reaction took place in one of two ways or a combination of both. In many of the defects the subperiosteal callus arose from

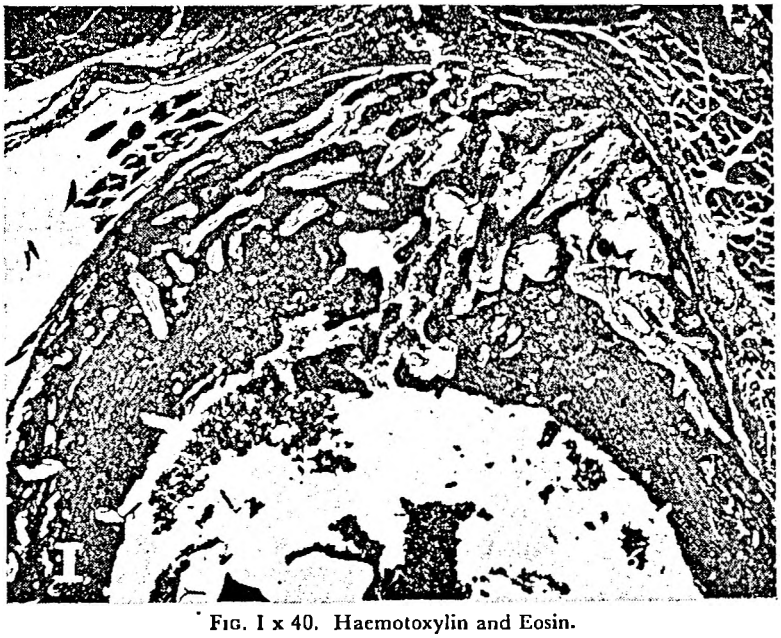


FIG. 1 x 40. Haemotoxylin and Eosin.

Cross-section of femur three weeks post-operatively. Part of the defect is still visible but the alginate mixture which at first acted as a physical barrier was now being replaced by bone. In this specimen the endosteal response was minimal while the marked periosteal bone formation has bridged the defect completely.

an area of the cortex far more remote from the defect than commonly occurs. The outward proliferation of the subperiosteal callus varied from usual to markedly exaggerated proportions, the latter of which contained very large amounts of cartilage. In only one defect was the endosteal response inordinately profuse, and this included the formation of cartilage (Fig. 2).

The Resorptive Response

A consistent finding in the alginate implanted femora was a resorption of the cortex in a manner not unlike that which occurs in a healing fracture (Fig. 3).

The implant materials were apparently well tolerated by the host tissues. Where small fragments of the material were present in the medullary cavity these were often covered by new bone. When the material protruded from the defect, foreign body giant cells were observed in the vicinity.

Many methods and materials have been investigated in an endeavour to find an inductor of new bone, Levander (1945), Lecroix (1945), Urist and McLean (1952), Johnson and McMinn (1956), and Bridges and Pritchard (1958). The reasons for Blum's choice of the enzyme alkaline phosphatase and the substrate calcium glycerophosphate are obvious. The results of the present investigation, however, show that the presence or absence of these two substances in the alginate gel does not materially affect the end result. It must be concluded that any stimulating effect that the implant may have on new bone formation is due to the presence of the alginate gel.

The periosteal response, which arises at a greater distance from the defect than has generally been observed, was, on two occasions, of such magnitude that it resembled that which is usually associated with a healing fracture.

The subperiosteal response could possibly be mediated from the alginate gel as a physical or chemical stimulus, the physical stimulus may be in the form of osmotic pressure built up in the alginate and exerted on the endosteal aspect of the shaft of the femur. This pressure may then be transmitted to the regional blood vessel and lymphatics and thence, as a pressure

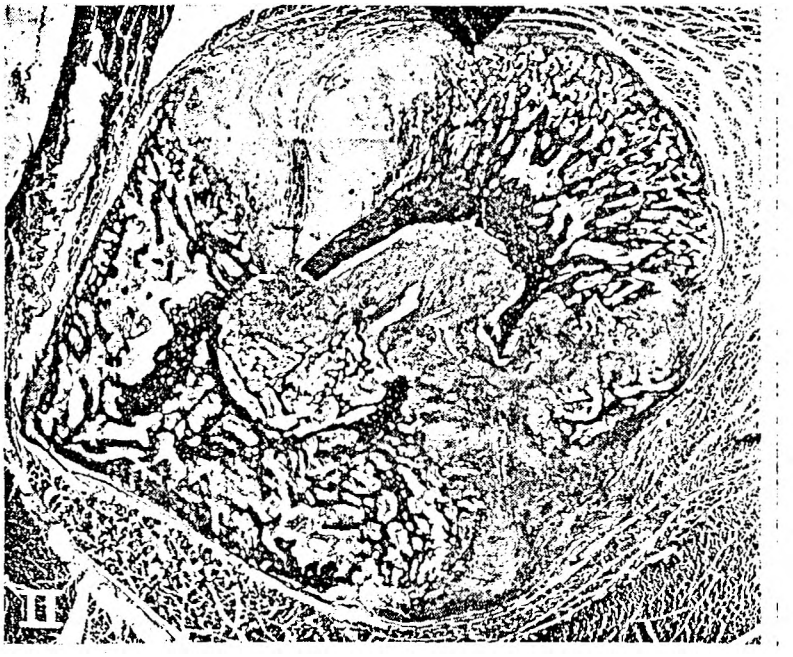


FIG. II. x 10. Papenicolaou.

Cross-section of a femur three weeks post-operatively. In this specimen the endosteal and periosteal response, which included the formation of cartilage, has been so marked that only a small portion of the original shaft with an edge of the defect was visible.

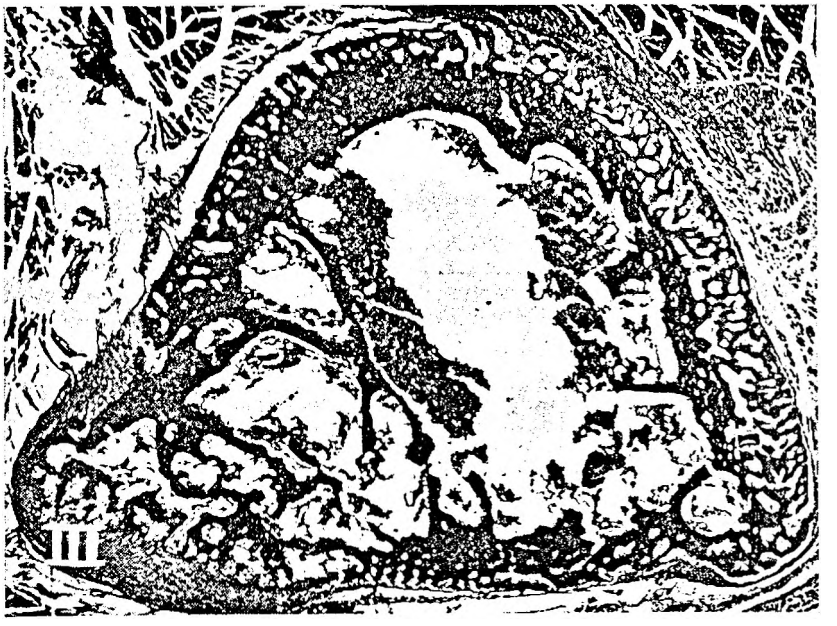


FIG. III x 25. Haematoxylin Eosin.
One week post-operatively. This cross-section of the femur taken immediately above a defect demonstrates the characteristic resorption of the cortex associated with alginate implants.

or circulatory stimulus, to the overlying osteogenic tissue.

A chemical stimulus arising from the alginate is also possible and may explain the more remote reactions. However the usual lack of endosteal reaction in relation to the alginate and the absence of bone formation round collections of alginate in the soft tissues, is not entirely compatible with a chemical stimulus.

The endosteal reaction as seen in the normal healing of a defect in the femur of a rat varies considerably. This may be related to the amount of damage inflicted on the endosteum and inner surface of shaft while preparing the defect with a drill. Melcher (1960) did not find any cartilage in the endosteal callus of 82 untreated healing defects. Similar observations were also made by Phemister (1935). In one instance, where the defect had been packed with Ca. glycerophosphate in an alginate gel, extensive cartilage formation occurred in the region normally occupied by endosteal callus.

Amies (1959) has described the response of the tissues when alginate is injected subcutaneously. Our own findings on intramuscular alginate injections are in agreement with this author and thus have not been described.

Remodelling of the bony callus normally occurs in the healing defect, but resorption of the bone cortex, similar to that which takes place in a healing fracture has not been previously observed (Melcher, 1960). In the majority of defects in the present experiment however, resorption of the shaft of the femur has been marked and widespread, even when the formative reaction has been minimal. This resorptive phase usually commences about a week after the operation and may last from one to two weeks, and is then followed by a redeposition of bone in the shaft. The phases seem to occur independent of the continued presence of alginate in the medullary cavity.

CONCLUSION

This investigation has not confirmed the observations made by Blum (1944) on rabbits. Consistent stimulation of bone proliferation in healing defects in rats has not been obtained. A larger area of subperiosteal callus was laid down on the femoral cortex than is generally observed in similar defects or defects grafted with

other materials; but the volume of the proliferation was so inconsistent that, although on occasions the subperiosteal callus reached enormous proportions, it is not possible to say with any degree of certainty that the experimental implant will hasten healing or provide an appreciably greater volume of bony callus than would have occurred in its absence. It is thought that any osteogenic stimulus which might occur may arise out of the presence of alginate and not because of the influence of alkaline phosphatase or calcium glycerophosphate.

The enormous resorption of the femoral cortex which has taken place in this experiment has not, as far as is known, previously been observed in the healing of this type of defect. It is thought that the osmotic effect of the alginate may play some part in this mechanism, but this suggestion requires further investigation.

The authors wish to thank Mr. J. B. Cuthbert who first drew their attention to the paper by Dr. G. Blum.

REFERENCES

AMIES, C. R. (1959). The use of topically formed calcium alginate as a depot substance in active immunization. J. Pathology and Bacteriology, **77** : 435.

BLUM, G. (1944). Phosphatase and the Repair of fractures. Lancet, **247** : 75.

BRIDGES, J. B., and PRITCHARD, J. J. (1958). Bone and cartilage induction in the rabbit. J. Anatomy, **92** : 28.

JOHNSON, F. R., and MCMINN, R. M. H. (1956). Transitional epithelium and osteogenesis. J. Anat. London, **90** : 106.

LECROIX, P. (1945). Recent investigation on growth of bone. Nature, **156** : 576.

LEVANDER, G. (1945). Tissue induction. Nature, **155** : 148.

MELCHER, A. H. (1960). The Healing mechanism of bone. M.D.S. Dissertation, University of the Witwatersrand, Johannesburg.

MELCHER, A. H., and DREYER, C. J. Protection of the blood clot in healing circumscribed bone defects (in the press).

PHEMISTER, D. B. (1935). Bone growth and repair. An. of Surgery, **102** : No. 2, 261.

URIST, M. R., and MCLEAN, F. C. (1952). Osteogenetic potency and new bone formation by induction in transplants to the anterior chamber of the eye. J. Bone and Joint Surgery, **34** A., 2nd April, 443.

STRESS BREAKING FOR PARTIAL DENTURES

JACK L. KABCENELL, D.D.S.

Reproduced from the J. Amer. D.A., 63 : 5, with due acknowledgments to the author and publishers

Many and varied stresses are imparted to the hard and soft tissues which bear a distal extension partial denture. The dentist must incorporate into the design of such a denture those factors which best help the tissue of the patient to remain in a healthy state. Some type of stress-breaking action must be incorporated into a Class II partial denture, particularly when the patient has weakened abutment teeth. The ideal stress-breaker does not exist. The dentist must evaluate the three main types of stress-breakers and select that type which provides the patient with maximum masticatory function and comfort and minimum damage to the supporting structures.

A FIXED prosthesis is the treatment of choice for the replacement of missing dental units. Such a prosthesis often is not possible, however. One of the chief contraindications to use of a fixed prosthesis is the absence of posterior abutments and the absence of more posterior units than could successfully be replaced by a cantilever fixed bridge. Such cases comprise the Class I and II (Kennedy) classification, and the treatment plan calls for the distal extension partial denture[1].

Any denture represents the imposition of an inanimate object onto a most animated region where it will be subject to countless multidirectional forces. It is not possible to analyse accurately all the mechanobiologic forces that will act on the denture and, therefore, the dentist cannot, with any great exactitude, eliminate or ameliorate all the harmful components of these forces. He can, however, be wary of the most serious offenders and attempt to design the denture so as to