EXCISION AND REIMPLANTATION OF THE EPIPHYSEAL CARTILAGE OF THE RABBIT

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The postnatal growth of a typical long bone is associated with activity within the epiphyseal plates, and within the articular cartilage which surmounts the ends of the bone. The early enlargement of the epiphysis takes place at the expense of both epiphyseal and articular cartilage, as the illustrations of Gottesleben (1939) clearly show. Later enlargement of the epiphysis occurs solely on its articular aspect (Boerema, 1942). The epiphyseal cartilage thus shows an early bipolarity, although its contribution to shaft elongation is always greater than its contribution to epiphyseal enlargement. With the formation of a terminal plate of bone upon its epiphyseal aspect, the epiphyseal cartilage loses this bipolarity, and its activities are restricted to new bone formation in the metaphyseal region. Payton (1933) has suggested that the region of this terminal plate is the site of absorption of epiphyseal bone. His deductions, however, from the madder-fed pig are circuitous and unsupported by the radiological evidence of Siegling (1941). The growth of the epiphysis itself has been fully discussed by Lacroix (1951).

In the present investigation the polarity of the epiphyseal cartilage has been investigated by experiments in which the plate has been excised and replaced in its normal site, either orientated normally, or rotated through 180° to bring the diaphyseal surface into contact with the epiphysis. The further growth of the bone, the extent, and the nature of the contribution from each end have been studied.

METHOD

Rabbits aged from 2 to 5 weeks were used. Each animal was anaesthetized with ether, and the bones of the forelimb approached by a longitudinal incision. The distal end of the ulna was identified, and exposed for most of its circumference by freeing and retracting the adjacent tendons. The epiphyseal cartilage was isolated by transverse cuts passing through the immediately adjacent metaphyseal and epiphyseal bone, and this growth cartilage, together with its perichondrium, and a thin sliver of adjacent bone was removed. The excised portion was then replaced, either in its normal position, or reversed. With the latter procedure, the metaphyseal aspect of the cartilage with its adjacent bone lay against the epiphysis, and the epiphyseal aspect against the diaphysis. The tendons were allowed to fall together to hold the fragment in position, and the skin was sutured. No immobilization was necessary.

Each animal was radiographed after the operation and thereafter at weekly, and later fortnightly intervals. Animals were removed from the mother at 6 weeks and subsequently kept in a large wire run. They received a normal mixed diet supple-

mented by pellet feeding. At varying periods up to 28 weeks biopsies were performed. The animals were killed with Nembutal and the limbs radiographed. The bones of the forelimb were fixed in formol saline or Susa, and later decalcified. Following decalcification the distal ends of the radius and ulna were removed and sectioned.

All radiographs were taken with the animal in the prone position, each limb flexed at the elbow and the paw outstretched to give a standard dorso-ventral view of the distal part of the limb, and a lateral view of the proximal part. The tube-film distance was constant at 100 cm.; it can be demonstrated that at this distance the error due to magnification is less than 0·5 mm. and is equal on both sides. Each ulna was measured from its most proximal point to the centre of the distal articular surface to give its total length. The length of the distal epiphysis was measured from the centre of the translucency representing the epiphyseal plate to the centre of the articular cartilage. This measurement, when the epiphyseal plate is broad in the young animal, is about 1 mm. greater than that of the bony epiphysis.

OBSERVATIONS

(*a*) *Excision and reimplantation in normal position*

The postoperative growth of the sixteen animals in this series is recorded in Table 1. The radiographs of thirteen of these animals show fusion of the metaphysis and epiphysis with the bony edges of the reimplanted epiphyseal cartilage within the

Table 1. *Excision and reimplantation of distal ulnar epiphyseal cartilage*

No.	Survival (weeks)	Growth (mm.)		Shortening (mm.)
		Operated	Control	
195	1	1	1	0
198	1½	3	3	0
197	2	4	4	0
219	2	6	7	1
220	2	5·5	7·5	2
196	3	5	6	1
296	3	8	9·5	1·5
222	5	10	10	0
143	7	22	24	2
104	8	13	26	13
90	9	12·5	27	14·5
140	11	28·5	29	0·5
142	11	26	27	1
84	19	20·5	32·5	12
141	24	39·5	40	0·5
75	28	36	41·5	5·5

first 14 days. Subsequent growth was, on the whole, undisturbed, shortening in the operated limb being less than 2 mm. in twelve animals and totalling 5·5 mm. in the thirteenth. The slight shortening occasionally observed was mainly apparent only towards the end of the period of growth.

The three animals in which little or no postoperative growth occurred merit consideration in detail. Failure of growth in rabbit 104 was associated with absorption of the graft followed by bony union between epiphysis and diaphysis. The graft in rabbit 90 at first appeared to grow in a normal manner, but subsequently flakes of calcified tissue appeared within the epiphyseal cartilage and premature epiphy-

seal fusion followed. The early radiographs of rabbit 84 showed the graft in position, and normal growth occurring. After 5 weeks, however, cross-union was apparent between the ulnar epiphysis and the shaft of the radius, probably due to damage of the periosteum of the latter during the operation. As a result of this cross-union further growth of the ulna resulted first in a dislocation of the radius, and secondly in a dislocation of the ulna at the elbow (Text-fig. 1). The strain to which the epiphyseal cartilage was subjected was probably responsible for its subsequent fusion.

Text-fig. 1. Cross-union between radial shaft and ulnar epiphysis has produced dislocation of the radius at the elbow.

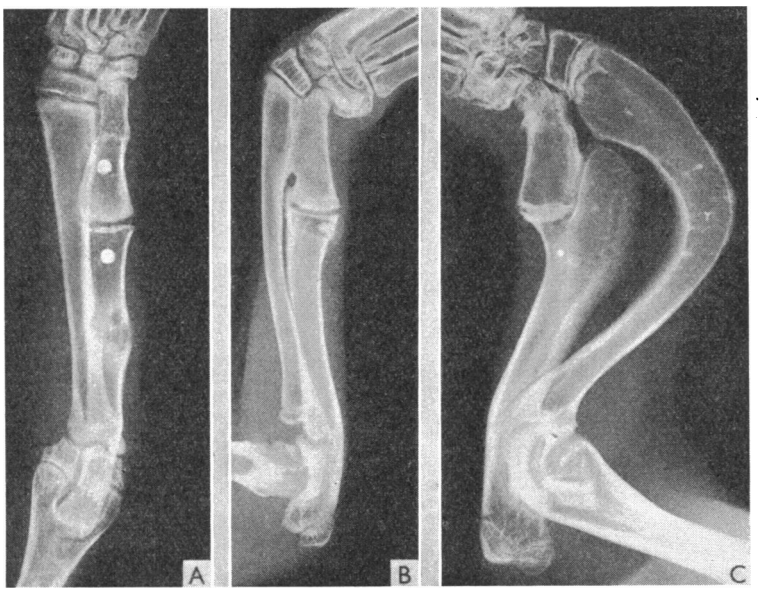
Examination of the histological sections showed that the epiphyseal cartilage remained intact. The bone of the ulnar epiphysis and diaphysis united with the thin plates of bone on either side of the reimplanted cartilage within the first 2 post-operative weeks. The site of the union was for some time marked by an irregularity in the lamellae of the bone. The epiphyseal cartilage was at first considerably longer than normal, mainly due to an increase in length of the cartilage columns. This change was most marked during the third postoperative week. Sections from animals killed after a longer interval showed that the cartilage returned to its normal proportions, but in most animals showed a pronounced irregularity in the arrangement of its columns. In the centre of the epiphyseal cartilage an irregularity and enlargement of the cells of the reserve zone was often apparent, and was often associated with bone invasion from the epiphysis (Pl. 1, fig. 1).

Sections were available from two of the three animals which failed to grow. Rabbit 104 showed no trace of an epiphyseal cartilage. Sections of rabbit 90 showed a thin quiescent epiphyseal plate surrounded by irregular deposits of bone.

(*b*) *Reimplantation in reversed position*

The measurement of the radiographs of the nineteen animals falling into this group are recorded in Table 2. Animals followed for more than 2 weeks showed a shortening which in general increased as the age of the animal advanced. The total postoperative growth of the ulna increased to a maximum of 18·5 mm. This growth included contributions from both proximal and distal ends of the bone. Comparison of the size of the distal epiphysis on both operated and control limbs showed that in each case the epiphysis was longer than its fellow (Text-fig. 2A). Direct comparison of the radiographs showed that the epiphysis was also broader. The increase in size of the distal epiphysis occurred mainly in the first 6 weeks after reversal, and subsequent growth was slight or absent. Since shortening was present throughout the whole series, in no case was the activity of the reversed epiphyseal cartilage as great as that of the normal. Cross-union of radial shaft and ulnar epiphysis was seen in seven animals, but growth of the distal epiphysis was not sufficiently vigorous to produce a complete dislocation at the elbow (Text-fig. 2B).

In most animals the epiphysis started to fuse with the shaft after 5 or 6 weeks, although occasionally a thin epiphyseal line was demonstrable until biopsy many weeks later. The retardation of growth was associated with an outward curvature of the radius. Where this curvature of the radius was particularly pronounced, considerable new bone formation was seen on the inner aspect of the curve. It was often associated with a spur of bone on the radial aspect of the ulnar metaphysis (Text-fig. 2C).



Text-fig. 2. A. Elongation of the ulnar epiphysis following reversal of the cartilage. The lead markers were inserted some weeks after operation and do not indicate the rate of bone growth. B. Cross-union between radial shaft and ulnar epiphysis following reversal of ulnar epiphyseal cartilage. Further growth of the ulna has only produced slight displacement of the radius at the elbow. C. Marked shortening of the ulna and curvature of the radius following reversal of the ulnar epiphyseal cartilage.

The contribution of the reversed epiphyseal cartilage to shaft elongation is difficult to assess, although in certain animals the insertion of shaft markers permitted comparisons with the control. During the first two postoperative weeks, the distance between the marker and the epiphyseal cartilage occasionally increased; this effect was always slight, but was a little more conspicuous in the younger animals. No valid shaft growth from the distal epiphyseal cartilage was demonstrable radiologically after this period.

Examination of the sections of these animals demonstrated that union of the bony edges of the reversed fragment with the adjacent bone of the ulna was complete within 2 weeks. Six days after operation the epiphyseal cartilage was united proximally by fibrous tissue to the shaft of the ulna. The cartilage itself was longer than normal, mainly due to an increase in the length of the cartilage columns, but the fragment of metaphysis left attached to the cartilage after the operation was no

longer visible (Pl. 1, fig. 2). Sections from animals killed during the following week showed an increasing irregularity of the cartilage columns, and a patchy re-organization of the epiphyseal bone into a new 'metaphysis'. Irregularity of the epiphyseal cartilage ceased when this 'metaphysis' was fully organized, and by the fifth post-operative week the epiphyseal cartilage had returned to its normal proportions. Sections at this time showed a normal epiphyseal plate, although structurally and functionally reversed. The metaphysis lay on the epiphyseal aspect and gave rise in the usual way to a perichondrial ring (Pl. 1, fig. 3).

Table 2. *Excision of distal ulnar epiphyseal cartilage and reimplantation in reversed position*

No.	Survival (weeks)	Growth (mm.)		Shortening (mm.)	Growth of distal epiphysis (mm.)	
		Operated	Control		Operated	Control
190	1	0·5	1	0·5	*	*
191	1½	2·5	2·5	0	*	*
192	2	1	3	2	*	*
145	3	7	9	2	5	1·5
147	3	6·5	9	2·5	3	1·5
194	5	4·5	7·5	3	4·5	0·5
193	6	6	10·5	4·5	4·5	0
57	8	5·5	13	7·5	4·5	0·5
129	8	12	25	13	7	3
65	9	7·5	15·5	8	7	0·5
78	10	8·5	25	16·5	5·5	1·5
128	10	10	29	19	4·5	3
62	11	10·5	18	7·5	7	0·5
76	11	11·5	26·5	15	8	1
61	13	14·5	31·5	17	9	0·5
67	13	7·5	18	10·5	6·5	0·5
144	20	16	40	24	7·5	1
130	22	14	50·5	36·5	9·5	3
131	22	18·5	46·5	28	10	3

* Not measured.

In the later sections the epiphyseal cartilage was seen to become narrow, and finally quiescent, although in most animals it still showed a reversal of its normal polarity (Pl. 1, fig. 4). In three animals complete bony fusion appeared to have occurred between epiphysis and diaphysis.

DISCUSSION

The growth which occurs after excision and reimplantation of the epiphyseal cartilage was first described by Helferich in 1899. In these experiments the distal epiphyseal cartilage of the ulna of the dog was excised and replaced, and normal growth was observed to follow. The histological changes in Helferich's material were described in some detail by Enderlen (1899). The bone cells attached to the reimplanted fragment died, but the cartilage, although for some time showing a broadening and an irregularity of its structure, remained to contribute to the normal growth in this area. No growth was detected if the epiphyseal cartilage was boiled in physiological saline before reimplantation. The studies of Helferich were amplified by Heller (1914, 1917) who also described the enlargement of the epiphysis which follows reversal of the epiphyseal cartilage. A further contribution to this problem was made by Sousa

Pereira (1937) who confirmed the earlier observations and also demonstrated the failure of the epiphyseal cartilage to grow when transplanted to the shaft of the ulna. The experiments of Haas, however, which were first reported in 1915, and later repeated in 1931, show no growth on reimplantation or reversal of the cartilage. Haas suggests that the elongation after operation may be due to incomplete removal of the epiphyseal cartilage, but the weight of the evidence is clearly against this view.

In an examination of the growth which follows simple reimplantation of the epiphyseal cartilage it is apparent that the operation causes little disturbance of bone growth. Since growth does not follow if the epiphyseal cartilage is boiled prior to reimplantation, it would appear that it is the original cartilage which contributes to bone elongation. This view is confirmed by the histological studies which show persistence of the original transplant, and no evidence of cartilage regeneration.

If the epiphyseal cartilage is replaced in the reversed position, it retains for some time its original polarity, and an enlarged bony epiphysis is produced. The well-organized 'metaphysis' which is produced within the epiphysis suggests that this region is organized by the epiphyseal cartilage, or at least that its structure is determined by the activities of the cartilage. Most of the animals used in these experiments were of an age in which the epiphyseal cartilage has lost its original bipolarity, but in three animals aged 3 weeks at operation there was no evidence that the reversed epiphyseal cartilage made any significant contribution to shaft elongation. Whilst reversal of the epiphyseal cartilage allows growth in length to continue, the rate of growth is always slower than that of the control, and terminates after 6–8 weeks. There is no direct evidence in these experiments to suggest a constant cause for the cessation of growth. Cross-union between radial shaft and ulnar epiphysis is common, and leads to considerable compression of the ulnar epiphyseal cartilage. It has been shown by Blount & Clarke (1949) that pressure across the epiphyseal cartilage restricts its growth, but animals in which cross-union did not occur still failed to grow after the first few weeks. It may well be that the blood supply of the distal epiphysis of the ulna is inadequate to maintain the nutrition of the cartilage and of the new 'metaphyseal' area. As the epiphysis increases in size its cartilage retreats further from the vessels which supply it. Examination of the radiographs suggests that the epiphysis attains a length of 12–16 mm. under these conditions before growth stops.

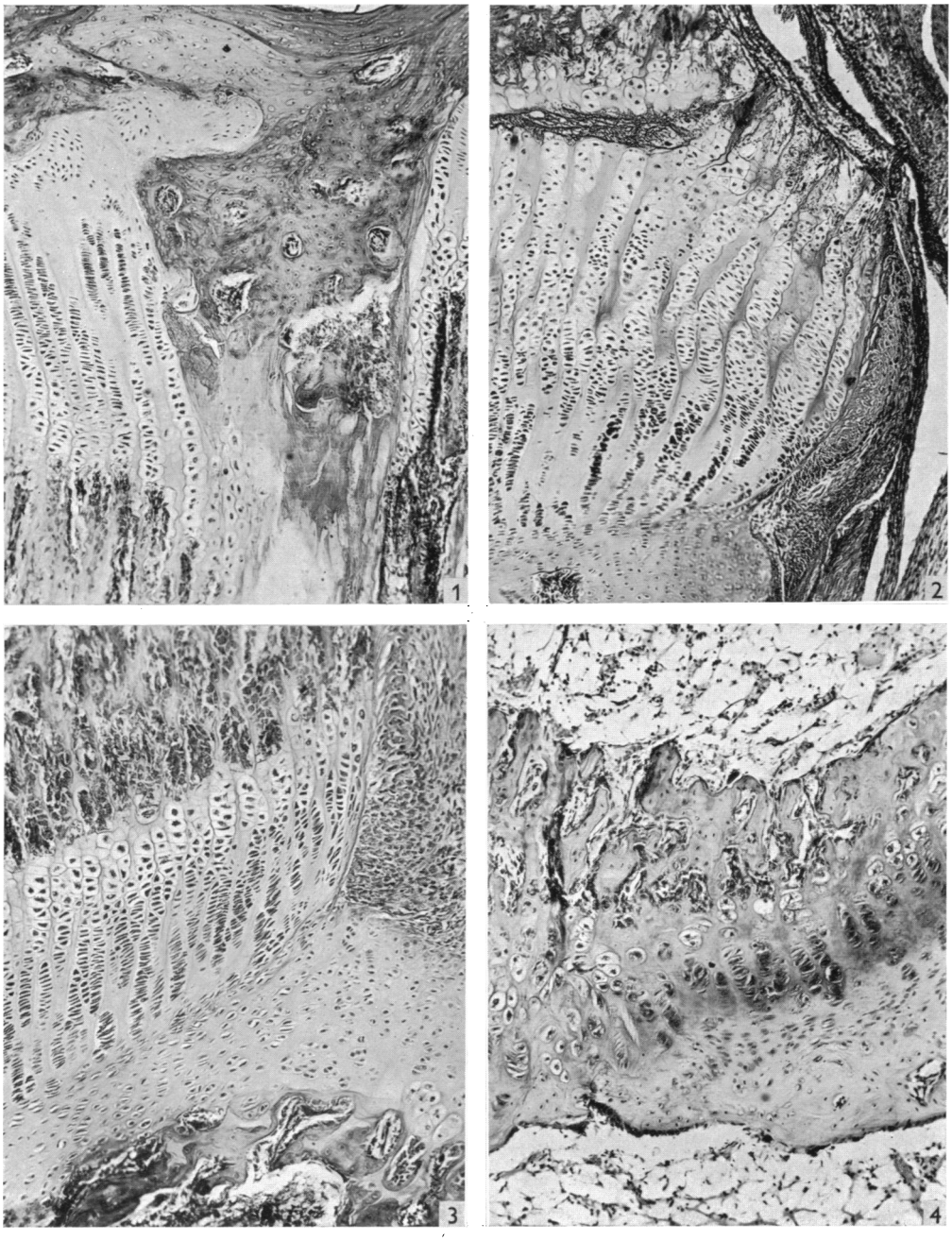
These experiments confirm that the activities of the epiphyseal cartilage are largely predetermined, and that its polarity is determined by its structure rather that its site. Whilst the mode of growth is uninfluenced by changes in its orientation, the rate of growth is restricted when the cartilage is reversed.

SUMMARY

1. The effect upon bone growth of excision and reimplantation of the epiphyseal cartilage has been studied.

2. If the epiphyseal cartilage is replaced in its normal position, the subsequent growth of the bone is undisturbed.

3. Excision and reversal of the epiphyseal cartilage leads to enlargement of the bony epiphysis, the cartilage retaining its original polarity.



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4. Growth of the bone following reversal of the epiphyseal cartilage is slow, and ceases after 6–8 weeks.

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REFERENCES

BLOUNT, W. P. & CLARKE, G. R. (1949). Control of bone growth by epiphyseal stapling. *J. Bone Jt. Surg.* **31**A, 464–478.

BOEREMA, I. (1942). Über das Knochenwachstum. *Acta neerl. morph.* **4**, 365–377.

ENDERLEN, E. (1899). Zur Reimplantation des resecirten Intermediärknorpels beim Kaninchen. *Dtsch. Z. Chir.* **51**, 574–598.

GOTTESLEBEN, in SCHINZ, H. R., BAENSCH, W. & FRIEDL, E. (1939). *Lehrbuch der Röntgendiagnostik*, **1**, 63–65. Leipzig: G. Thieme.

HAAS, S. L. (1915). The experimental transplantation of the epiphysis with observation on the longitudinal growth of bone. *J. Amer. Med. Ass.* **65**, 1965–1971.

HAAS, S. L. (1931). Further observation on transplantation of the epiphyseal cartilage plate. *Surg. Gynec. Obstet.* **52**, 958–963.

HELFERICH, H. (1899). Versuche über die Transplantation des Intermediärknorpels wachsender Röhrenknochen. *Dtsch. Z. Chir.* **51**, 564–573.

HELLER, E. (1914). Experimentelle Untersuchungen über die Transplantation des Intermediärknorpels in Form der halbseitigen Gelenktransplantation. *Arch. klin. Chir.* **104**, 843–954.

HELLER, E. (1917). Versuche über die Transplantation Knorpelfuge. *Arch. klin. Chir.* **109**, 1–62.

LACROIX, P. (1951). *The Organization of Bones.* London: Churchill.

PAYTON, C. G. (1933). The growth of the epiphyses of the long bones in the madder-fed pig. *J. Anat., Lond.*, **67**, 371–381.

SIEGLING, J. A. (1941). Growth of the epiphyses. *J. Bone Jt. Surg.* **23**, 23–36.

SOUSA PEREIRA (1937). Le renversement du cartilage du conjugaison et son influence sur le travail d'ossification enchondrale et la croissance de l'os. *Lyon chir.* **34**, 513–541.

EXPLANATION OF PLATE

All sections are displayed with the distal end of the ulna to the top of the plate.

Fig. 1. The reimplanted epiphyseal cartilage 11 weeks after operation. The centre of the cartilage is ossified, but this bone does not completely unite epiphysis and metaphysis. ×80.

Fig. 2. Six days after reversal of the epiphyseal cartilage. The cartilage columns are long and irregular, and are calcified distally. ×72.

Fig. 3. The periphery of the epiphyseal cartilage 5 weeks after reversal. Reversal of structure and function now appears complete. The metaphysis lies on the perichondrial epiphyseal aspect of the cartilage. The perichondrial ring is continuous with the periosteum of the epiphysis. ×96.

Fig. 4. The epiphyseal cartilage 8 weeks after reversal. The cell columns are still directed towards the epiphysis. Absence of a new 'metaphysis' suggests that growth has ceased and this is confirmed by radiological measurements. ×88.