THE VASCULAR CONTRIBUTION TO OSTEOGENESIS

IV. The Effect of Pressure upon the Epiphysial Cartilage of the Rabbit

JOSEPH TRUETA, OXFORD, ENGLAND and ANTONI TRIAS,† CHICAGO, UNITED STATES OF AMERICA

From the Nuffield Orthopaedic Centre, Oxford

In the previous paper of this series (Trueta and Amato 1960) the effects of reducing or suppressing the blood flow to either the epiphysial or the metaphysial side of the growth cartilage were reported. The most striking findings were the extreme dependence of the life of the chondrocytes—the only known agents of longitudinal growth of long bones—on the blood supply of the epiphysis and the lack of participation of the metaphysial blood supply in maintaining the life of the cells of the epiphysial cartilage. It was found that, on the contrary, the vascular activity of the metaphysial side of the growth cartilage was responsible for the death and removal of hypertrophic cartilage cells, for when the blood flow of these metaphysial vessels was discontinued calcification was prevented and the hypertrophied chondrocyte survived for a long time.

When this finding was made, early in our research, it became interesting to investigate the part that pressure plays in the alteration of the normal rate of blood supply adjacent to the growth cartilage. It also was considered important to collect further data on the influence of pressure on the mechanism of growth apart from its action upon the vessels.

MATERIALS AND METHOD

For this work, as for the preceding one, the rabbit only was used. All the animals were from six to eight weeks old (except numbers 14 and 15 which were ten and twelve weeks respectively); their weights ranged from 1·25 to 1·5 kilograms. The rabbits were prepared by the technique described by Trias (1961). As in his work, compression of the epiphysial cartilage was produced by the use of a clamp with springs and pins aseptically placed above and below the growth cartilages of the right distal femoral and proximal tibial epiphyses (Fig. 1), the left hind limb being used as a control. To prevent flexion at the knee, and consequent decrease of pressure across the growth cartilage, a plaster reaching from foot to groin was applied in most experiments. In a few the knee was allowed to flex, so that the area of greatest pressure across the epiphysial cartilage shifted progressively backward. This displacement of the areas of greatest pressure permitted a study of the changes produced in the growth cartilage by the constricting force in varying positions. Radiographs of both hind limbs were taken at the end of the procedure.

† Formerly Girdlestone Scholar in Orthopaedic Surgery.
The animals were killed with an overdose of ether. This was followed by an intra-arterial injection of a mixture of equal parts of 2 per cent Berlin blue and Micropaque (barium suspension) or, in some animals, Indian ink. The amount varied according to the weight of the animal: on average some 40 millilitres of the mass were perfused.

After removal of the soft tissues and disarticulation of the tibia and femur of both limbs the four bones were radiographed again. Finally, the distal ends of the femora and the proximal ends of all the tibiae were embedded in celloidin, and histological sections were cut and prepared by the methods described by Truea and Harrison (1953).

Of the thirty-one rabbits used in this experiment, two died from anaesthesia at operation and another was discarded because the position of the clamps was unsatisfactory.

Table I shows the number and duration of experiments and the survival time after removal of the pins and clamp.

<table>
<thead>
<tr>
<th>TABLE I</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DURATION OF COMPRESSION</strong></td>
</tr>
<tr>
<td>Days</td>
</tr>
<tr>
<td>Number of animals</td>
</tr>
</tbody>
</table>

Nine of these rabbits were allowed to survive for a varying length of time after the removal of the pins and clamp at different stages. The shortest survival time was two days and the longest seventy-five days. In describing our findings we will refer particularly to this group.

**Measurement of pressure**—The use of springs between the head of the pressure clamp and the pin nearest it causes a compressing force which we have attempted to measure. It also allows for some expansion of the area between the pins. This precaution was considered necessary because of the excessive increase in pressure which would be caused by the division and growth of the cells of the growth plate.

A force of between 3.5 and 4.5 kilograms was required to shorten our springs, unloaded, to half their length. Since each clamp contained two similar springs, the compressing force acting on the pins varied from 7 to 9 kilograms (15 to 20 pounds) when the springs were reduced to half their length. Since the pins were placed at an almost constant distance from the growth cartilages, the pressure upon these was estimated from the formula \( P = \frac{F}{S} \) where \( P \) = pressure, \( F \) = force and \( S \) = surface area. The area of contact between the two opposing epiphyses was considered of the order of one-third of a square centimetre. Therefore \( P = \frac{8 \times 3}{1} \) (average) = 24 kilograms per square centimetre. Since the growth cartilages were situated much closer to the pins than was the area of pressure in the joint surfaces, a substantial increase in the area of distribution of pressure at the level of the growth cartilages had taken place (Fig. 2). This meant that the cone of pressure had increased to at least twice its size, thus making the formula \( P = \frac{8}{1} \) centimetres = 8 kilograms per square centimetre or somewhat less. This resulted in less damage to the growth cartilage than that caused to the articular...
Fig. 3
Triangular thickening of the plate limited to the areas of greatest pressure. (×20.)

Fig. 4
Widened epiphysial femoral cartilage with a central area of degeneration. (×120.)
Thinning of growth plate on the left while on the right the widening still persists. (× 25.)

Vascular bridge preceding the bone formation across the bone plates. (× 240.)
cartilage by the same compressing force. Nevertheless, it maintained the greatest changes in that part of the growth cartilage which was intersected by the cone of pressure. In the study of these changes it was fortunate that in a number of animals the position of the tibia changed during the experiment, for it allowed us to study the changes of the epiphysial cartilage in every position, including extreme flexion.

RESULTS

After the application of persistent pressure of the nature described, the most common finding in the epiphysial cartilage was an increase in its height, frequently limited to the areas of greatest pressure. The earliest increase detected was found to occur after about three days and affected both the femoral and the tibial growth cartilages. At this early stage the increase affected only the restricted area of greatest compression—that intersected by the cone of pressure (Fig. 3). After seven days of pressure the whole area of the epiphysial cartilage became widened in almost all cases. In some rabbits signs of disorganisation of the columnar arrangement of the growth plate were observed in the areas of strongest pressure (Fig. 4); different segments of the columns began losing their boundaries and it became difficult to distinguish between the proliferative and the hypertrophic zones. The nuclei were not stained and finally the cell limits were no longer visible.

In animals with the clamps left for longer periods the whole of the compressed area of the epiphysial cartilage broke down and in these rabbits in which the pressure was maintained for durations up to a maximum of twenty-one days, the growth plate became thinner and thinner (Fig. 5), until it was seen invaded by vessels and eventually cut by a long bridge of bone between epiphysis and metaphysis (Fig. 6).

Mechanism of epiphysial cartilage thickening—It was of particular interest to investigate the way in which the growth cartilage increased in height. As mentioned above, this increase was the earliest change detectable in the growth cartilage under pressure. It was not due so much to the chondrocytes getting bigger but to their getting more numerous. Repeated counts in all the animals studied gave a constant histological picture of the part taken in the increase by the different sections of the epiphysial cartilage. No change was found in the number of germinial and proliferative cells remaining within the normal limits of twenty to thirty (Trueta and Morgan 1960). On the other hand, the number of hypertrophic cells varied in accordance with the increase in total thickness of the cartilage, amounting in some cases to as many as eighty or ninety (Fig. 7) instead of the normal ten to twelve. Beyond that number we could not find a further increase. It appeared that a point was reached beyond which the cells of the hypertrophic zone could not remain alive as they became squashed against each other and against a fibrous barrier which appeared at the distal end of the columns (Fig. 8) until the cells degenerated and finally died.

After the examination of some hundreds of sections it became apparent that those thin growth cartilages examined in the late stages of degeneration and disorganisation in all probability had been preceded by an earlier phase of cartilage thickening.

When the hypertrophic cartilage cells remained for a longer time without being removed the enormously enlarged columns often lost their ability to grow straight and became curved with their convexity towards the periphery of the metaphysial region (Fig. 9).

The metaphysial end of these enormous columns was covered by a band of degenerate fibrous tissue well supplied with vessels at its deepest side. As this fibrous band was only found where the hypertrophic cells were firmly compressed against each other, we believe the death of these cells to be due to their inability to carry on their required interchanges in such a confined area. Sometimes chondrocytes could still be detected in their fibrous band and even beyond (Fig. 10).

The examination of sections from areas of growth cartilage affected by severe disorganisation and vascular invasion showed that, without exception, the compression had
FIG. 7
Enormously long columns of hypertrophic cells caused by compression. (×300.)

FIG. 8
The fibrous barrier at the ends of the columns of hypertrophic cells. (×90.)
affected the epiphysial side of the plate even more than the metaphyseal side. These areas of epiphysial damage included severe necrotic changes of the epiphysial trabeculae and particularly of the bone plate (Fig. 11). The osteocytes of these areas were dead or showed clear signs of degeneration, and the continuity of the bone plate, which in the normal is only interrupted by the narrow vascular canals, had large gaps through which profuse vessels penetrated deep into the growth cartilage proper (Fig. 12). In all cases free from degeneration, irrespective of the thickness of the growth cartilage, the germinal and proliferative layers of the columns were completely normal, both in their staining and reproductive ability. It was also noted that while the metaphyseal side of the epiphysial cartilage always became convex, the bone plate covering the growth cartilage at its epiphysial end usually remained flat.

When finally the vascular penetration gave place to the formation of a bone bar, it was always after the vascular progression from both ends had bridged the height of the epiphysial cartilage.

Histological and vascular changes after removal of the compressing clamps—This study was based on the nine rabbits which were allowed to survive for from two to seventy-five days after the clamp had been in position for twelve, fifteen or twenty-one days.

The most striking feature in most of the animals was the rapidity of calcification-ossification after removal of the clamp. The columns became incorporated with the primitive bone (Fig. 13)
Severe damage to the bone plate covering the epiphysial cartilage, with profuse vascular invasion. (×250.)

The vascular invasion across the dead bone plate reaches the area of germinal layer. (×400.)
Fig. 13
Rapid ossification of the columns of hypertrophic cells after removal of compression. (×70.)

Fig. 14
Great vascular activity at the metaphysial side of the growth cartilage. (×300.)
FIG. 15
The fibrous band at the end of the columns of hypertrophic cells remains free for some time from being the site of preliminary bone. (x 700.)

FIG. 16
Cell capsules in the process of being converted into bone. (x 160.)
precisely up to their normal level for the upper tibial growth plate; that is, leaving the first eight to twelve hypertrophic cells untouched. In some sections of well injected material, we have been able to witness the rapid progress of the vessel loops back towards their normal level in the growth cartilage (Fig. 14). The rate of vascular progress could be better appreciated in those rabbits in which progressive flexion of the knee caused a steady displacement of the cone of greatest pressure towards the back of the growth cartilage, situated anteriorly to the zone of greatest pressure at the time the animal was destroyed; this showed how the vessels progressed towards their normal place of occupation. One circumstance was, nevertheless, indispensable for the rapid incorporation of the enormous mass of hypertrophic cells, namely, that no obvious signs of degeneration were present. When evident fibrotic or necrotic changes of the cells were observed, the affected area remained free for some time from being the seat of preliminary bone (Fig. 15).

The pattern of the new bone was always the same (Fig. 16). Small irregular spaces, enlarged remains of the cell capsules, were surrounded by newly calcified cartilage without, as yet, any osteoblasts and even less osteocytes appearing in it.

Metaphysial trabeculae adjacent to the growth cartilage were found often to have dead osteocytes. This gave evidence of the amount of compression under the growth cartilage. But in this, as well as in the animals with only mild changes in the hypertrophic layer, the limits of the enlarged growth cartilage were clearly seen, even several days after the area of hypertrophic cells had become calcified.

Growth changes after continual compression—All the rabbits had radiographs of both hind limbs taken at the beginning and end of the experiments. For this investigation only the tibia was considered suitable. In those animals in which the length of the tibia had not been altered during preparation, both tibiae were compared for length after removal of soft tissue.

It was found that a compression of less than seven days does not permanently affect growth. Compression for from seven to ten days had shortened by one millimetre all the tibiae except for one in which there was no alteration of length. All the tibiae subjected to compression for over twelve days had two or more millimetres of shortening, with the exception of two rabbits (numbers 12 and 38) which showed two millimetres of shortening after only seven and eight days respectively. The greatest shortening found was four millimetres after sixteen days of compression.

Several of the animals allowed to survive for varying durations after removal of the clamp showed increasing deformity, particularly of the tibia. The deformity varied, but flexion was the most frequent; this was due to the growth of the anterior part of the epiphysial cartilage which still remained active when the posterior side was not growing any more. It was rare to find varus or valgus deformity from unbalanced growth (Fig. 17). In some animals fine metal markers in the bone aided detection of changes in growth responsible both for the shortening and the deformity.

The study of the vascular pattern by the perfusion method described earlier, showed that the vascularity at the metaphysial end of the growth cartilage was prevented from progressing along the columns of hypertrophic cells while it remained normal at its epiphysial side (Fig. 18).

In all growth cartilages which showed degeneration the epiphysial side of the cartilage
Figure 18—Vessels of the metaphysial side of the growth cartilage unable to progress along the columns of hypertrophic cells. (× 250.) Figure 19—Vessels from the epiphysial side of the cartilage advancing towards the depths of the columns in severe degeneration. (× 250.)

showed a vascular disorder, characterised by early ischaemia followed by profuse, irregular vascular proliferation (Fig. 19).

Calcification—We had special interest in the effect of compression on calcification. We examined particularly the metaphysial side of the columns with the help of the light microscope, the electron microscope and microradiography. In all the animals in which this study was made it was found that the area of calcification remained constant at about the level reached at the time the clamps began to work. Thus, compression caused interruption of the progressive calcification which normally precedes the first phase of osteogenesis.

DISCUSSION

To emphasise the main points for discussion, it seems appropriate first to summarise our findings. The present investigation has shown that continuous severe compression of the epiphysial cartilage of the rabbit alters its normal function in the following manner.

Stage one: up to seven days of compression there is an inhibition of degeneration of the hypertrophic cells at the end of the growth columns. This allows the cells to survive for an, as yet, indefinite number of days: in some of our experiments, up to more than twelve days. During all this time cells from the proliferative section of the columns continue to develop into hypertrophic cells while the rate of cell division at the proliferative level of the columns remains apparently normal. In these circumstances the growth cartilage at the area of greatest pressure may develop to about four times its normal height.
Stage two: at approximately eight to ten days after the initiation of compression, the continuous accumulation of the new cartilage cells and their lack of subsequent removal at the metaphysial end of the epiphysial cartilage causes the compression to increase. This occurs despite the increase in the distance between the pins, because of compression of the springs of the clamp. At about ten days changes appear in the epiphysial end of the growth cartilage. From then on, the suffering of the cartilage cells and their organisation appears progressively greater until the next phase is reached.

Stage three: at about fourteen days or more the disorganisation is, in general, severe and mostly irreversible. The bone plate covering the epiphysial side of the cartilage has dead cells and appears broken into fragments. The new addition of cells by division of the proliferative section is interrupted and shortly after this, irregular vascular invasion occurs from both ends, despite the continuation of compression.

Stage four consists of the fusion of the growth cartilage by bone formation following the vascular invasion of the previous phase, beginning by establishing a narrow bone bridge across the growth plate.

It is interesting that compression has two early features, namely the “protection” of the hypertrophic cells and the lack of action upon cell reproduction in the proliferative section of the cartilage.

It is also interesting that the normal regular progression of the vascular loops at the metaphysial end of the growth plate is interrupted from the first stages of compression. Another important observation is the prevention of calcification of the matrix along the intercolumnar spaces. It appears that the first row of hypertrophic cells, not yet surrounded by a calcified matrix at the beginning of compression, remains alive until the increasing compression causes them to disintegrate in the last stages of the experiment.

The return of the growth cartilage to normal function after compression has been discontinued depends on the severity of the damage suffered by the epiphysial side of the growth cartilage. Where no serious damage has been caused to the epiphysial side of the columns, total regeneration takes place very rapidly and is always preceded by the realignment of the vessels of the metaphysial side of the cartilage. Calcification in the intercolumnar matrix up to the normal levels recurs within four days of the removal of the compression. Concurrently, the vessels of the metaphysial side of the growth cartilage penetrate the cartilage columns to their normal level.

In the preceding paper of this series (Trueta and Amato 1960) it was reported that the interruption of the vascular supply at the metaphysial side of the growth cartilage caused calcification to cease and allowed the hypertrophic cells to survive for a long time. On the other hand, the interruption of the blood flow at the epiphysial side of the cartilage caused severe permanent damage which, on many occasions, ended by fusion of the growth plate.

The present work shows that similar changes are produced by constant pressure. This suggests that the mechanism of action is alike in both cases, namely, by interruption of blood flow.

It has been shown by the use of compression that the epiphysial blood supply is much better protected against excessive pressure than the metaphysial blood flow. Probably the solid roof constituted by the bone plate protects the net of vessels underneath it responsible for the nourishment of the germinal and proliferative section of the epiphysial cartilage.

CONCLUSIONS

From this work it may be concluded that persistent compression affects the growth plate by interference with the blood flow on one or both sides of the growth cartilage.

Despite exertion of the same pressure upon both sides of the growth plate, only the metaphysial side was readily affected in the early stages, for, as long as no damage was caused to the epiphysial side of the growth cartilage, the lesions were fully reversible.
Interference with growth was directly proportionate to the damage caused by compression to the epiphysial side of the growth plate and, in general, to the duration of compression. The first signs of interference with the metaphysial side of the plate were the lack of vascular progression and concomitant retardation of calcification.

When severe degeneration was not present the growth cartilage recovered within four days. The matrix was ready for calcification all the time, as shown by the extremely rapid calcification occurring soon after the compression had ceased and the vessels were able to reach their proper place.

It seems justified to believe that the first hypertrophic cell not to be calcified after removal of the clamp is the one around which the matrix has not yet changed sufficiently to have an affinity for the apatite crystals. As in moderate compression, the division of the proliferative cells continues and it seems it must be the age, or even more likely the distance from the transudate coming from the epiphysial side of the growth cartilage that conditions the maturity of the cell, which prepares the field for calcification and thus initiates the osteogenic process.

Views similar to this have been advanced by Ham (1957) and his school.

REFERENCES


