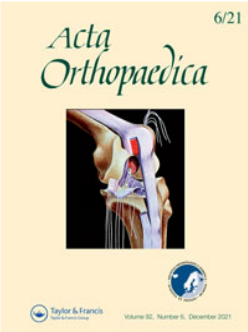

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The Morphology of Growth Cartilage Using the Scanning Electron Microscope

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THE MORPHOLOGY OF GROWTH CARTILAGE USING THE SCANNING ELECTRON MICROSCOPE

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The growth cartilage remains the centre of attention of many authors (Sundén 1967). The possibility of its observation using the transmitting electron microscope has been an outstanding scientific advance (Trueta 1968). However, due to the complicated spatial organization of the growth cartilage, information on the three-dimensional configuration, which would objectively show the morphology of epiphyseal growth, has been missing. This can be afforded by scanning electron microscopy (Horn et al. 1972, Fujita et al. 1971, Köhler 1973) as used by the present authors, who studied an experimentally induced traumatic epiphyseolysis in rabbits.

MATERIAL AND METHODS

The authors carried out their experiments on 20 domestic rabbits (albinos), 4 weeks old. The distal ends of the femurs were isolated, immediately post-mortem, by rapid dissection and bent to imitate the separation of the epiphysis. Both ends of the specimens were immediately fixed in 10 per cent formaldehyde, and then dehydrated in acetone of graduated concentrations. Finally they were adhered to an observation table and shadowed with gold using a Balser apparatus. The specimens were then observed under the scanning electron microscope (Cambridge), 10–25,000 × magnification. The specimens were fixed simultaneously, and studied by the usual histological methods.

FINDINGS

The transverse break of the epiphyseal plate most often ran through the area of hypertrophied cells. To obtain other sections of the epiphyseal plate in the break, the authors were obliged to break a larger number of specimens and sometimes even cut the cartilage in the appropriate zone before breaking it. Thus they managed to obtain breaks of different layers; for example, the layer of proliferating cells,

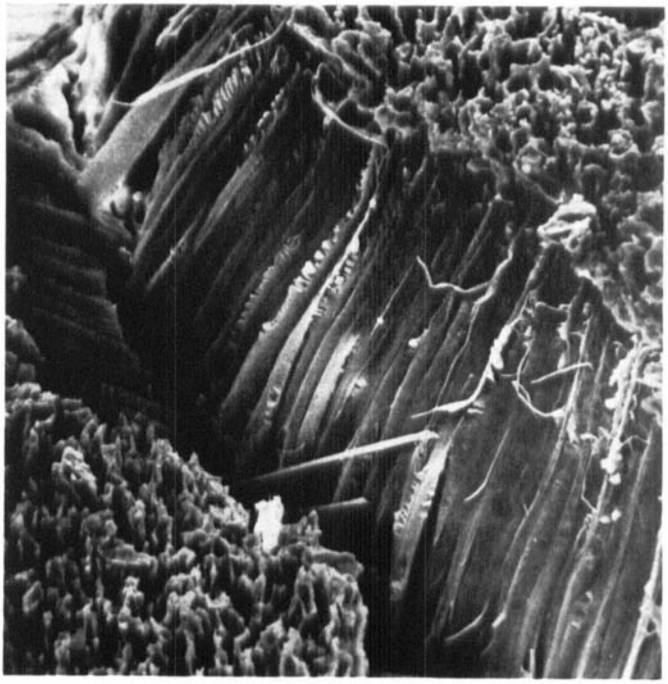

Figure 1. A longitudinal section of epiphyseal cartilage with a layer of proliferating cells and the cells of the hypertrophic zone above. × 160.

the germinal layer of cells, or the boundary between the germinal and terminal plates.

In some cases the line of epiphyseolysis ran first horizontally and then continued vertically, parallel with the column of cells, as is seen in clinical practice (Holland's triangle—Blount 1955). These longitudinal breaks give a clear picture of the chondrocyte structure as well as the collagen structure of the matrix (Figure 1).

There can be seen many impressions of various depths, originating from previously attached chondrocytes of the germinal zone and appearing on the border of the terminal plate. Where some of these cells of the germinal zone remained attached to the terminal plate, they were easily distinguishable from the neighbouring empty lacunae. In other places the impressions (Figure 2) occurred as honeycomb-like structures, creating the basis for the columnar arrangement of the chondrocytes in further layers of the growth cartilage.

The proliferative zone in the longitudinal break showed a great gathering of inset and flattened chondrocytes (like piled coins) (Figure

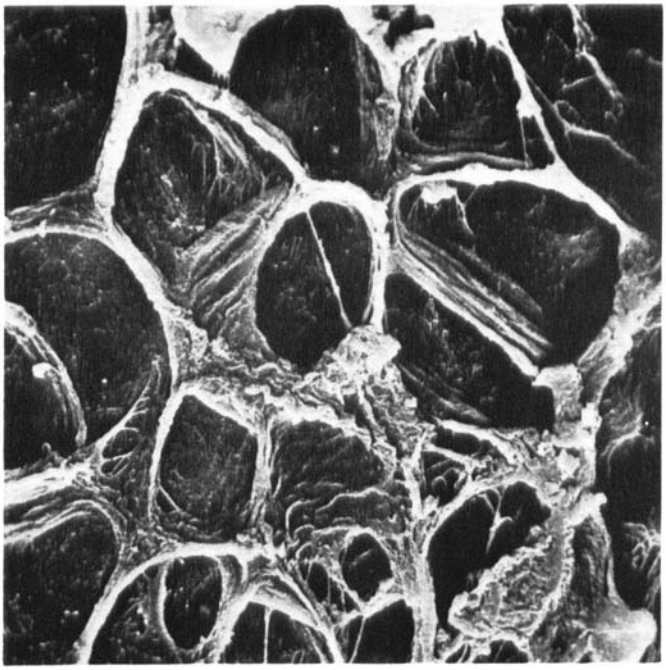

Figure 2. Honeycomb-like structure of the intercellular matrix in the epiphyseal part of the growth cartilage in the area of the germinal cells. × 1,600.

3). The transverse septae were strikingly narrow, whereas the longitudinal ones were markedly wider. Numerous protruberances occurred on the surface of the flattened chondrocytes by which they are anchored to the wall of the lacunae. Further towards the metaphysis, the chondrocytes enlarged and the transverse septae became more marked. The chondrocytes took on a spherical or oval shape and their attachment to the wall of the lacunae was clearly seen. Here (in the longitudinal break) the passage of collagen fibres in the intercolumn and transverse septae was well manifested (Figure 4). When the longitudinal break did not run directly through the column of chondrocytes but through the septum, the lacunae occurred as protruberances of the collagen matrix. In some of these breaks it was found that the cells remained in the other part of the specimen and that the lacunae were empty. Here the structure of the lacunae was particularly well seen.

In the zone of degenerating chondrocytes (in the zone of capillary invasion) empty lacunae occurred, thus forming, by resorption of the transverse septae, connecting tubular spaces without any remnants of

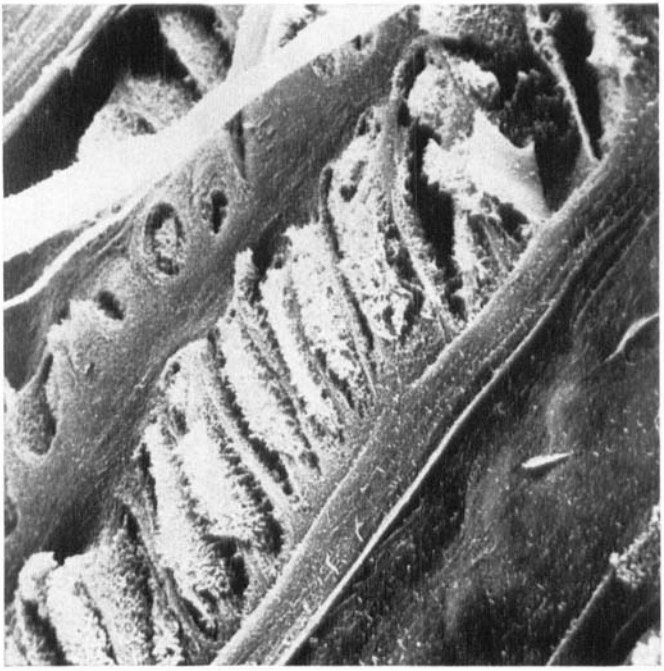

Figure 3. The proliferating zone of the epiphyseal cartilage with columns of cartilage cells and typically arranged intercellular substance. × *2,500.*

the chondrocytes (Figure 5). In some columns net-like structures occurred partly containing erythrocytes, which may be considered as vascular areas (Figure 6).

DISCUSSION

The organization of the epiphyseal growth cartilage is the consequence of complex evolutionary processes already originating in the skeletal cartilage. This organization, however, enables the longitudinal growth of the bone in spite of the mechanical load, the rather unfavourable nutritive conditions (Trueta 1968), the influence of pressures in the growing bone, and also in spite of the action of the surrounding muscles, which do not always work evenly and rectilinearly. The fertility of the cartilage cells depends on the number of cells in the column. The terminal cartilage cells on the metaphyseal side are swollen and are gradually replaced by vessels, loops of the metaphyseal capillaries, which form the borderline of erosion. The intercellular fibrillar

Figure 4. A longitudinal break through a cartilage cell column of the epiphyseal cartilage closer to the metaphysis. The cells are more swollen, the transverse septae are thinner. × 3,160.

matrix is relatively thin and calcified between the hypertrophic cartilage cells. The calcification influences and organizes the penetration of the metaphyseal vessels into the bone plate, thus effecting enchondreal ossification. This layer of the growth cartilage is at the same time also the most frequent site of traumatic epiphyseolysis, which again, under certain conditions, can exert an unfavourable influence upon the further development of the damaged epiphyseal cartilage.

Each layer of the growth cartilage, including the sites of epiphyseolysis, were observed by scanning electron microscopy. This method clearly showed the course of the collagen fibres in the longitudinal (intercolumnar) and transverse (intercellular) septae. Especially clear is the gradual disappearance of the transverse septae towards the metaphysis.

The chondrocytes send out a number of protruberances directed towards the wall of the lacunae on which there are correspondingly reflected depressions. The terminal bone plate appears as an isolated and

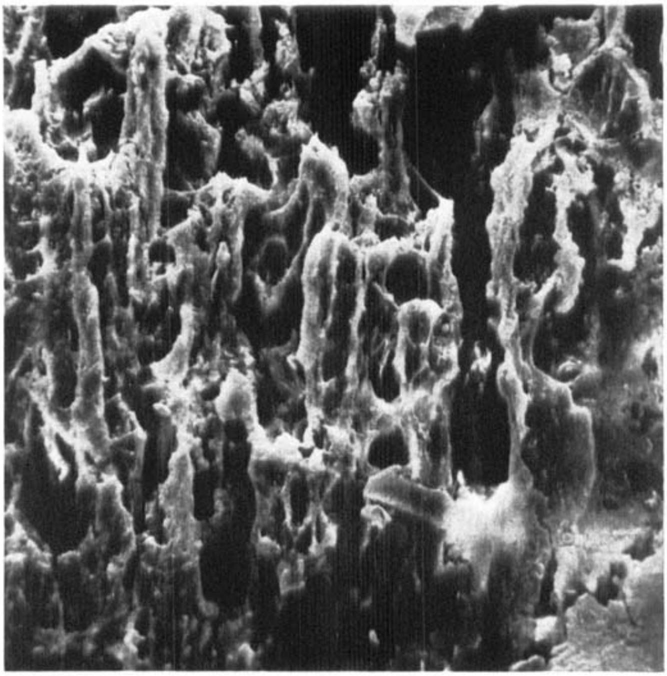

Figure 5. The degenerative zone showing only the remnants of the longitudinal intercellular septae. × *350.*

separate structure onto which, however, cartilaginous elements (germinal zone of the epiphyseal cartilage) continually attach themselves.

The degenerating cells with the invading capillaries proved to be the most interesting layer. Here originate the wide tubular cavities, in between which the remaining matrix is fairly thin and is represented by remnants of the septae. This explains why this zone is mechanically also the most labile and why it has the highest frequency of traumatic epiphyseolysis. Here the matrix loses its fine granular or fibrous character (as shown by the breaks in the proliferating cartilage) and takes on a more homogenous bone-like appearance. By scanning electron microscopy it is not possible, of course, to determine whether it is bone or calcified cartilage and this complicates the more precise differentiation of the primary zone of calcification. This area would call for further study, especially in regard to the exact spatial arrangement of the remaining septae and cartilaginous cells in relation to the penetrating vessels.

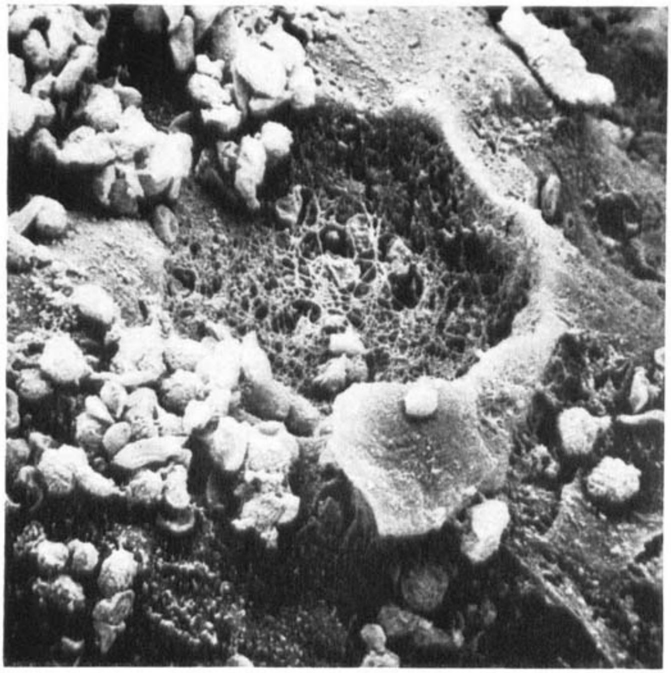

Figure 6. Capillary spaces with blood cells in the zone of capillary invasion. × *1,460.*

CONCLUSIONS

It may be said that the method used by the authors improved the spatial image of each layer of the growth cartilage and explained the mechanism of traumatic epiphyseolysis in places of least mechanical resistance in the plate. From these pictures it also follows that insufficient treatment of epiphyseolysis may very easily reflect itself in defects of the enchondral growth of the bone.

SUMMARY

The authors undertook a scanning electron microscopic study of the layers of the growth cartilage in the distal end of the rabbit femur in longitudinal and transverse fractures. The relationships between the various morphological structures were determined. The three-dimensional spatial depiction facilitates the better understanding of some mutual relationships between various growth cartilage components. Especially interesting are the regressive processes in both the cells and the matrix which explain the reduction of mechanical strength in the

zone of the hypertrophic cells. Also interesting is the way in which the cells are placed and fixed in the lacunae. Moreover, the spatial picture of the terminal plate shows an unexpectedly compact structure.

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