THE VASCULAR CONTRIBUTION TO OSTEOGENESIS

I. Studies by the Injection Method

J. TRUETA and J. D. MORGAN, OXFORD, ENGLAND

From the Nuffield Orthopaedic Centre, Oxford

In 1727 Stephen Hales noted that the long bones grew in length only at their extremities. Placing markings in the bones of animals he, and later Duhamel (1742) and Haller (1757–66), demonstrated the lack of any substantial interstitial lengthening in the normal process of growth. This was again confirmed by John Hunter (1837), two of whose experiments are still preserved at the Royal College of Surgeons of England, London (Fig. 1). The extent of understanding of the mechanism of growth in the middle of the eighteenth century may be judged by the description given in the 1750 edition of Cheselden's *Anatomy*, which reads as follows: "The cylindrical bones and all others whose fibres are nearly parallel, begin (to ossify) about the middle of each fibre, and whence shoot forth to their extremities; not always in continued lines but frequently beginning new ossification which soon join the former; and by the continued addition to this ossifying matter the bones increase till their hardness resists a further extension." Enchondral ossification in growing bones was apparently not mentioned until 1836 when Miescher made the first substantial reference to this basic mechanism of the growth process. The earliest complete account of the growth plate was that of Todd and Bowman (1845) which was soon followed by the painstaking study of Sharpey, appearing in the fifth edition of Quain's *Elements of Anatomy* (1848), the notes and references of Leidy of Philadelphia (1849) and the extensive study of Tomes and De Morgan (1853). But it was not until 1858 when Müller published his now classical work on the mechanism of epiphysial cartilage calcification that the first full account of the basic mechanism of growth was published. In the succeeding twenty years since the publication of Müller's work the detailed anatomy and function of the epiphysial cartilage became nearly as well known as they are to-day. In some respects, such as those which refer to the part that the vascularity adjacent to the epiphysial cartilage plays in this process, some authors of past generations seem to have collected more information than is usually shown in current text-books today. A good example of this old interest may be found in Ranvier's *Traité technique d'histologie* (1875) where repeated references to the part that the vessels play in osteogenesis appear. Ranvier, like many of his contemporary anatomists, prepared his specimens by vascular perfusion before sectioning for microscopic examination. Present day studies of injected materials under the light microscope have become rare, even though satisfactory methods of perfusion of the finer vessels are now available.

In this centre for the last ten years procedures of vascular perfusion for the study of the bone tissues have been developed and a number of papers referring to the vasculature* of bone have been published, but with one exception (Trueta 1958) only a passing reference to the role of the vessels in the mechanism of calcification and in osteogenesis proper has been published. The present work refers to the vasculature adjacent to the growth plate, and at the time it was planned by the senior author (J. T.) it was decided that the data that might be collected would not be published until the work was considered as near its completion as possible. The investigation was divided into five main sections:

1. Study by injection methods of the normal vascular supply adjacent to the growth plate.

* For some time a word referring to the vascular system as a whole as opposed to its constituent parts has been found necessary. We consider that the word *vasculature* would cover this requirement as does the word *musculature* for the muscular system. Professor Kenneth Franklin supports its use in the United Kingdom as it is sometimes used in the United States of America.

2. A similar study using the electron microscope.

3. Changes produced in the epiphysial cartilage by the experimental suppression of either the epiphysial or the metaphysial blood flow.

4. Effect of compression and of distension upon the epiphysial cartilage and its blood supply.

5. Study of the vascular pattern of the plate in conditions such as rickets, hypothyroidism, pituitary removal and others which could be experimentally reproduced in laboratory animals.

For this study a total of over four hundred rabbits, two hundred rats, sixty guinea-pigs, twenty dogs and fifty human specimens were available for vascular perfusion. Direct light

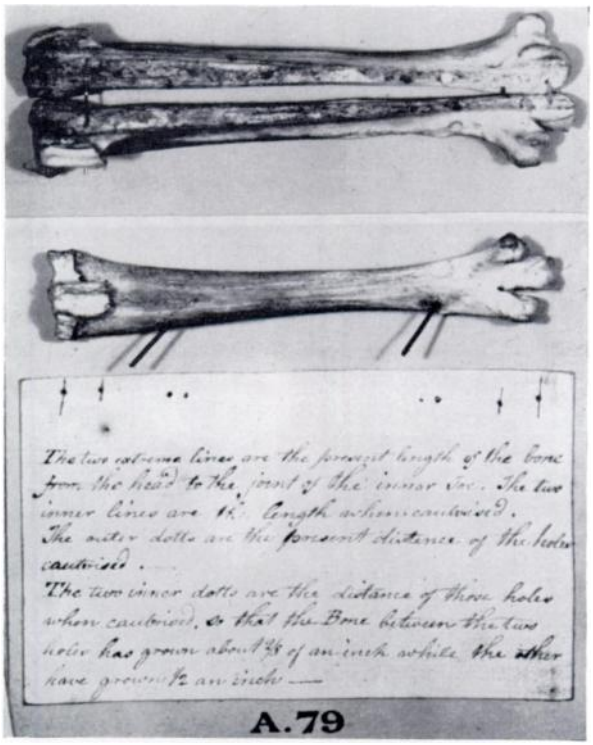

FIG. 1

The famous tarsal bone of a chicken in which John Hunter demonstrated by the inclusion of two lead shots that growth occurs in the long bones at their extremities. From the Hunterian Museum, Royal College of Surgeons of England, London. (*By kind permission of the President and Council of the Royal College of Surgeons of England.*)

and phase microscopy, microradiography and macrophotography by the method of Spalteholz were used in most specimens; when necessary, electron microscopy and x-ray diffraction were also available and on a smaller number of occasions biochemical studies were performed. In the present paper we refer to the data obtained in our first study.

STUDY OF THE NORMAL VASCULAR SUPPLY OF THE GROWTH CARTILAGE BY PERFUSION

The purpose of this investigation was to collect data on the pattern of the vessels adjacent to the growth plate, with the object of using the information as control for the subsequent investigations and also for the direct study of the relationship between the cartilage cells and the vessels during enchondral ossification.

MATERIALS AND METHODS

The proximal epiphysial plate of the femur and the proximal and distal epiphysial plates of the tibia in children, dogs, guinea-pigs, rabbits and rats were studied. A total of well over two thousand sections were examined under the light microscope, the dissecting binocular microscope or by microradiography. Although we think that some small difference in the vascular pattern of the growth plates of several of the specimens studied do exist, we believe that they are not sufficient to justify a separate description of the vessels of any particular species; we shall thus assume that the data given below refer to all the species mentioned above and, if we may judge by the results of a few complementary investigations in other animals, to all mammals in general.

Some substantial differences are present in all the examined species between the plates near the knee and that of the proximal femoral epiphysis, but, while we believe that these are important in explaining the peculiarities of growth at the upper end of the femur, we will not describe them here. Only bones of healthy animals of all ages from early foetal life to pre-maturity have been used; the human specimens were only included after we were satisfied that the cause of death had no detectable influence upon the integrity of the growth cartilage.

Our methods of vascular perfusion have been described before (Trueta and Harrison 1953). Alternating thin (10 μ) and thick (200 to 300 μ, and on occasions to 400 μ) sections of the injected materials were obtained; this was considered essential to establish the relationship between cellular structure and the circulatory system at its first ramifications. Serially numbered sections cut at right angles to the direction of the columns of cartilage cells were also examined. Usually fixation was obtained by formol saline, and decalcification by formic acid; haematoxylin-eosin for basic staining, 1 per cent toluidin blue for metachromasia of the cartilage. Gomori and an azo-dye method were used for the detection of alkaline phosphatase and P.A.S. reaction for mucopolysaccharides and mucoproteins.

For this particular study we used microradiography to detect early calcification rather than to visualise fine vessels. Perfusion of 2 per cent Berlin blue followed by the method of Spalteholz gave the clearest detail of the fine vessels.

The object of the present work was to study the vascular characteristics of the growth cartilage assuming that if its two opposite surfaces had different functions then the shape, size, direction or other characteristics of the blood vessels supplying them might provide sufficient data to suggest what part each plays in the complex process of growth. Detailed description of the epiphysial cartilage cells is common enough to preclude enlarging further on this subject but, as our papers will repeatedly refer to particular parts of the growth plate and its surroundings, it seems correct to introduce the subject by giving a short account of the region under study. We will, therefore, refer here to the well developed growth cartilage once the epiphysial bone nucleus, either primary or secondary, is fully grown. The vascular and cellular changes occurring during the formation of the bone shaft and preceding the final organisation of the epiphysial cartilage have been investigated and will be published separately at a later date.

DESCRIPTION OF FINDINGS

Fine anatomical structure of the growth cartilage—Although the following description may apply to any growth cartilage from any of the species we have studied, we have selected the upper tibial epiphysis of the three-months-old rabbit to illustrate our text (Fig. 2). These figures could have been substituted by others from our human material without any substantial change in the description.

The growth plate has its epiphysial and metaphysial surfaces or " borders "; to abbreviate we shall refer to the *epiphysial* side as the E-side and the *metaphysial* as the M-side.

The cancellous lamellar bone of the weight-bearing epiphysis during the first growth period has hardly any predominant orientation. In man at about four to five years the so-called

weight-bearing trabeculae will make their appearance reaching the surface of the epiphysis in increasing numbers until they form a robust wide column at the age preceding the fusion of the plate. Independent from these thickened trabeculae we find the bone plate (Fig. 2, zone a), a sort of rudimentary cortex, limiting the epiphysial cancellous bone immediately adjacent to the first row of cartilage cells. This corresponds to the bone shell first described under the joint cartilage by Toynbee (1841), and known as the zone of calcified cartilage, but it is thicker than this zone and has openings through which the vessels penetrate, an event which in the calcified zone of the joint cartilage only occurs in certain pathological conditions such as degenerative arthritis. The bone plate is formed by a variable number of lamellae, usually from six to eight, and it constitutes approximately a fifth of the thickness of the growth cartilage proper. The bone plate present at the time of epiphysial closure remains visible until late in life and is known as the epiphysial scar.

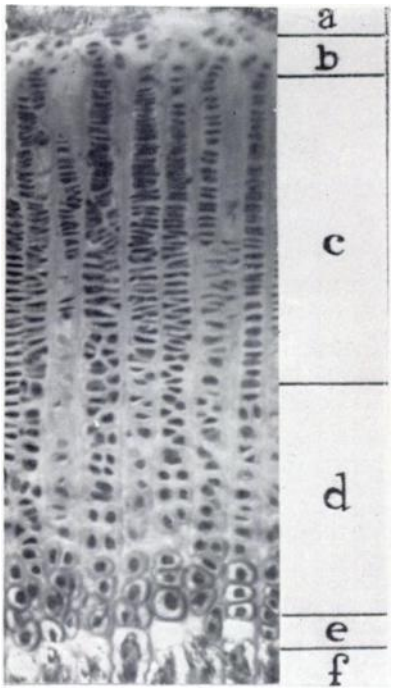

FIG. 2

Growth plate from the upper tibial epiphysis of a rabbit. The illustration shows the several zones into which the plate is divided: a—Bone plate. b—Zone of "resting" cartilage cells. c—Zone of proliferation. d—Zone of hypertrophic or giant cells. e—Zone of cell degeneration. f—Zone of bone formation. (× 40.)

Immediately under the bone plate there is a zone of amorphous material, mainly a mucopolysaccharide, perhaps chondroitin sulphuric acid and some protein, in which the first cartilage cells are found (Fig. 2, zone b); these are usually either single or in couples facing each other along their longest axis. When this occurs they are surrounded by a limiting membrane as if they were, in fact, cells which had recently undergone division. But by light microscopy no cells are seen in mitosis. This part of the growth cartilage is sometimes called that of the undifferentiated or resting cartilage cells. Immediately below this area appear the tops of the columns of cartilage cells; these cells are arranged in close order and flattened against each other. They have little cytoplasm (Fig. 2, zone c). The nucleus is seen displaced towards the periphery as if being squeezed out by the pressure to which these cells are subjected. In a sequence of ten to twenty such cells their shape scarcely alters although they are actively reproducing themselves. There is a constant relation between the number of cells and the activity of the growth plate, the greater their number the higher the fertility of the plate. The slow growing vertebral epiphyses do not have more than six to eight such cells in each column. This is the zone of proliferation or palisade, and together with the zone of germinal or "resting" cells corresponds to approximately half of the growth cartilage.

Towards the end of this part of the column the cells begin to enlarge to form the zone of hypertrophic or giant cells; this zone contains from four to twelve large, spherical or cuboid, oedematous cells with a central nucleus (Fig. 2, zone d). Altogether, the zone of giant cells, despite their smaller number, is frequently larger than that of the proliferative cells. The giant-cell segment is followed by the zone of degeneration (Fig. 2, zone e). Only one, or exceptionally two, degenerating cells are found at the end of each column; they represent the last stage of cellular disintegration before the first bone elements appear (Fig. 2, zone f).

It may be worth remembering that each cell we have described above represents no more than a stage in the evolution of a single cartilage cell, from birth to senility and final death, with which the cartilage cell makes its contribution to the genesis of bone.

It is of interest to refer briefly to the intercellular substance on which the cartilage cells

are anchored. In the area of the so-called resting cells the intercellular substance is almost amorphous, with the cells widely scattered apart and the collagen not completely orientated, even if there is some predominance of the fibres directed towards the cell columns. This predominance increases at the level of the proliferative cells where all the collagen fibres arrange themselves parallel to the cell columns.

As the M-end of the columns is approached the space left between the cells becomes narrower, the cells of each column almost touching their neighbours in the next columns. As a consequence of this arrangement the intercellular substance is greatly reduced but the remaining fibres continue in the direction of the columns. The earliest sign of calcification is found in the space just above the last two giant cells, but from this point onwards the

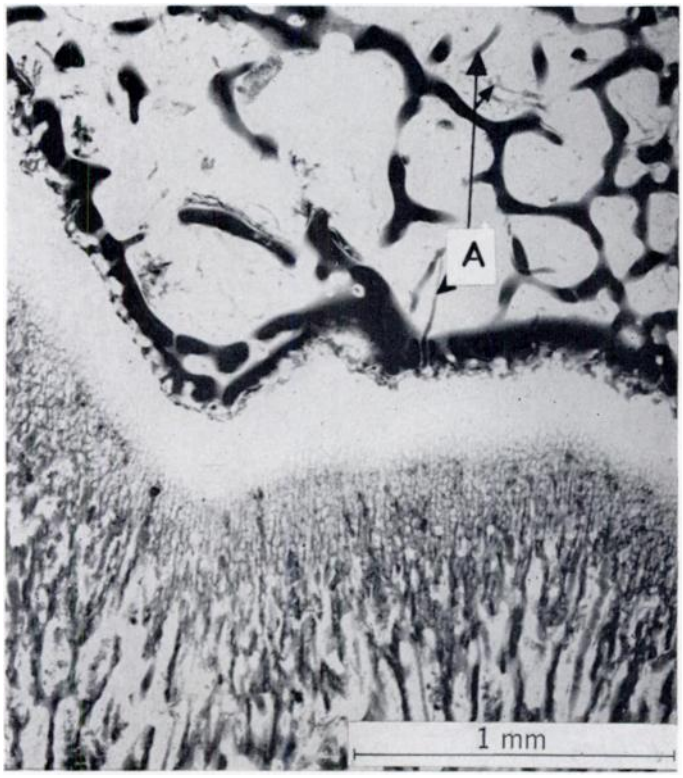

FIG. 3

Microradiograph showing the hydroxyapatite deposit adjacent to the epiphysial cartilage. Note the mould of cell capsules at the metaphysial border of the growth plate. In A a vessel crosses under the bone plate.

calcification of the intercolumnar ground substance progresses rapidly until beyond the level of the first degenerative cell only calcified columns are now present (Fig. 3).

This is a summary of the course of events in the evolution of the cell and matrix of the epiphysial cartilage. Many important points are still to be clarified, such as the role of the vessels in calcification, the origin and destiny of the " resting " cell, the cause of the cartilage cell growth and final degeneration, what part it takes in forming the ground substance, and finally whether cell division may occur in the columns apart from the initial division of the cells of the proliferative segment. It would be important also to elucidate whether resorption of the E bone plate takes place, to allow space for the constant reproduction of most of the cells, as has been asserted by Payton (1933) and others, or whether the whole epiphysis is " lifted " to keep the necessary distance with the zone of calcification advancing from the metaphysial end of the column, as Lacroix (1951), Ham (1957) and many others believe.

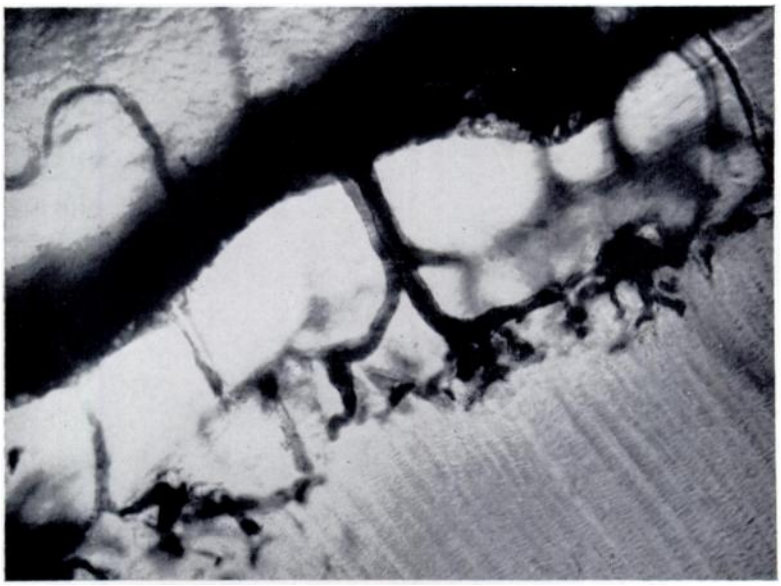

FIG. 4

Epiphysial vessels penetrate the bone plate towards the first row of cartilage cells. Note that not all the arteries and veins penetrate the bone plate through the same canals. (×40.)

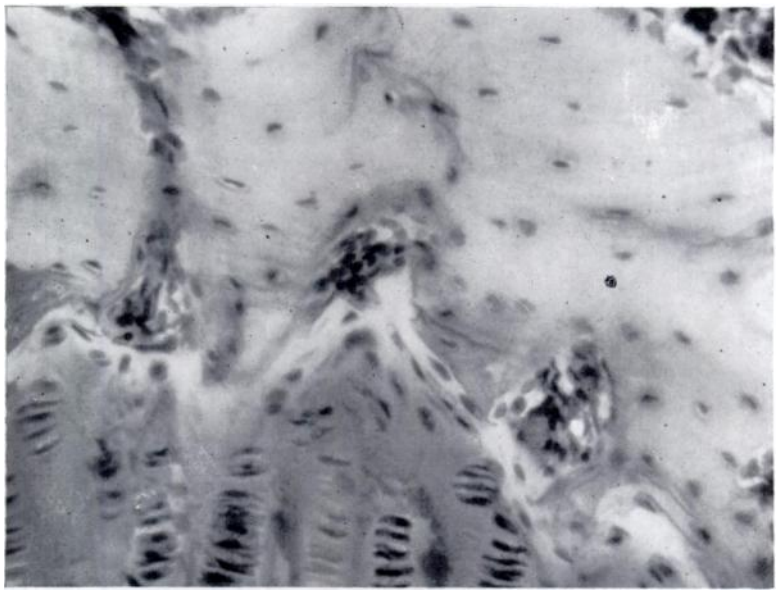

FIG. 5

E-vascular ends just under the bone plate. Each bulging vessel covers the space corresponding to from four to ten cell columns. It is striking how many cartilage cells appear to be very close to the vessels. (× 240.)

NORMAL VASCULAR PATTERN

Epiphysial side—The arteries penetrate the epiphysis close to the capsular insertion near the growth plate. The distribution of the main E-vessels, except in a few decidedly individualistic epiphyses like the proximal femoral (Trueta 1957), is that described by Morgan (1959) for the rabbit's tibia. By progressive branching and anastomosing, fair sized arteries reach the bone plate and largely communicate between them. Through canals in the bone plate

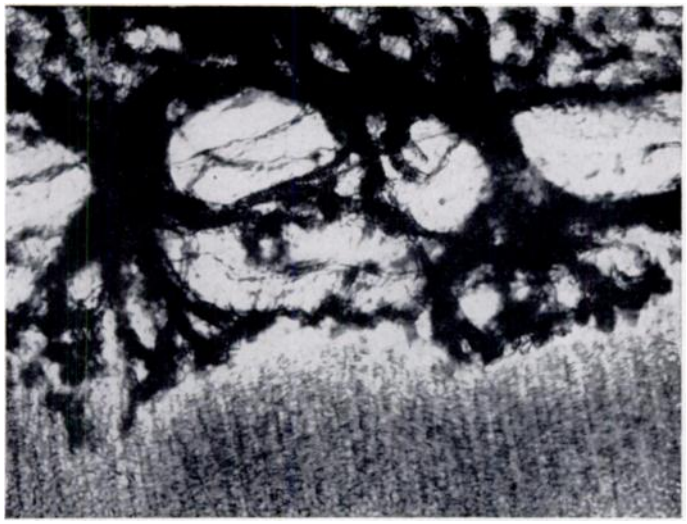

FIG. 6
Well injected E-vessels expanding underneath the bone plate giving the appearance of a rake. (×35.)

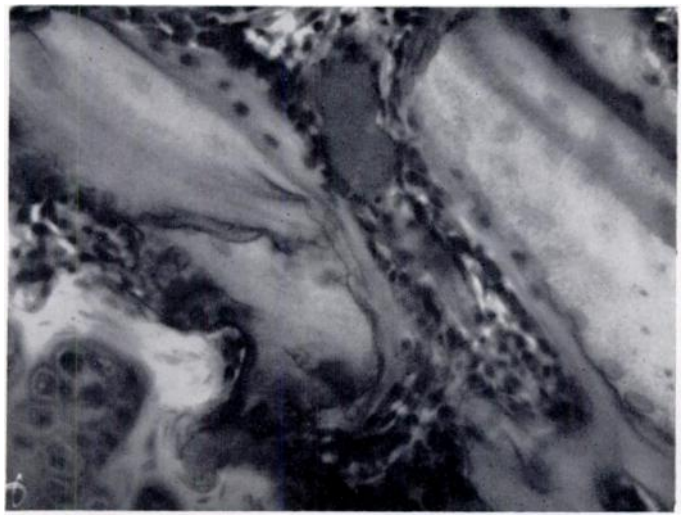

FIG. 7
Injected E-vessel penetrating the bone plate. Profuse osteoblast apposition may be seen close to the vessel wall. (×210.)

these arteries cross the barrier and expand into terminal spurs before turning back as large veins, not always through the same canal (Fig. 4). Each terminal expansion covers the space corresponding to from four to ten cell columns and it is frequent to find " resting " cells, or even columns, which begin very close to the vascular endothelium (Fig. 5). We have never found a vessel that penetrates between two cartilage cell columns, even though it is common to find vessels turning back at different levels depending on the diversity of depths at which the columns begin. In a transverse section immediately under the bone plate a very rich vascularity is seen, the whole of the vascular expansions forming a ceiling under the roof of the bone plate.

There is apparently no difference between the vascularity of the periphery and that of the central part of the space under the bone plate even though many vessels from the surface

penetrate at the point of insertion of the capsule. We have not been able to demonstrate anastomoses between them once they have crossed the bone plate; on the other hand, as stated above, anastomoses in large numbers do occur above on the E-side of the plate. It is common to find a large epiphysial vein immediately over the bone plate formed by the confluence of four or more venules, each of which has penetrated across the plate through a different passage, giving to the vessel the appearance of a rake (Fig. 6). Considering the total

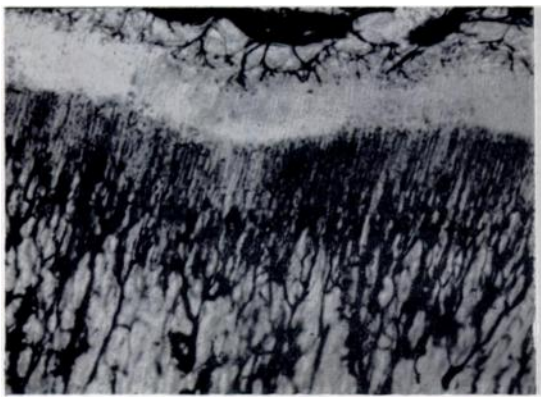

FIG. 8

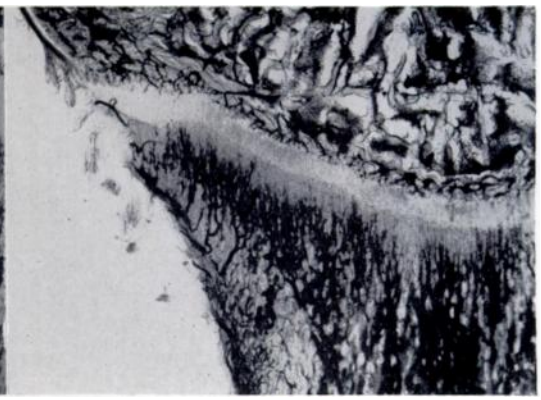

FIG. 9

Figure 8—Contrasting vascular shape at both sides of the growth plate. The loops of the distal branches of the nutrient artery reach the growth plate from its metaphysial side and cover the three central fourths or more of the plate. (· 8.) Figure 9—The outer fringe of the growth plate at its M-side is supplied by the robust periosteal vessels known as the perforating vessels of the metaphysis. (· 15.)

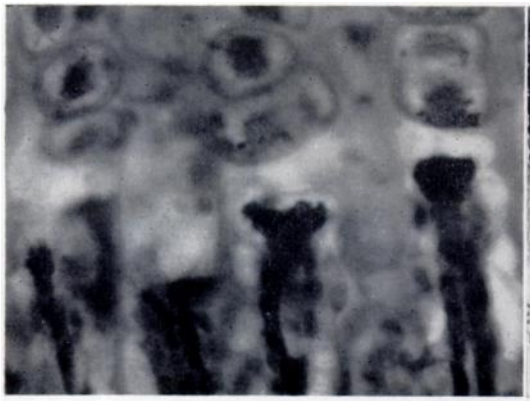

FIG. 10

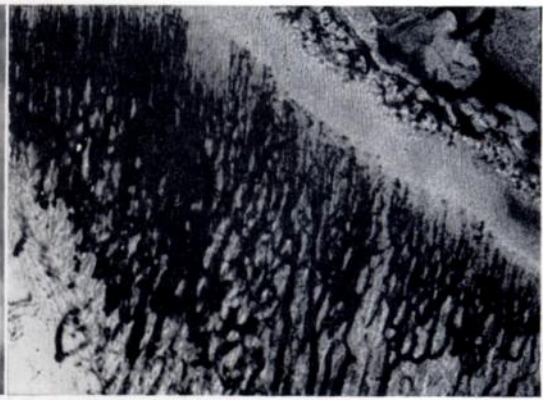

FIG. 11

Figure 10—M-end of the growth plate columns. The vascular loops turn back without reaching the last intercellular septum. The last normal looking hypertrophic cell is separated from the vessel by a dying "degenerate" cartilage cell. (· 320.) Figure 11—Hairbrush appearance of the M-vessels of the growth cartilage looping back at the level of the last row of cartilage cells. (· 32.)

number of vessels which expand under the plate it is fair to assume a very rich fluid supply for the cells of this area. Thus the current view that a rich blood—and so oxygen—supply exists only at the M-side of the plate is not supported by our findings.

Despite the assertion made above that resorption of the bone plate covering the germinal area is supposed to occur, we have been unable to detect any osteoclast there, a fact which is of interest whatever concept of the resorptive mechanism of bone may be adhered to. Neither have we seen any signs of trabecular erosion.

As stated before, the arteries may be seen on occasions penetrating the bone plate (Fig. 4) across a passage different from that of the veins.

Bone formation by osteoblast apposition may be profuse (Fig. 7). Some of the vessels are reminiscent of those found in the head of the femur before the secondary ossicle has appeared (Trueta 1957).

On none of the normal fully developed growth plates from any of the species we have studied, have vessels been seen passing from the epiphysial to the metaphysial side across the plate or *vice versa*. Thus the belief (Gill 1940) that vessels penetrate the plate seems totally untenable.

Comment—The vascular pattern is similar throughout the whole of the periphery of the epiphysis with two exceptions: firstly, the vessels close to the bone plate which covers the epiphysial cartilage are larger than and of different shape from those under the calcified cartilage surrounding the rest of the epiphysis; secondly, whereas no vessels penetrate the normal calcified zone of the joint cartilage, large vessels pierce the bone plate to expand underneath it before returning, frequently through different passages across the bone plate.

A rich blood supply is available underneath the bone plate directly in the midst of the intercellular substance surrounding the germinal cells and the reproductive part of the columns of cartilage cells.

Metaphysial side—Approximately four-fifths of the vessels reaching the growth plate from the M-side consist of the last ramifications of the nutrient artery. They are very evenly distributed over the central part of the growth plate extending over the three central fourths or more of the growth plate (Fig. 8). The outer fringe of the plate is supplied from the system of large perforating M-arteries, robust periosteal vessels which act as minute nutrient arteries (Fig. 9). There is no detectable distinction between the end vessels from these two sources. Both repeatedly divide into ever finer arterioles until at about the level of the last trabeculae they are arranged in straight parallel vessels, advancing towards the last hypertrophic cells of the columns (Fig. 10). They all turn back at approximately the same level, the descending leg of the loop being indistinguishable from the ascending or arterial one. The whole of the loop system of vessels resembles the hairs of a brush (Fig. 11).

Studied in detail, these vessels resemble the vasa-recta-spurea of the renal circulation (Trueta, Barclay, Daniel, Franklin and Prichard 1947), but in distinction from these, they never anastomose with each other. They terminate in the pool system of sinusoids at the level of the first cancellous space of the bone marrow with all the characteristics of the haemopoietic vessels (Doan 1922, Trueta and Harrison 1953). The venous sinusoids drain into large collecting vessels which in joining together form the bulky emerging veins described by Marneffe (1951) and by Morgan (1959).

Of particular interest for the understanding of the process of calcification is the relationship of the vascular loops with the cartilage cells of the plate. The two legs of the looping vessels once well injected almost, but not completely, fill the spaces left by the removal of the cartilage cells; the walls limiting this space consist of the calcified cell capsules together with the intercolumnar substance and form a long tube which represents the beginning of the bone marrow cavity.

It is always found that even in the best injected specimens (Fig. 10) there is no direct contact between the vascular loop and the last intercellular septum. This is because the end of this space is filled by erythrocytes which have been displaced and accumulated by the injection mass (Fig. 12). The great frequency with which this peculiar accumulation of erythrocytes is found pressing against the last remaining intercellular system may be interpreted in two ways. Either it represents vascular bursts caused by the injection mass or, much more probably, vascular extravasations caused by the sudden opening of the capsules which precedes the progression of the M-vessels towards the epiphysis. If this were confirmed, the existence of open vessels in bone which had been suggested by Stricht (1892) and denied by Doan (1922), Drinker, Drinker and Lund (1922) and Trueta and Harrison (1953) among many others, should be admitted as real. In the normal there is a constant relationship between the vessel

and the earliest deposition of hydroxyapatite. As the advancing vessel is stopped by the intercellular septum limiting the last recognisable but degenerate cell, the crystals of hydroxyapatite are found at the top of the capsule immediately preceding it; that which still

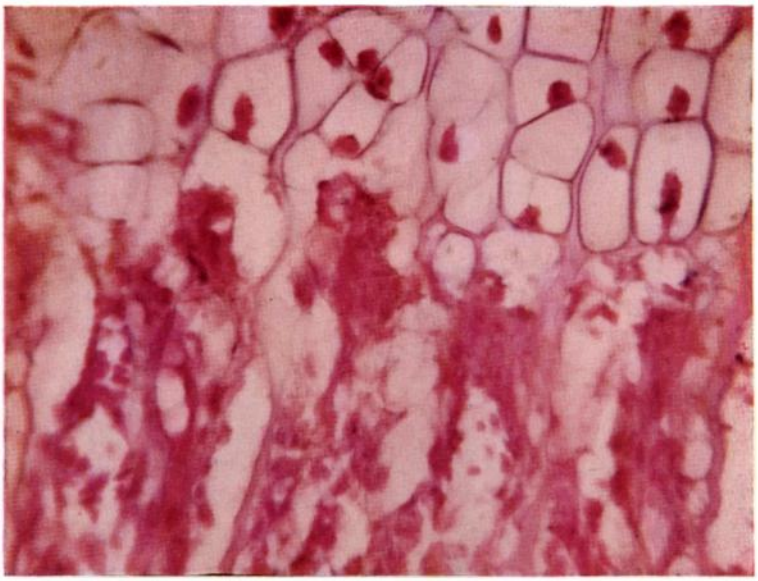

FIG. 12
Colour microphotograph showing the degenerating giant cell being invaded by erythrocytes from the vascular loops. (× 400.)

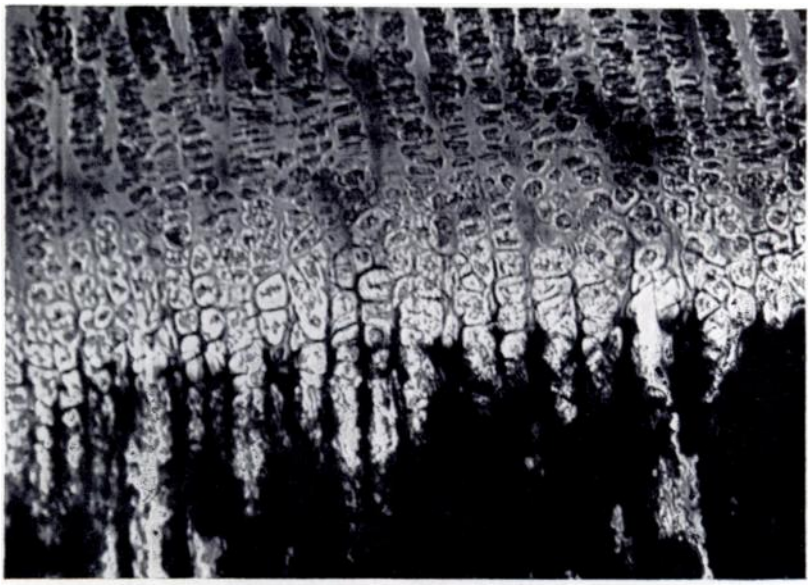

FIG. 13
Calcification of the intercolumnar spaces initiated at the top of the last but two normal looking giant cell. Section cut without decalcification; stained by von Kossa's method. (× 200.)

contains the remnants of a giant cell. The earliest signs of calcification have never been found in the normal beyond the intercolumnar spaces corresponding to the top of the last but two normal looking giant cell (Fig. 13). Thus, there appears to be a close relationship between

the open end of the M-vessels and the area of calcification, but finer methods are required to elucidate this most important point. This is now being done with the help of the electron microscope.

Proceeding towards the metaphysis the vessels acquire a more consistent structure and are surrounded by the osteoblasts which are responsible for the laying down of the first lamellar bone (Trueta 1958). At this level bone reabsorption and bone formation go hand in hand in the normal, and thus the presence of osteoblasts is paired, even if in a much lesser extent, with that of the osteoclasts, even if we are sceptical about the part they may play in the active removal of bone.

Alkaline phosphatase—In all sections stained for alkaline phosphatase we have confirmed, as many before us since the early finding by Robison (1923), that alkaline phosphatase appears first in the nucleus of the giant cells and later in the intercellular substance surrounding

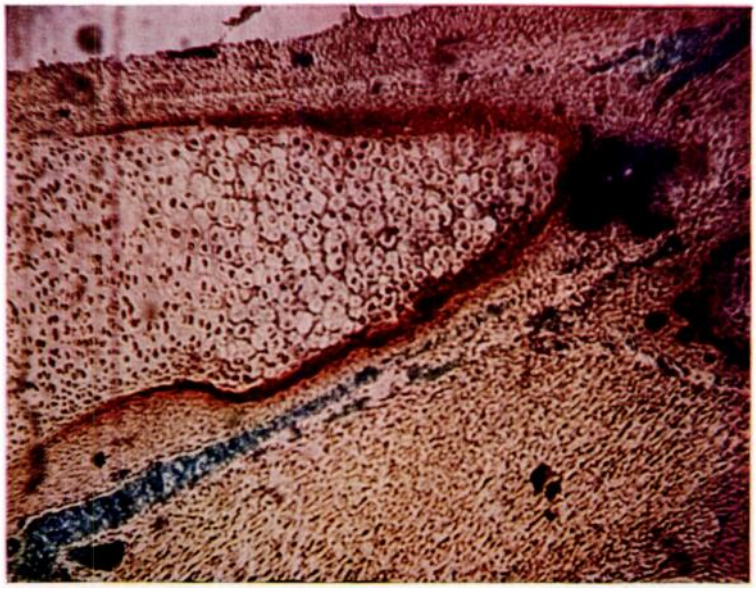

FIG. 14

Colour microphotograph showing the perfect correspondence between (*a*) the giant cartilage cell, (*b*) alkaline phosphatase (*in brown*) and (*c*) the injected vessels (*in blue*). (· 50.)

them. As with calcification, there is a close relationship between the vascular loops, the giant cells and the placement of alkaline phosphatase. The correlation has been studied in detail during the earlier periods of bone growth preceding the organisation of the epiphysial cartilage (Fig. 14) and will be published in detail elsewhere. In this figure the perfect correspondence is seen between the giant cartilage cells (*a*), alkaline phosphatase (*b*) and the injected vessels (*c*). It may be of interest that when the giant cells are present but not the vessels, the characteristic stain of alkaline phosphatase does not appear.

Comment—The vessels of the metaphysial side of the plate are in a very large proportion the last ramifications of the nutrient artery. They appear regular in shape and progress, by penetrating, one after the other, the last recognisable giant cell of each column of the growth plate. Even in the best injections the loops are rarely in contact with the last intercellular septum but an interposition of red corpuscles accumulates between the loop and the septum. This forces us to consider that the opening of every one of the last capsules by the progressing vessel causes a sort of microhaemorrhage which may leave the vessel permanently open at its most distal part.

The area of cartilage calcification has been found to start always within two or three capsules of the one just invaded by the vessel. This implies that, after a suitable matrix has been laid down by the cells, the near approach of blood is necessary before calcification occurs. One or both of two mechanisms may be at work. In the first place it may be necessary to increase the local phosphate concentration (and red corpuscles are known to contain a high proportion of phosphoric esters (Gutman 1951)); secondly, it might be that it is necessary to convey the alkaline phosphatase which some believe to be necessary for the destruction of an inhibitor of the crystallisation process (Neuman and Neuman 1958). We have observed that alkaline phosphatase, giant cartilage cells and the vessels appear intimately related, and, at least in early calcification such as in the formation of the perichondral ring of the long bones, only those giant cells near the vessels appear to have, and be surrounded by, alkaline phosphatase.

But in the growth cartilage the whole mechanism of growth is implicit in the giant cartilage cell—vessel—calcification. Growth occurs first by the division of the proliferative cartilage cells with which their number repeatedly doubles; secondly, by the increase in size of these cells, and, last but not least, by the calcification of the matrix round the last three cells of the columns, a process which is conditioned by the penetration of the vessels. Consequently, while all three factors contributing to the mechanism of growth seem essential, bone appears to be laid down only in the presence of an appropriate type of vessel. A transverse section of the recently calcified part of the metaphysis appears as a honeycomb. In reality the lifting of the epiphysis—and thus of the whole body—which is the consequence of bone growth, is effected over a large number of calcified tubes which work like the pillars under the artificial islands used in recent times to perforate the bed of the sea.

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REFERENCES

BRODIN, H. (1925): Longitudinal Bone Growth: the Nutrition of the Epiphyseal Cartilages and the Local Blood Supply. *Acta Orthopaedica Scandinavica*, Supplementum 20.

CHESELDEN, W. (1750): *The Anatomy of the Human Body*. Sixth edition. London: C. Hitch and R. Dodsley.

DOAN, C. A. (1922): The Circulation of the Bone-Marrow. *Contribution to Embryology, Carnegie Institution of Washington*, **14**, 27.

DRINKER, C. K., DRINKER, K. R., and LUND, C. C. (1922): The Circulation in the Mammalian Bone-Marrow. *American Journal of Physiology*, **62**, 1.

DUHAMEL, A. L. (1742): Sur le développement et la crue des os des animaux. *Histoire de l'Académie royale des Sciences*, p. 354.

GILL, A. B. (1940): Legg-Perthes Disease of the Hip; its Early Roentgenographic Manifestations and its Cyclical Course. *Journal of Bone and Joint Surgery*, **22**, 1,013.

GUTMAN, A. B. (1951): Current Theories of Bone Salt Formation, with Special Reference to Enzyme Mechanisms in Endochondral Calcification. *Bulletin of the Hospital for Joint Diseases*, **12**, 74.

HALES, S. (1727): *Vegetable Statics*. London: Innys and Woodward.

HALLER, A. von (1757–66): *Elementa physiologiae corporis humani*. Lausanne: Marci-Michael Bousquet.

HAM, A. W. (1957): *Histology*. Third edition. London: Pitman Medical Publishing Co. Ltd.

HUNTER, J. (1837): *The Works of John Hunter, F.R.S.* Edited by J. F. Palmer. Vol. 4. London: Longman, Rees, Orme, Brown, Green, and Longman.

LACROIX, P. (1951): *The Organization of Bones*. London: J. & A. Churchill Ltd.

LEIDY, J. (1849): Observations on the Development of Bone, the Structure of Articular Cartilage, and on the Relation of the Areolar Tissue with Muscle and Tendon. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **4**, 116.

MARNEFFE, R. de (1951): *Recherches morphologiques et expérimentales sur la vascularisation osseuse*. Brussels: Editions Acta Medica Belgica.

MIESCHER, J. F. (1836): *De Inflammatione ossium eorumque anatome generali*. Berlin: G. Eichler.

MORGAN, J. D. (1959): Blood Supply of Growing Rabbit's Tibia. *Journal of Bone and Joint Surgery*, **41–B**, 185.

MÜLLER, H. (1858): Ueber die Entwickelung der Knochensubstanz nebst Bemerkungen über den Bau rachitischen Knochen. *Zeitschrift für wissenschaftliche Zoologie*, **9**, 147.

NEUMAN, W. F., and NEUMAN, M. W. (1958): *The Chemical Dynamics of Bone Mineral.* Chicago: The University of Chicago Press.

PAYTON, C. G. (1933): The Growth of the Epiphyses of the Long Bones in the Madder-fed Pig. *Journal of Anatomy*, **67**, 371.

QUAIN, J. (1848): *Elements of Anatomy by Jones Quain.* Fifth edition. Volume 1. Edited by R. Quain and W. Sharpey. London: Taylor, Walton, and Maberley.

RANVIER, L. (1875): *Traité technique d'histologie.* Paris: F. Savy.

ROBISON, R. (1923): The Possible Significance of Hexosephosphoric Esters in Ossification. *Biochemical Journal*, **17**, 286.

STRICHT, O. van der (1892): Nouvelles recherches sur la genèse des globules rouges et des globules blancs du sang. *Archives de Biologie*, **12**, 199.

TODD, R. B., and BOWMAN, W. (1845): *The Physiological Anatomy and Physiology of Man.* Volume 1. London: J. W. Parker.

TOMES, J., and DE MORGAN, C. (1853): Observations on the Structure and Development of Bone. *Philosophical Transactions of the Royal Society of London*, **143**, 109.

TOYNBEE, J. (1841): Researches, Tending to Prove the Non-vascularity and the Peculiar Uniform Mode of Organization and Nutrition of Certain Animal Tissues. *Philosophical Transactions of the Royal Society of London*, **132**, 159.

TRUETA, J. (1957): The Normal Vascular Anatomy of the Human Femoral Head during Growth. *Journal of Bone and Joint Surgery*, **39-B**, 358.

TRUETA, J. (1958): La vascularisation des os et l'ostéogénèse. *Revue de Chirurgie Orthopédique*, **44**, 3.

TRUETA, J., BARCLAY, A. E., DANIEL, P. M., FRANKLIN, K. J., and PRICHARD, M. M. (1947): *Studies of the Renal Circulation.* Oxford: Blackwell Scientific Publications.

TRUETA, J., and HARRISON, M. H. M. (1953): The Normal Vascular Anatomy of the Femoral Head in Adult Man. *Journal of Bone and Joint Surgery*, **35-B**, 442.