A PRELIMINARY COMMUNICATION ON THE DEVELOPMENT AND GROWTH OF BONE AND THE RELATIONS THERETO OF THE SEVERAL HISTOLOGICAL ELEMENTS CONCERNED.

By T. WINGATE TODD, M.B., F.R.C.S., *Lecturer in Anatomy, University of Manchester.*

DURING an investigation on bone, normal and pathological, which has extended over the last four years and is still in course of prosecution, many facts have come to light which suggest that some modification is necessary of our present views concerning the subject of osseous tissue in general. Upon a subject so difficult as that of bone it is impossible to speak until experience has given confidence in the interpretation of results. For this reason I have hitherto desisted from writing that which seems to me to be the logical inference from the appearance which bone exhibits under various methods of treatment. This paper is of the nature of a preliminary note. It embodies observations on stages in development of bone and on the interstitial changes which occur in this tissue during life. It also includes certain views on the nature of the histological elements composing bony tissue.

In stating these views I am fully aware that in the light of further experience and research they may require some modification, for the subject is not an easy one. They are, however, in accord with observations made, not only in histology but also in the course of a considerable pathological, clinical, and radiographic experience.

Both human and mammalian material has been used in the preparation of tissue for examination. The bone has been of varying ages, from its earliest appearance during fœtal life to extreme old age. The use of formalin as a fixative, while very good for most purposes, has two disadvantages, even though it be used in a normal saline solution. These are the action of formic acid in decalcifying bone, and, more important, the contractile effect of the formalin itself. Many of the appearances seen in prepared sections of bone, notably the space around the nucleus of the osteoblast and the gaps in the wall of the chondroblast, seem to be entirely due to the fixative. Such appearances do not occur in fresh material, and hence I consider them to be artefacts. The decalcifying action of the formalin has been tested by taking successive portions of the same fœtal tissue from the formalin at intervals of some weeks and comparing sections stained and mounted in the same manner. The alteration of tissue by the fixative has been checked by the observation of fresh material which has been untouched by any fixative or decalcifying agent, but simply frozen, cut, and examined immediately in glycerin. The following observations are given in condensed form. Each subject will be discussed in detail at a later date, together with its technical difficulties and the methods used in its investigation.

Most of the ordinary stains have been used, and certain special methods

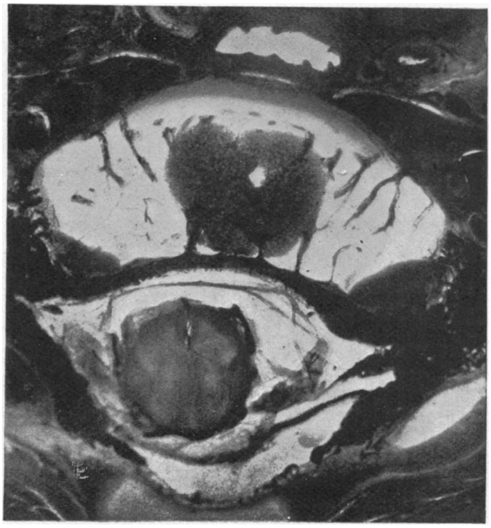

FIG. 1.—Transverse section through the lower part of the neck of a five-months human fœtus. (Not decalcified.) To show ossification commencing in the vertebra. The body is still cartilaginous except at its centre, where ossification is commencing. This ossification is not perivascular. Blood-vessels can be seen penetrating the cartilage from its periphery.

of staining, such as that of Mallory, have also been undertaken. With this latter stain, however, the success attained by Professor Geddes has not been secured by me.

Fig. 1 represents a transverse section through the lower part of the neck of a five-months human fœtus. The major part of the body of the vertebra is as yet cartilaginous, but ossification is occurring in the centre of the body. Vascularisation of the cartilage is in progress, but it is to be noted that the formation of bony tissue does not occur primarily around the blood-vessels. This fact is borne out by the occurrence of blood-vessels apart from ossification in other situations, such as the upper cartilaginous extremity of the humerus, at the same period of fœtal life. Ossification is not a direct result of vascularisation of cartilage, or it would be first exhibited along the course of the vessels. It would appear that osteoblasts do not travel along the blood-vessels to their ultimate destination. These cells are produced in quite another manner, and their appearance in cartilage or "*membrane*" is not directly due to vascularisation, although indirectly an increased blood supply has some association with ossification.

Vascularisation occurs in cartilage some time before ossification commences. Since this piece of work was done I find that Retterer has pointed out the same fact (4).

I have not thought it necessary to introduce at this stage a photograph of the microscopic appearance of ossifying cartilage. The study of such sections only confirms the view suggested by the appearances noted in fig. 1.

In the light of experimental and pathological evidence it is difficult to see how an increased vascularisation could be the direct cause of ossification. Most of the experimental studies on calcification and ossification have been performed on the kidney. An important communication on this subject is that of Wells, Holmes, and Henry (1). Any previously semi-necrotic material will take up calcium. But in such tissue the calcium is present almost wholly as phosphate. The article just quoted shows that calcium deposited thus pathologically is probably of urinary origin and not from the blood. It occurs in the renal epithelium and tube casts, and with this variety of calcification we have nothing to do. A very different type of calcification is that appearing in the interstitial tissue of the kidney, especially in the region of the pelvis, after ligation of the renal vessels. Such calcification may go on to ossification—(2), (1, pp. 3 and 8)—in from sixteen to twenty-one days.

For these phenomena some collateral circulation seems to be necessary. The blood supply of the part undergoing calcification or ossification is rather diminished than otherwise, at least for a time. Interference with the normal blood supply is also seen in a fractured bone, and here again the callus begins to ossify in about the same time. The calcium in the renal connective tissue is present mainly as carbonate. This is shown in some of the cases described by Wells, Holmes, and Henry (1).

It is the latter type of calcification which is analogous to that occurring normally in skeletal tissues. Osteoblasts do not travel by the blood-stream or along the coats of blood-vessels to their destination. We may now consider the possibility of the change in character of cells *in situ*.[1]

[1] Since the above view was expressed I note that Professor Benjamin Moore has formed somewhat similar conclusions with regard to bone formation, and has stated them in the abstract of his opening paper for the discussion on the rôle of calcium at the British Medical Association meeting at Liverpool (see *British Medical Journal*, 6th July 1912).

Fig. 2 shows a section of an ossifying occipital bone from a fœtus of eleven weeks. This section is taken from the previously cartilaginous supra-occipital portion of the bone. The central cells of the bone are seen to be quite different in character from those on the surface. These central cells resemble cartilage cells, except that they contain in their peripheral zone ossein in place of chondrin.

On examining carefully my sections of ossifying cartilage, I was impressed with the fact that the nuclei and peri-nuclear granular protoplasm of the cartilage cells never disappear. The matrix alone is lost. Retterer has shown that the matrix of both *chondroblasts* and *osteoblasts*

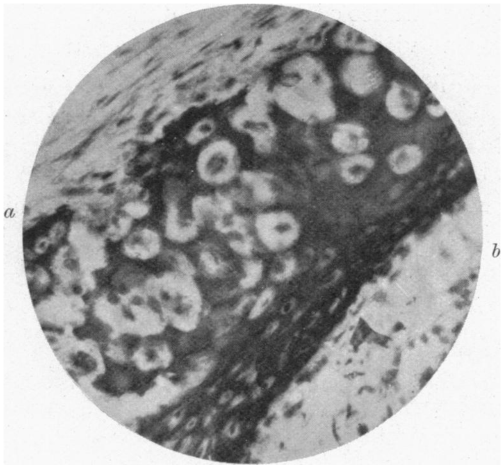

Fig. 2.—Vertical section of the occipital bone of a human fœtus of eleven weeks. (Not decalcified.) The previously cartilaginous portion is becoming ossified. Ossification is also taking place in the fibroblasts. *a*, chondroblasts in transition; *b*, fibroblasts in transition.

is really the peripheral portion of the cells abutting on each other, and that this zone consists of non-granular or clear protoplasm and forms the ground-substance of the tissue (3), (4).

In transforming cartilage, only the material in the clear peripheral zone disappears. The nucleus and peri-nuclear granular protoplasm remain. These never disappear; but when all the characters of a cartilage cell have been lost by the disappearance of the material in the clear peripheral portion of the cell or ground-substance, it takes on the character of an osteoblast, and calcium in combined form as ossein appears in place of the chondrin in the peripheral zone. Cartilage cells may be directly transformed into osteoblasts, and the breaking-down appearance of transforming cartilage cells, apart from the disappearance of the chondrin, is entirely an artefact.

The cells composing the surface layers of the bone in fig. 2 resemble fibroblasts which have undergone ossification. This is precisely what has happened; and as Retterer has already fully worked out this subject, it will be sufficient merely to recapitulate his views, which are thoroughly borne out by my own sections. The transforming fibroblast undergoes the following changes:—Its nucleus and peri-nuclear protoplasm stain less deeply.

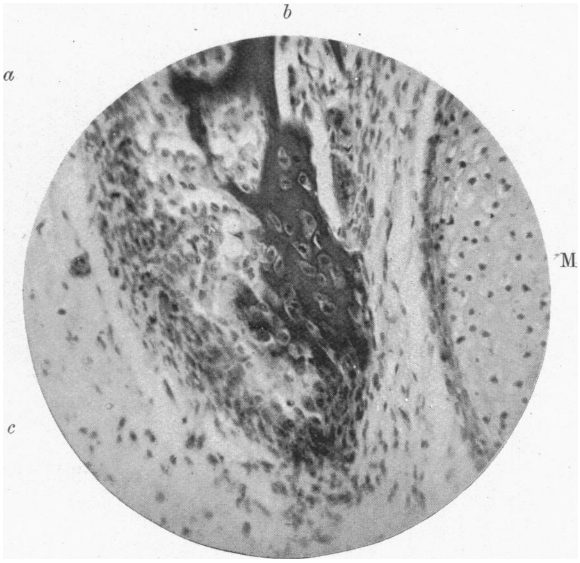

FIG. 3.—Sagittal section of the mandible of a fœtus of eleven weeks to show the formation of pre-osseous tissue. (Not decalcified.)

a, pre-osseous tissue; *b*, bone; *c*, surrounding connective tissue; M, Meckel's cartilage.

The peripheral zone of clear protoplasm increases in amount and the definite cell borders become indistinct. The fibroblast has now reached the stage of pre-osseous tissue. It corresponds to the chondroblast which has lost the chondrin from its peripheral zone, and is illustrated in fig. 3. This is a section of a developing mandible from a fœtus of eleven weeks. Calcium is next deposited in the peripheral zone of the protoplasm, and thus the osteoblast is produced.

Osteoblasts are fibroblasts or connective-tissue cells which have undergone certain characteristic modifications and may or may not have passed through a "chondroblast" stage.

The non-disappearance of cartilage cells in ossification is borne out by certain general facts enunciated by Leutert.

This author states that completely necrotic epithelium does not calcify. The cells calcify while still living and attached to the basement membrane (5).

Between pathological and normal calcification there would seem to be the following distinction. Pathological calcification occurs in injured cells, in which the calcium occurs mainly as phosphate and in an amorphous condition. In physiological calcification the cells are still healthy, and they form a protoplasmic compound with the calcium, which can be recovered as

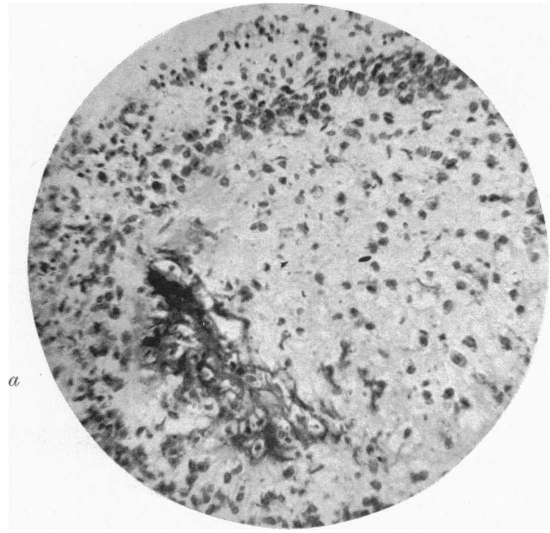

FIG. 4.—Oblique section through the outer third of the non-decalcified left clavicle in a sixty-days human embryo (8½ weeks). The greater part of the tissue is of the nature of muco-cartilage. Ossification is taking place at *a*.

carbonate, and which is present in the peripheral zone only of the cell, where it possibly exists in colloidal condition.

There would appear to be no distinct difference between physiological calcification and ossification.

In considering the transformation of more primitive skeletal tissue into bone some important facts have come to light regarding special bones.

Fig. 4 represents a section through the outer third of the left clavicle in a sixty-days human embryo. It will be seen that calcification has as yet occurred only in one portion of the section. The majority of the tissue is similar to cartilage, though not identical with the cartilage of the humerus and scapula. That the clavicle is in reality to a large extent a cartilage bone is not a new idea. It has already been thus described by several authors, of whom the most recent is Fawcett (9).

This brings the clavicle into line with the other bones of the limbs as regards its development. Moreover, cartilage is not one single entity in itself, but presents numerous modifications, the different forms of which exhibit variations in the relative proportion of cells to matrix and in the constitution of the matrix. The subject of variations in cartilage and their relation to ossification is under investigation at the present time.

The character of formed bony tissue varies with the age and situation.

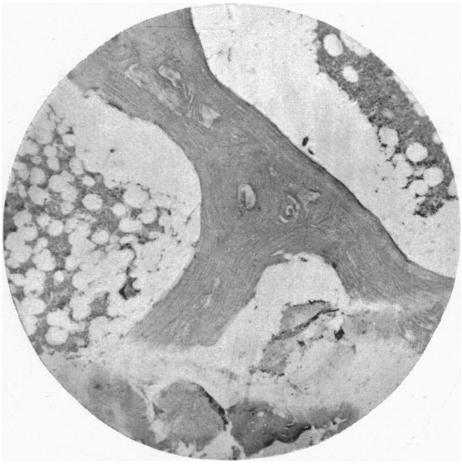

FIG. 5.—Transverse section of the sternal end of the clavicle from a man aged 57. (Decalcified.) To show the occurrence of Haversian systems in cancellous tissue.

Von Eggeling (6) has divided bone into the following varieties:—

(1) Coarse-fibred tissue deposited from the periosteum.

(2) Fine-fibred and cartilage bone.

(3) Haversian systems.

He admits, however, that the separation of bone into different varieties is to a large extent artificial.

All bone first develops according to the patterns shown in figs. 2 and 3.

At a later date Haversian systems appear. That is to say, rearrangement occurs among osteoblasts in such manner that they become grouped around the blood-vessels. It is one of the essential distinctions of bone from cartilage that it requires a much greater blood supply. This may be owing to the fact that it is not pervious to the body fluids as is cartilage (see (4)). The changes in the appearance of bony tissue which are represented by the occurrence of Haversian systems are of slow development. They are not brought about necessarily by the absorption of previous osteoblasts, but through rearrangement of these cells, from the peripheral zone of which the ossein can disappear exactly as the chondrin disappeared from the chondroblasts in their transformation into osteoblasts. Haversian systems are not confined to compact tissue, but also occur in spongy or cancellous bone, as shown in fig. 5. This is from a transverse section of the sternal end of the clavicle from a man aged 57.

The breaking down of Haversian systems is illustrated by fig. 6. This

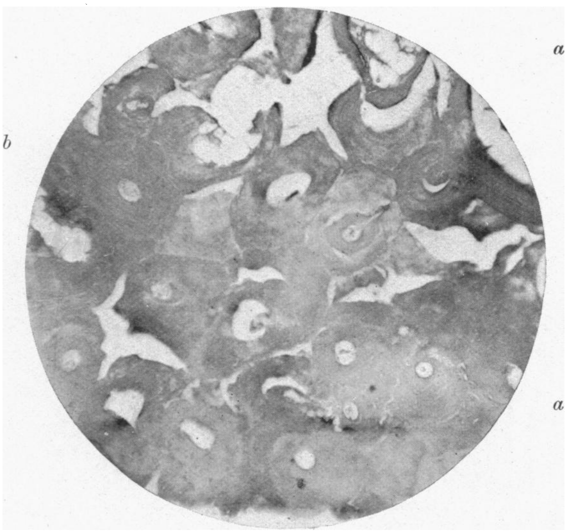

FIG. 6.—Transverse section through the decalcified compact tissue of the sternal end of the clavicle from a man of 57. Ossein is disappearing from certain of the Haversian systems, *a*. A new Haversian system, partially taking the place of the old ones, is shown at *b*.

represents another transverse section through the compact tissue at the sternal end of the clavicle of a man of 57. It will be observed that the ossein is disappearing from some of the systems, which are becoming fragmentary in character and are being replaced by other more recently formed systems. Probably a similar process is going on throughout life.

In pathological conditions this disappearance of ossein from bony tissue occurs to a much greater extent.

Fig. 7 is a section of the tibia from a case of ostitis fibrosa, kindly lent me last year by Mr Charles Roberts and Dr Loveday. The case has not developed sarcomatous features, so that the diagnosis may be certainly established. A point of importance to which reference will later be made is that no osteoclasts are present. The same transformation of bone into fibrous tissue occurs in leprosy and in syringomyelia. In these diseases the stumps of fingers or toes are naturally rounded. The bone does not protrude as in a case of gangrene. This is due to the fact that the bone has previously undergone a fibrous change.

In leprosy, again, no osteoclasts are found. I have not had the opportunity of examining histologically a stump from a case of syringomyelia. Such fibrous change in bone has, I find, already been noted by Retterer (10).

Changes in formed bony tissue may and do occur through resorption of ossein, and this without the intervention of so-called osteoclasts. That

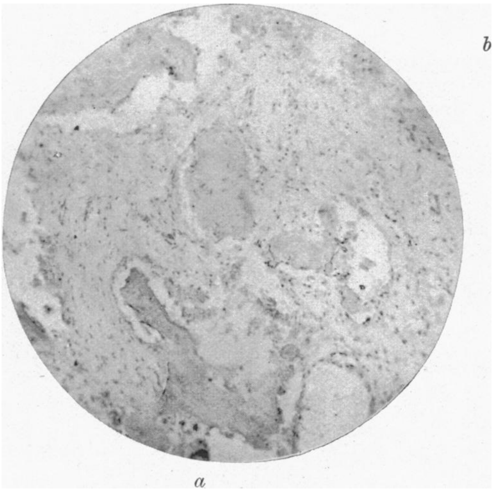

FIG. 7.—Non-decalcified section of the tibia from a case of ostitis fibrosa in youth. The ossein has almost entirely disappeared, and the bone is transformed into "fibrous" tissue.

a, portion of bone still remaining; *b*, "fibrous" tissue.

some thermolabile substance, probably a ferment, does exist in bone, and can be investigated by autolysis, has been shown by Morpurgo and Satta (7). This brings the calcium stored in osseous tissue into line with the glycogen of the liver and the fat of connective-tissue cells.

Throughout the discussion of the formation and transformation of bone there has been no need to have recourse to the cells known as osteoclasts, examples of which are shown in fig. 8. This section is from the alveolar part of the mandible in a fœtus of eleven weeks, and represents the appearance which these cells usually assume under the microscope. Full discussion of them cannot be entered into in the present paper, but a few observations may not be out of place at this juncture. They certainly are not present at sixty days (eight and a half weeks) in fœtal heads, in which situation they do not appear as early as osteoblasts. They are large multinucleated masses of protoplasm, whose nuclei display a striking similarity to those of osteoblasts. Somewhat similar giant-cells are also to be found in the connective tissue apart from bone. They occur on the surfaces of the bone—that is to say, between bone and the surrounding tissues. They lie in depressions on the bony tissue. This last feature would seem to be an artefact, as it is not to be found in fresh tissue.

FIG. 8.—Sagittal section of non-decalcified mandible from a fœtus of eleven weeks. To show two typical "osteoclasts" and the Howship's lacunæ from which they came.

a, "osteoclasts"; *b*, developing bone.

Fig. 9 shows a condition which occurs occasionally in the sections. It is from the mandible of a fœtus of eleven weeks, and shows a giant-cell whose tissue is in continuity with that of the bone. This section should be compared with fig. 3. The striking similarity between so-called osteoclasts and masses of what Retterer has called pre-osseous tissue (3) then becomes apparent.

Bloodgood has suggested that "osteoclasts" are the proliferating tips of developing blood-vessels (8). I have not found this to be the case. Indeed, the area round a blood-vessel is usually wholly devoid of these giant-cells.

One is therefore inclined to consider "osteoclasts" to be masses of pre-osseous tissue artificially separated from the fully ossified bone during its preparation for histological examination. This view is fully borne out by the examination of fresh bone.

Summary.

In the light of the present research, on which this paper is preliminary in character, the following conclusions seem to be justified:—

(1) Osteoblasts do not enter skeletal tissue along the blood-vessel tracks, but are fibroblasts or connective-tissue cells which have undergone certain

FIG. 9.—Sagittal section of mandible of human fœtus of eleven weeks. (Not decalcified.) This is considerably magnified but should be compared with fig. 3. It shows an "osteoclast" which has not been separated from the osseous tissue by the fixative, and represents the condition exhibited by sections of fresh bone when examined at once.

a, "osteoclast"; *b*, developing bone.

characteristic modifications, and may or may not have passed through a "chondroblast" stage.

(2) Calcification may occur as the deposit of phosphate or carbonate mainly. The latter type is more nearly allied to normal ossification.

(3) Haversian systems occur equally in cancellous and compact bone.

(4) Interstitial changes occur in bony tissue through absorption of ossein apart from the action of osteoclasts.

(5) The cells known as osteoclasts are masses of developing pre-osseous tissue, and do not possess chondroclastic or osteoclastic properties.

The sections and micro-photographs for reproduction in this paper have, as in previous papers, been prepared by Mr H. Gooding, our departmental histologist. My best thanks are due to Mr Gooding for the care which he has taken and the successful manner in which he has produced the illustrations.

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