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A THEORY OF BONE FORMATION

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Over two hundred years of clinical observation and experimental research have failed to clarify the mechanism of bone formation despite the undeniable progress made in the study of many of the factors involved. It has been the writer's privilege to be able to concentrate on the study of the vasculature of bone and on the part the vascular system plays in osteogenesis. As a result of most of these works (*Trueta, Trueta et al.)* a concept of bone formation has progressively emerged which, as new evidence has been collected, has approached near conviction.

In a few words the concept is that the wall cells of the bone capillaries and sinusoids – endothelial cells – are responsible for the formation of osteoblasts, directly or by the intervention of one or more intermediate cells. In the following pages are given some of the facts which may contribute to the understanding of the way in which the endothelial cell becomes a bone forming cell.

The data in support of the osteogenetic properties of the bone vessels has been accumulated during the investigations of the vasculature of the callus of fractures and of the growth cartilage, the incorporation of bone grafts and the osteoporosis following muscle inactivity. We have concluded that the factors underlying the unexplained Wolff laws of trabeculae deposition and orientation (1870) are mediated by the particular positioning of the osteogenetic vessels (*Trueta* 1956b, 1958, 1960, 1961b).

As reported elsewhere (*Trueta,* 1958), the progression of the vessels is towards the ischaemic area at the centre of the fracture site (Fig. 1). But what seems relevant here is that the deposition of new bone in the form of provisional trabeculae is also found in radiating form, moulded onto the vascular patterns (Fig. 2).

The following is a summary of the way in which we believe the ves-

Fig. I.
Experimental fracture of the radius (rabbit). The perfussed vessels all point towards the ischaemic ends of the bone fragments and the mature cartilage. The vascular stimulating factor (V. S. F.) is produced here.

Fig. II.
Radiating orientation of provisional trabeculae in the callus of an experimental fracture in the rabbit. These trabeculae are exactly moulded into the vascular pattern.

sels enter into osteogenetic activity. The endothelial cells of the walls of the advancing vessels divide not only at their extending ends but along large sections of their wall and lay down a progeny of either osteoblasts or their near predecessors (*Trueta*, 1961). All these cells, from endothelium to osteocyte, remain attached by intercellular cytoplasmic connections. After the osteoblasts have laid down the collagen and polysaccharide matrix and have subsequently become buried by the deposition of apatite, the cytoplasmic attachments to their ancestors prevent the deposit of mineral from becoming an isolating wall. Canaliculae will thus appear round each of the cell expansions and the original syncytium will be preserved by the rigid mineral structure characteristic of bone.

We have studied with the help of the electron microscope the suc-

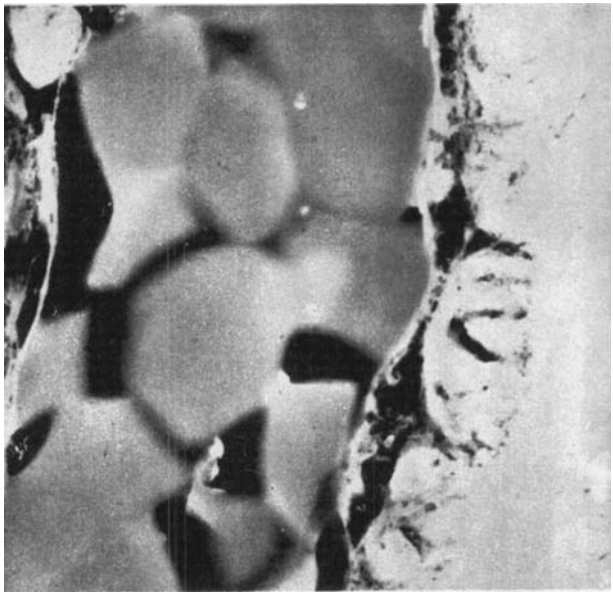

Fig. 3.
Electron microscope photograph. Vessel of a Haversian canal full of erythrocytes cut longitudinally. Its endothelium is becoming incorporated into bone. × 3000.

cessive stages by which these vascular cells become incorporated into bone and with the collaboration of Dr. K. Little we have been able to illustrate this point to our satisfaction. The reason why we have been able to collect all this data on the vessel wall is because our sections, including most of those for the electron microscope, have been obtained from perfused specimens in which the vascular lumen remains patent (*Trueta & Little*, 1960). Our data of the area of active bone formation in the calcified tubes of the metaphysis during growth is abundant and *Rigal* (1961) in our laboratories has found by cell labelling with tritiated thymidine that many of the endothelial cells of the vessel wall enter the synthetic phase of their mitotic cycle, i.e. are preparing to divide.

Questions may be raised as to the validity of this concept of bone formation when applied to the origin of the haversian system of the osteones. Consequently, it was thought necessary to elucidate the role the vessels play in the organization of the haversian system of cortical bone.

The data presented here was obtained by the use of the electron

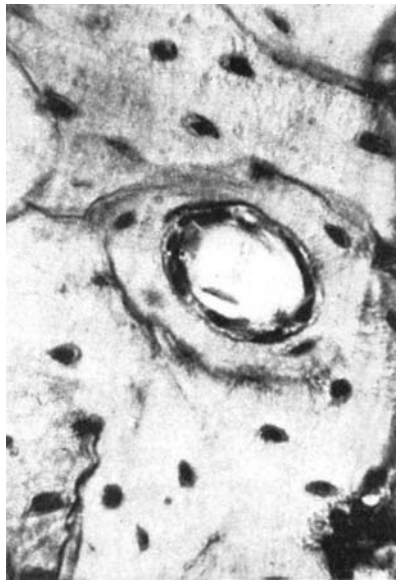

Fig. 4.
Optic microphotograph. One single row of osteoblasts separates the wall of the central vessel from the calcified canal. The syncytium constituted by all these cells and the osteocytes round them is well seen. × 700.

microscope and particularly by microscopy under ultraviolet light following the injection of tetracycline and the subsequent perfusion of the animal – mainly the guinea-pig or rat – with the 2 per cent solution of Berlin Blue.

The part the periosteal vessels play in nourishing the outer layers of the cortex has been mentioned before; we must point out that the vascularity of the periosteum is much greater during growth than after its cessation. But in both young and adult, the vessels of the periosteum appear as responsible for the origin of the osteoblasts as are those of the trabecular system.

The deposition of subperiosteal bone is made, like geological strata, in successive layers each of which is vascular in origin. On close examination of the single or double vessel of the Haversian canal after perfusion it appears frequently that the endothelial cell is in immediate connection with the osteoblasts either directly or by the intermediation of a single cell (Fig. 3). With ultraviolet microscopy, the tetracycline deposition – which indicates the area of previous labour of the osteoblasts – so perfectly corresponds with the outer perimeter of the vessel

that the only rational conclusion seems to be that both of them are very intimately related to each other.

In the light of these findings one cannot but admire the perfect arrangement by which the transudates from the central vessel can reach the cells at the depth of the osteone. Each row of osteocytes keeps its syncytial link by means of its cytoplasmic prolongation with the row it encircles until the more central line of osteocytes embraces and connects the osteoblasts and these the cells of the vascular wall (Fig. 4). This material is now being prepared for publication with the collaboration of Dr. Rios Leal.

BONE REMOVAL

We have elsewhere referred to a mechanism of bone removal (*Geiser & Trueta*, 1958) and have pointed out that the normal process of bone resorption in this experiment depended on vascular changes. It seems unnecessary to mention that either reduced apposition of bone or its increased resorption can be produced by withdrawing from the blood some of the essentials required for bone formation such as O_2 and vitamins A, C and D, calcium and phosphate salts.

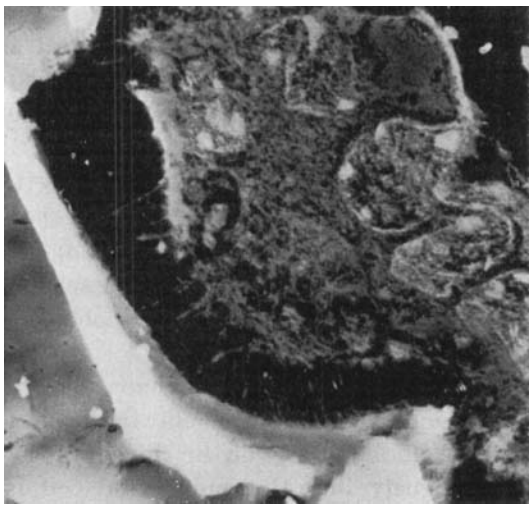

Fig. 5.

Electron microscope photograph. Osteoclast with a number of its component cells seen still attached to the cells of the resorbing bone by cytoplasmic prolongations. × 2500.

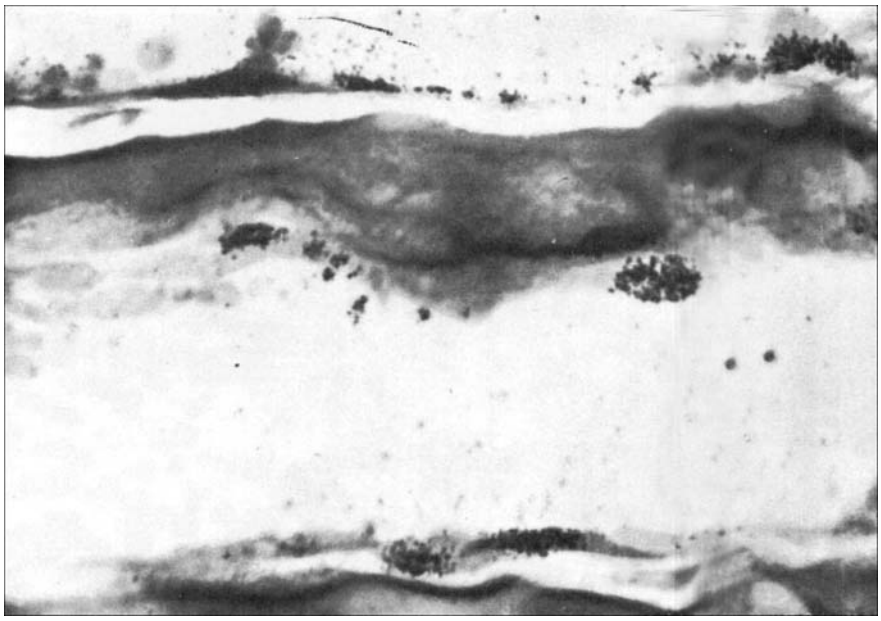

Fig. 6.
Endothelial vascular cells labelled by tritiated thymidine in the phase preceding cell division (tissue culture). × 800.

We shall now discuss in the light of our findings the possible role of the osteoclasts as the agents of bone removal.

We have been able to obtain microphotographs of osteocytes connected to neighbouring osteoclasts by cytoplasmic expansions of exactly the same nature as those linking osteocytes with each other or with the osteoblasts of their vicinity (*Trueta,* 1958). No other explanation was found that the osteoclasts include osteocytes and nearby osteoblasts from the same area of syncytium and probably intermidiate cells also. In this *Tonna* (1960) and *Young* (1961) coincide, and the evidence with tritiated thymidine obtained in our laboratory further supports the view that osteoclasts are formed by osteocytes, osteoblasts and/or intermediate cells.

In bone resorption caused by muscle inhibition (*Geiser & Trueta,* 1958) the number of osteoclasts present is enormous. This does not prove other than in this type of osteoporosis, osteoclasts are formed in large number. But are they the initiators of bone removal – as *Kolliker* (1873) thought they were – or are the osteoclasts a simple consequence of the resorption of bone, as *Recklinghauser* (1891) preferred

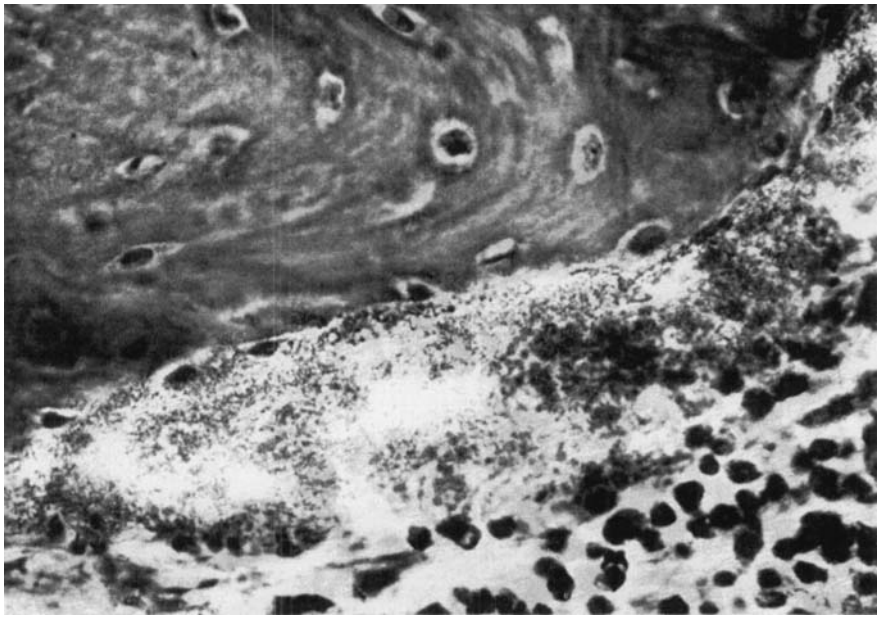

Fig. 7.
Perfussed vessel (Micropaque) demonstrating the active elaboration of bone by its endothelium. × 600.

to think? This is not an academic question for if it were possible to clear up this point the control of such a severe disability as clinical osteoporosis would be nearer.

We have little doubt about this. Dozens of works and papers on the origin of the osteoclasts by people who assign a specific nature to such cells had failed to discover from where they come and what causes them to appear. Again, no light had been thrown on the final destination of the bone cells and their syncytial connections which must be left to withstand conditions for which they were not prepared, once the crystals of apatite have been washed away in the early stages of bone removal (Fig. 5). In one point we may differ from the workers who doubted the agressive nature of the osteoclasts and denied any osteolytic power to them (Fig. 6). It has been widely recognised that degenerating or dead osteocytes liberate some substance that is responsible for the removal of the mineral limiting the bone lacunae. It may well be that the accumulation of defective osteocytes preserves in the osteoclast, even perhaps enhances, the agressive property of the osteocytes from which they are mainly formed. But this does not detract

us from the conviction that what makes the osteoclast appear is the initial bone removal caused by the vascular mechanism to which we have referred. *Kelly, Little & Courts* (1959) have shown the part that an alteration of the matrix plays in causing osteoporosis.

Many important aspects of the osteogenetic process remain still a mystery and thus are subjected to theorising and controversy. Such is the case with this constant attendant at osteogenesis which is alkaline phosphatase. But of one thing at least we are certain, namely that bone is an organized "soft" tissue of which only part has been made rigid by the deposit of calcium salts. The organiser is the osteogenetic vessel from which springs the syncytial frame of cells and their connections on which the bone architecture is established. Endothelial cell – intermediate cell – osteoblast – osteocyte – osteoclast, constitute the normal sequence of cellular philogeny in the constant elaboration and removal of the bone substance. The initial cells on which the whole process rests are those of the capillary-sinusoid vessel (Fig. 7) which is responsible for providing the transudate on which the life and health of the whole syncytium depends.

SUMMARY

A concept of bone formation based on the reproductive activity of the endothelial vascular cells of bone capillaries and sinusoids has been explained and some of the data on which this concept is based adduced. An intimate connection amounting to cell continuity is postulated between the endothelial cell, the intermediate cell, the osteoblast, the osteocyte and the final conglomeration of most of them in the cell we know as osteoclast.

RESUME

Une conception de formation osseuse basée sur l'activité reproductive des cellules vasculaires endothéliales des capillaires osseux et des sinusoïdes est expliquée et quelques-unes des données sur lesquelles cette conception est basée sont fournies. Il est prétendu qu'il existe une étroite liaison en ce qui concerna la continuité des cellules entre la cellule endothéliale, la cellule intermédiaire, l'ostéoblaste, l'ostéocyte et la conglomération finale de la pulpart de ceux-ci dans la cellule que nous connaissons comme ostéoclaste.

ZUSAMMENFASSUNG

Eine Auffassung der Knochenbildung, der die reproduzierende Tätigkeit von endothelialen Gefässzellen der Knochenkapillaren und Sinusen zu grund liegt, wurde erläutert und einige der Tatsachen auf denen diese Auffassung gegründet ist wurden angeführt. Ein enger Zusammenhang, der einer Zellkontinuitet zwischen den Endothelzellen, den intermediären Zellen, den Osteoblasten, den Osteozyten und der schliesslichen Verschmelzung der meisten von ihnen in die Zelle, die wir als Osteoklast kennen, gleichkommt, wird angenommen.

REFERENCES

Geiser, M. & Trueta, J.: J. Bone and Joint Surg., *40 B:* 282, 1958.

Harrison, M. H. M., Schajowicz, F. & Trueta, J.: J. Bone and Joint Surg., *35 B:* 598, 1953.

Kölliker, H.: Die normale Resorption des Knochengeweses. Vogel, Leipzig, 1873.

Kelly, M., Little, K. & Courts, A.: Lancet *ii*, 1125, 1959.

Recklinghauser, F. D. von: Festschrift f. R. Virchow, Berlin, 1891.

Rigal, W. M.: A study of Bone Development using Tissue Culture as the Main Technique. D. Phil. Thesis, Oxford, 1961.

Tonna, E. A.: Anat. Rec., *137:* 251, 1960.

Trueta, J., Barclay, A. E., Daniel, P. M., Franklin, K. J., & Pritchard, M. M. L.: Studies of the Renal Circulation. Blackwell Scientific Publications, Oxford, 1947.

Trueta, J.: Bull. Hosp. Joint Dis., *14:* 5, 1953.

Trueta, J. & Harrison, M.H .M.: J. Bone and Joint Surg., *35 B:* 442,1953.

Trueta, J. & Cavadias, A. X.: J. Bone and Joint Surg., *37 B:* 492, 1955.

Trueta, J.: Acta Med. Belgica, suppl. 1, 165, 1956a.

Trueta, J.: Lancet *i:* 585, 1956b.

Trueta, J.: J. Bone and Joint Surg., *39 B:* 358, 1957.

Trueta, J.: Rev. Chir. Orthop., *44:* 3, 1958.

Trueta, J.: J. Bone and Joint Surg., *41 B:* 671, 1959.

Trueta, J.: Nursing Mirror, 20. May, p. 5, 1960.

Trueta, J. & Morgan, J. D.: J. Bone and Joint Surg., *42 B:* 97, 1960.

Trueta, J. & Little, K.: J. Bone and Joint Surg., *42 B:* 367, 1960.

Trueta, J. & Amato, V. P.: J. Bone and Joint Surg., *42 B:* 571, 1960.

Trueta, J.: In press, 1961a.

Trueta, J.: J. Trauma, *1: 5*, 1961b.

Wiley, A. M. & Trueta, J.: J. Bone and Joint Surg., *41 B:* 796, 1959.

Wolff, J.: Virchow's Arch. f. path. Anat., *50:* 389, 1870.

Young, R. W.: Autoradiographic Studies on Thymidine – H_3 Metabolism in Bones of Young Rats. Program, Gordon Research Conference on the Chemistry, Physiology and Structure of Bones and Teeth, Meriden, New Hamshire, 1961.