The Function of Bone As A Calcium Reservoir With A Consideration of the Cellular Pictures Seen in Resorption With Particular Reference to the Significance of Osteoclasts

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Bone is not the fixed and permanent structure which its relatively dense composition might seem to indicate. It performs a dual function in the body by providing both a supporting structure and a calcium reservoir. Its architecture changes constantly throughout life, and the remodelling which occurs after fractures offers very good evidence of its ability to accommodate itself to the work placed upon it. As well as this ability to structurally adapt itself to stress and strain, bone also adapts itself to altered conditions of calcium metabolism and may provide large amounts to the blood under certain conditions. The cells of bone do not, however, remain quiescent to this depletion of the matrix, so the proliferation and differentiation of cells in bone may arise not only as a result of trauma or increased strain, but also because of a marked calcium loss. There is a fairly general tendency to regard the cellular pictures seen in bone resorption as causatory, but now that the calcium metabolism and the mechanism of calcification are better understood, it is becoming obvious that great care must be used in deciding whether the pictures seen in bone resorption represent cause or effect. This contribution therefore attempts to review briefly some of the more important features of the calcium metabolism and their relationship to the calcification and decalcification of bone. It is also concerned with a study of the cells in bone and their ability to react to these various stimuli, and deals particularly with the ability of the osteogenic cells to form osteoclasts whose function and significance are considered in detail.

In order to understand the way in which cells of bone react, it is first essential to realize the different types which exist and their capacity

for further differentiation. Briefly, the cells of bone are of two types, the adult bone cells which reside in lacunae and which are mature, fully differentiated elements, and the osteogenic cells which are found in the deep layer of the periosteum and which also make up the endosteum which lines the marrow cavity and the Haversian canals. The osteogenic cells have been discussed in detail, (Ham[1,2]), and have the capacity for division and for differentiation into bone, cartilage, or osteoclasts. Their capabilities are well demonstrated in the healing of experimental fractures, and there they are seen, in the early phases of the repair process, to differentiate into both cartilage or bone, the former developing in an avascular environment and the latter in a vascular one. Later in the process many of the osteogenic cells are seen to differentiate into osteoclasts. This phenomenon occurs largely as the cartilage, which originated in the callus, is being replaced by a second invasion of osteogenic cells and blood vessels. Whereas many of the osteogenic cells in this instance differentiate into bone, many others form osteoclasts, and it is thought by the author that this represents what might be termed a foreign body reaction, on the part of these cells, to the breaking down cartilage. In any instance it is seen that the osteogenic cells of bone possess a considerable amount of potentiality and may differentiate at least into cartilage, bone or osteoclasts.

Turning next to the function of the bone cell, it is evident that during the phase of differentiating into this entity, the cell creates a matrix which consists of collagen, together with a mucoid and an albumoid. Many observers have thought that the osteoblasts or bone cells secreted the calcium salts into the matrix but this theory seems to be entirely disproven by certain experimental findings which have lately been recorded. In the first place Wells[3] showed some time ago that boiled cartilage, when transplanted into the abdominal cavity of animals, would calcify, which shows that the matrix itself possesses the ability to take up calcium salts by its physico-chemical properties. It was later shown by Robison, MacLeod and Rosenheim[4], however, that if a slice of bone was killed by chloroform and then placed in tissue culture, where there were available calcium salts, it would only partially calcify, but if the enzyme phosphatase, which is a normal product of hypertrophied cartilage cells and bone cells (Robison and Soames[5]) was added to the tissue culture, the matrix would calcify despite the fact that the cells were dead. Thus it is seen that the cells do not actually deposit the calcium in the matrix but that the calcification of matrix depends upon its physico-chemical properties which allow it to take up calcium salts from the tissue fluid, and also upon the presence of enzyme

phosphatase which apparently has a local action in rendering more phosphate ions available for the calcification process.

The calcification of bone, however, depends on other factors than the physico-chemical properties of bone matrix and the production of phosphatase by the bone cells. It is evident that in order for this mechanism to function properly, calcium and phosphorus must be present in the blood in certain amounts, because the mechanism is designed to operate at normal levels of these substances. Consequently the general factors pertaining to the maintenance of normal blood calcium levels have to be considered together with those which are concerned with the local removal of calcium from the blood to the bones.

The general factors which are related to the maintenance of proper levels of calcium and phosphorus in the blood are rather complex. It is difficult to separate theory from fact and in order to postulate a good working hypothesis, it is necessary in some degree to draw upon the former. In the first place there is more calcium and phosphorus in the blood than can be explained by the laws of simple solution. Furthermore it has been shown by Collip[6] that an extract may be prepared from the parathyroid gland which possesses the ability to raise the blood calcium level when injected, and that this same preparation will keep dogs out of tetany after parathyroidectomy. As it has been observed that the cerebro-spinal calcium level was normally considerably under that of the serum calcium level, Cameron and Moorhouse[7] performed experiments in which they found that, after parathyroidectomy, the serum calcium level fell more rapidly than the cerebro-spinal fluid level. If it can be assumed that the cerebro-spinal calcium represents that portion of the serum calcium which is diffusible, it would thus appear that the non-diffusible calcium of the serum is tied up in some fashion with the hormone from the parathyroid gland, so that after the parathyroids are removed, most of the non-diffusible portion disappears from the serum. The latter in this instance would be chiefly composed of diffusible calcium so that the serum level would tend to approximate that of the cerebro-spinal fluid. Cameron[8] stated, "We believe that the parathyroids elaborate an internal secretion which controls the formation of a non-diffusible calcium compound in the blood, and that this, by a series of interlocked equilibria, keeps the diffusible calcium compounds of the blood to a constant ratio with itself. Greenwald[9], after several experiments in this field, proposed the following, "It is suggested that the calcium content of the plasma is normally at a constant level by

an equilibrium between inorganic calcium and an organic compound of calcium. Resemblences between this organic compound and calcium citrate are indicated but the substances are not identical. It is suggested that the parathyroid hormone is necessary to the preparation of this organic constituent." Recently Morgulis and Perley[10] found that after parathyroidectomy, although both the cerebro-spinal calcium and the serum fell to lower levels, the fall of the serum calcium was relatively greater. They also found that injections of parathyroid hormone would cause both the cerebro-spinal fluid calcium and the serum calcium to become increased, but that the rise in serum calcium was relatively greater than that of the cerebro-spinal fluid. These findings would tend to indicate that the parathyroid hormone was associated with the non-diffusible calcium of the blood; but, on the other hand, these same observers performed other experiments in which they dialyzed the serum against the cerebro-spinal fluid of the same animal, and found that even though the serum was obtained from animals which had received injections or parathyroid hormone plus calcium, on dialysis, the calcium content of the serum became lower, and that of the cerebro-spinal fluid higher. This last finding, however, instead of indicating that the parathyroid hormone does not control the formation of the non-diffusible calcium, could be interpreted, it is thought, to indicate the action of an injection of parathyroid hormone is only temporary, and that the compound (the non-diffusible calcium) almost constantly liberates calcium ions which could increase the ionic concentration on the serum side of the membrane so that calcium ions could therefor dialyze through to the cerebro-spinal fluid side of the membrane.

At present, the bulk of the evidence favors the theory that the blood calcium consists of two portions, the non-diffusible and the diffusible, and that the non-diffusable portion is controlled by the parathyroid hormone. Furthermore it seems that the two are in equilibrium not only with each other but also with the calcium of the bony reservoirs. Thus an increase in the amount of parathyroid hormone in the circulation would theoretically lead to a shift of the equilibrium to the non-diffusible calcium, so that calcium ions would pass from the bones through the diffusible calcium of the blood to the organic compound controlled by the parathyroid hormone. This would have the effect of gradually depleting the bones if the condition was maintained and it is evident, from the cases of parathyroid tumor studied in man, that this result is accomplished. It should also be pointed out that the source of calcium for the institution of this hypercalcaemia may be either the diet, the bones, or both. In long continued hyper-

calcaemias of parathyroid tumors it seems that the bones are usually drawn upon. During this interval there is also an increased amount of calcium excreted from the body by means of the urinary tract. This problem is discussed in detail by Bulger, Dixon, Barr and Schregardus.[11]

As well as the hormone of the parathyroid gland, there is also a second agent which appears to bear a profound relationship to the calcium metabolism, namely vitamin D. On diets deficient in this vitamin, young growing rats will develop rickets. One must be careful in interpreting its action as being simply that of an assisting one to the calcification process because it has been found by numerous investigators that the administration of vitamin D in huge doses will lead to a hypercalcaemia, with a depletion of the bones of the animals, together with the deposition of calcium salts in various parts of the body. Concerning, first, the site of action of the vitamin, it should be pointed out that Shipley, Kramer and Howland[12], found that the cartilage from rachitic animals would calcify perfectly in tissue culture providing sufficient calcium and phosphorus were available. Thus it would appear that the helpful action in rickets exerted by the vitamin is not on the tissue, but rather on the calcium and phosphorus metabolism.

The question now arises as to whether the action of vitamin D on serum calcium levels is exerted through the agency of the parathyroid gland or its hormone, or whether it is independent. It would seem at first glance that this matter could be easily settled by administering the vitamin to parathyroidectomized animals. This procedure, however, has met with a considerable amount of difficulty and many diverse results have been obtained. The work of Taylor, Branion and Kay[13], however, showed that, after very complete neck dissections, animals could not be kept out of tetany by large doses of irradiated ergosterol, so that this finding would seem to point very strongly to the fact that vitamin D exerts its influence through the agency of the parathyroid or its hormone.

A great deal of rather general evidence also supports this viewpoint. Taylor, Branion and Kay pointed out that the findings at autopsies of animals dying from hypervitaminosis were similar to those of hyperparathyroidism. Furthermore, both agents are known to cause a hypercalcaemia, and to cause, if administered in sufficient quantities, pathological calcifications in various parts of the body. The reactions in the bones to prolonged dosages of either is similar, leading to the development of

osteitis fibrosa cystica. Very recently Grauer[14] has reported the development of this condition in animals subjected to prolonged dosages of irradiated ergosterol, and states that the findings in the bones were similar to those described by Jaffe and Bodansky[15] who produced osteitis fibrosa experimentally in animals by administering excessive parathyroid hormone over a rather long period of time. Consequently, although the problem is not definitely settled, there appears to be very good evidence to consider that vitamin D exerts its action through the pararthyroid gland or its hormone.

There are still other factors which enter into the problem of the maintenance of adequate calcium and phosphorus levels in the blood. The first of these is that there should be a sufficient supply of each in the diet. The problem, however, does not stop there, because many factors appear to pertain to the absorption, relative percentage, and excretion of these two entities. The matter of absorption is complicated because of the fact that the blood may draw upon the bony reservoirs in order to increase the concentration of calcium in the blood. Harris and Innes[16] found, on feeding experimental animals certain large doses of irradiated ergosterol, the first tendency was for the serum calcium to become elevated at the expense of calcium from the diet. But if the animals were placed on a diet deficient in calcium, and given large amounts of irradiated ergosterol, a hypercalcaemia would develop at the expense of the skeleton. Bulger, Dixon, Barr and Schregardus[11] showed that in cases of hyperparathyroidism with hypercalcaemia, the administration of orthophosphate resulted in an increase of the serum phosphate, and a decrease of the serum calcium. They pointed out the tendency of a reciprocal rise and fall of the phosphorus and calcium, and showed that a high calcium is apt to be associated with a low phosphorus and vice versa. It was furthermore indicated that this sort of finding was typical of phenomena which could be explained by the theory concerning the solubility product of electrolytes in solution. The problem is further complicated by the fact that the calcium of the blood is probably present in both a non-diffusible and a diffusible form, so only a portion of the serum calcium can be considered as ionized. The evidence obtained from the various experiments pertaining to the cerebro-spinal fluid would indicate that this portion of the blood calcium which is diffusible may be raised to some extent, so it can scarcely be assumed that the diffusible calcium of the serum is normally in a saturated state. If, however, one applied the theory of the solubility product of electrolytes in solution to this situation, it is evident that, theoretically, the lowering of the serum

calcium level by the administration of sodium orthophosphate could readily cause a precipitation of the salts of calcium. This possibility has been mentioned already by Bulger, Dixon, Barr and Schregardus[11], and it is easily seen that several disturbances of the concentrations of the electrolytes present in the serum might lead to the precipitation of the less soluble salts. The recent work of Hess, Benjamin and Gross[17] may be of interest in this regard, as they found that on frequent injections of solutions of sodium bicarbonate into animals with hypercalcaemia, pathological calcifications were produced in certain parts of the body. This precipitation might conceivably be explained by the above described mechanism.

Another factor which concerns the calcium carrying power of the serum and which is related to the problem of bone resorption concerns the acid-base equilibrium of the serum or tissue field. It has already been indicated that normally, the diffusible calcium of the serum is probably not quite up to the saturation point, but it is probable that in hypercalcaemia an almost saturated condition may be obtained. Thus serum of a high carbon dioxide tension almost completely saturated with calcium (in a case of hypercalcaemia) will probably be unable to retain all of its calcium if the carbon dioxide tension should fall. The metastatic calcifications of hyperparathyroidism have been explained by this theory, as three of the most prevalent regions for calcifications in this condition are the lungs, gastric mucosa and kidney, all of which are supposed to be areas of acid excretion, so that the tissue fluid of these areas should be alkaline, thus forcing a precipitation.

Although it is seen that the development of a low carbon dioxide tension may account for the precipitation of calcium salts from the serum in hypercalcaemia it does not appear to explain all the pathological calcifications encountered in hyperparathyroidism or hypervitaminosis D. Furthermore, it does not seem that the process leading to the attainment of a hypercalcaemia by the equilibrium hypothesis could lead to an increase of the diffusible calcium of the serum beyond the saturation point, except by the previously outlined mechanism. It has already been pointed out that the increase in blood calcium levels is probably brought about by the increase in the amount of the non-diffusible calcium, which is in the nature of an organic compound, and that this increase is associated with the removal of ions through the diffusible calcium from either the gut or the bones. This organic compound, however, gives no evidence of being a permanent structure, because an injection of parathyroid hormone produces only a temporary

effect on the blood calcium level. Therefore, if in hypercalcaemia the non-diffusible element is built up to a considerable degree, one is faced with the problem of visualizing the result of the breakdown of this greatly increased compound. It seems only logical that this phenomenon should be associated with the liberation of a large amount of diffusible calcium which could raise the serum calcium to the saturation point and then cause precipitation. This hypothesis would infer that pathological calcifications would be more apt to occur, not when the blood calcium was being built up by a shift in equilibrium to the non-diffusible element, but when the equilibrium shifted from the non-diffusible calcium to the bones, as a result of the disintegration of the non-diffusible element. It is of interest in this regard to point out that Laas[18] found, after large single doses of irradiated ergosterol, that calcifications did not occur in animals until a period of four days had elapsed. This theory of the causation of pathological calcifications in hypercalcaemia is, of course, not yet substantiated and has only suggestive evidence to support it. Experiments, in which serum calcium curves will be correlated with the time of appearance of pathological calcifications in animals, are under way in this laboratory and will be reported on shortly.

As the mechanism by which calcium salts are deposited in bone has been reviewed it has become obvious that the older ideas of the calcification of bone placed too much stress upon the secretory ability of the osteoblasts and too little upon the chemical and physico-chemical aspects of the problem. It is only logical to suppose that this same type of misconception may have arisen in the field of bone resorption, and indeed it is found in this particular field that great stress has been placed upon one particlar cell known as the osteoclast. When one realizes, however, that the calcium salts of bone are in equilibrium with those of the blood, and that they may be shifted about between the two regions with ease, one becomes suspicious of the necessity for cellular activity to remove calcium from the bones. It would seem that if the deposition of calcium salts in bone matrix depends upon chemical and physico-chemical reactions, the removal of these salts from matrix would largely depend upon the same type of mechanism, particularly when the equilibrium between the calcium of the blood and bones appears to be reversible.

As it is now evident that a widespread removal of calcium salts from the skeleton may be readily obtained by the administration of a sufficient quantity of either parathyroid hormone or vitamin D, one must be prepared to explain the cellular picture obtained in the bones subsequent to the

calcium removal. It is known that, through its widespread system of canaliculi, the matrix of bone is in very close association with tissue fluid, and as it is quite evident that the calcium salts of the bones are in equilibrium with those of the blood, it seems scarcely necessary to postulate any other mechanism essential for calcium removal, under these circumstances, than a chemical one. In turning, therefore, to an explanation of the cellular picture encountered under these conditions, one must hesitate in postulating that any cells are the specific cause of the resorption. It should be pointed out that certain cellular pictures could originate as a result of calcium removal, so that one must be exceedingly careful in interpreting the circumstantial evidence regarding the activity of cells as specific agents of bone resorption. The problem, therefore, resolves itself into deciding upon the part that cells play in bone resorption and whether the characteristic cellular pictures of bone resorption can be considered as cause or effect.

Having thus suggested a different interpretation of the osteoclast picture, it is now possible to approach the matter of bone resorption with at least this proposed theory in mind. The problem is very complex, there being so many types and theories of various resorptive phenomena, that an intensive study always serves to indicate that the matter cannot possibly be dismissed with a simple explanation which will hold in all cases. In the first place it is well to realize that bone consists of cells and matrix, and that the latter possesses two components, one of which is organic and the other inorganic. The former consists of collagen, an albumoid and a mucoid. As bone is usually studied with microscopical sections it is of interest to know that many fallacies may be built up, particularly with regard to the staining of the material. There is a widespread idea that calcified material will take a deep stain with haematoxylin in decalcified preparations. In this connection it should be remembered that cortical bone in decalcified preparations, representing the portion of the section which formerly contained the greatest amount of calcium, stains with eosin much better than it does with haematoxylin. Furthermore, it is extremely dubious whether haematoxylin stains calcium in sections which are not decalcified. The recent work of Cameron[19] has thrown grave doubts upon the efficiency of both haematoxylin and silver preparations as stains for calcium. In any event it is readily obvious that one cannot hope to stain the calcium of the matrix in decalcified preparations after it has been completely removed by the decalcifying agent. The staining of decalcified preparations depends to a great extent upon the organic constitutents of the matrix and it is not unlikely that the deep blue

staining sometimes obtained with haematoxylin in decalcified preparations depends upon the mucoids of the matrix, and this type of staining is obtained more readily with cartilage matrix than with that of bone. Curiously enough the interpretation of this reaction as indicating calcification in decalcified preparations of cartilage, just as the depiction of calcified areas with haematoxylin in specimens which are not decalcified, although primarily based on fallacy, is often fairly accurate, but of course is not dependable and may lead one, on occasion, far from the truth. It must be granted, therefore, that one may determine little about the calcium content of bone in decalcified sections, and any deductions based on findings of this nature may be very misleading. Consequently, in a study of the histological picture in decalcified preparations one must remember that only the organic structure persists.

Many cells have had the function of bone resorption ascribed to them. Osteoclasts have received much consideration in this regard, but many authors have also ascribed this same sort of ability to osteoblasts. Furthermore, it has also been known that tumor cells, on invading bone, appear to possess this same sort of ability and in this connection many sorts of cells have been described. Considering first the osteoclasts, one does not find agreement between various authors as to their origin, which has been ascribed to osteoblasts by Kolliker[20], Howell[21], Arey[5] and others, endothelial leucocytes by Mallory[23] and Haythorn[24], osteoblasts, reticular cells, and in some instances hemoblasts by Jordan [25 26], and fusing cartilage cells by Kaczander[27] and Geddes[28]. It could be assumed from their supposedly phagocytic properties that the osteoclasts arose from reticulo-endothelial cells but this does not seem to be usually the case, as it has been demonstarted by Shipley and Macklin[29], Cappel[30], and by the author (unreported), that the osteoclasts do not take up vital stains and hence may be differentiated from the reticulo-endothelial system. It is also evident that the specificity of the osteoclast as a cell type hinges largely on its being found in bony regions and it is quite possible that this fact is one of significance in its origin. It should be remembered that the osteogenic cell found in the deep layer of the periosteum and in the endosteum is a relatively undifferentiated cell possessing the capacity for differentiation into both cartilage and bone. Consequently, it is not at all surprising that these cells could also give rise to multi-nucleated elements or osteoclasts and in this connection the work of Arey[22] is particularly illustrative, as he showed that masses of osteoblastic syncitia would shade off on occasion at one extremity to an eosinophilic syncitium indicative of an osteoclast. In this respect the healing fracture

is an excellent field for study regarding the origin of these cells. It has been pointed out by the author[2], that osteoclasts originate in this process in greatest numbers when the cartilage is being replaced by an ingrowth of osteogenic cells and blood vessels. Whereas many of the invading osteogenic cells differentiate into osteoblasts and later into bone cells, others differentiate into osteoclasts. It has also been suggested, Ham[1,2], that the calcified cartilage, which is being replaced, exerts a dual stimulus,—one which encourages bone formation and the other which incites a foreign body reaction. Thus the cartilage is invaded by osteogenic cells which differentiate both into new bone cells and giant cells. It is significant that the osteoclasts arise in greatest numbers when the differentiation of the osteogenic cells is at its height, and when there is considerable amount of foreign body (calcified cartilage matrix) present. In this sense the formation of osteoclasts could be thought of as a foreign body type of response to the breaking down calcified cartilage matrix. Their origin from the osteogenic cells is easily explained by the fact that the latter are present in large numbers, and as they are relatively undifferentiated, they may have the capacity to form giant cells of this type. On the other hand, there is no reason to suppose that other cells, possessing the proper potentiality, could not give rise to giant cells of a foreign body type in this location, but as osteogenic cells are present in such great numbers they form the most readily available source.

Phagocytosis by osteoclasts has been reported on by Arey[22] and by Jordan[31]. It is true that osteoclasts occasionally demonstrate phagocytosed material within their cytoplasm, such as dead bone cells, but on the whole Shipley and Macklin[29], Cappel[30] and the author (unreported experiments) showed that osteoclasts in animals do not take up vital stains. On the other hand, in giant celled tumors of bone, the giant cells are frequently seen to contain phagocytosed red blood cells. This somewhat contradictory evidence would indicate that the osteoclast, while possessed of phagocytic capabilities, is not as adept in this regard as the cells of the reticulo-endothelial system. In any event it would be extremely difficult to visualize the mechanism by which osteoclasts could phagocytose bone unless very small fragments were being considered. They form about bone in much the same manner as foreign body giant cells would form about injections of agar or paraffin. However, because of their location in Howship's lacunae, they have been thought to exert some destructive action on bone, so that other theories than a phagocytic one have been offered to explain the erosive action of the cell.

Theories regarding the elaboration of digestive substances by the osteoclasts have been, on the whole, quite popular to explain their action. The substances supposedly secreted have ranged from non-specific ones, such as carbon dioxide, to specific enzymes. The production of non-specific dissolving substances is, on the whole, preferable to the other hypotheses, because cells of different types may on occasion be present in bone at the site of resorption. The ability of cells to erode bone does not seem to be limited to any specific type, so that the dissolving property is also probably non-specific. In this regard the work of Hofmeister[22] merits special consideration, as he showed that the elaboration of carbon dioxide by any cell adjacent to bone could result in some dissolution of calcium salts from the latter. It has already been pointed out in this paper that the capacity of serum to retain calcium probably depends, in some degree, upon carbon dioxide tension. Furthermore, Robinson's[15] work has shown that the enzyme phosphatase, known to be of such importance in the deposition of calcium salts in bone, is active in a medium which is relatively alkaline, so that as the calcium retaining ability of matrix depends, to some extent, upon the same factors which encourage deposition, it is not unlikely that the matrix could lose calcium if the carbon dioxide tension in an area became higher. Consequently, with all these points in view, it seems most likely that the presence of any non bone-forming cell, directly adjacent to calcified matrix, would result in some solution of calcium salts from the latter.

In this connection it should be remembered that normally the matrix of bone is separated from other cell types by the osteogenic lining. This, of course, is the reason that osteoclasts are seen in close apposition to matrix, because, as they originate from the osteogenic lining cells of either periosteum or endosteum, they are then situated directly adjacent to the matrix. Even when a tumor invades bone there is a tendency for the osteogenic lining cells to persist and separate the tumor cells from the matrix. When the latter break through, however, they may then cause bone resorption. Bone must be considered as an entity complete with its lining cells, because, if it is deprived of the latter, it is very questionable whether the matrix is able to withstand the normal activities of any adjacent cells. It is probably because of this reason that the osteoclast, originating as it does from the osteogenic lining cells, but not partaking of bone building functions, can cause such marked indentations on the surface of the matrix. In other words, bone matrix, as such, shows little ability to withstand even the normal metabolic processes of adjacent cells, unless they are of the lining osteogenic type.

It is therefore thought better to visualize the extra-cellular substance of bone, not as a hard, permanent, indestructible sort of material, but rather as a calcium binding base which will only retain its mineral matter when kept in a very special sort of environment and even in this environment its calcium content is not in the nature of a permanent deposit but is in equilibrium with that of the blood. It also seems fairly evident that one does not have to place much stress upon the idea that bone resorption is limited to certain special cells. It would seem rather that the structure of bone is almost specially adapted to keeping any cells, except those of the osteogenic variety, away from bone matrix, and that, when the latter differentiate into osteoclasts instead of osteoblasts, their activity results in some dissolution of bone substance, not because they are specific bone resorbers, but rather because of their situation.

This conception, pertaining to the lack of ability of unprotected bone to maintain its form, is of great value in understanding other types of bone resorption which are observed. For instance, if no one follows the history of totally detached fragments of bone in the repair of fractures, many interesting pictures may be noted. On occasion, fragments are left bathed in tissue fluid or exudate for a few days before being completely encompassed by cells. In these conditions the fragments show, in a short space of time, the death of the adult bone cells and an increase in size of the lacunae. It would seem as if the exposure of dead bone to the action of the tissue fluid of the vicinity was sufficient to cause some resorption.

On the other hand, portions of dead bone, in the repair of fractures, frequently show on some surface new bone formation which apparently has the ability to advance into the old dead bone. This process, known as creeping replacement, has been discussed by Berg and Thalhimer[33], Mayer and Wehner[34], Ham[2], and others. It is not dissimilar from the process observed in the replacement of cartilage by new bone formation. In the latter, new bone is formed about the remaining cartilage matrix, which may be sharply demarcated from the new bone by its staining reaction, and the further history of such specimens, show that the enclosed cartilage gradually disappears by a process which Maximow and Bloom[35] have termed chondrolysis. It is obvious, in this type of resorption in both bone and cartilage, that the dead matrix melts gradually away ahead of the new, and that resorption occurs along a matrix-to-matrix line. The mechanism by which the new bone advances into the old is difficult to understand. Essentially it appears to hinge on the proliferative activity of the young

bone cells or osteoblasts of the advancing bone or osteoid tissue. Amitotic division has been suggested for them, but until many exceedingly good preparations have been searched for mitotic figures, this hypothesis should not be adopted. It is obvious that cell division in this situation would only occur infrequently, and the technical difficulties are great, so that the detection of mitotic figures in these osteoblasts, which are laying down matrix ahead of them, is a difficult problem.

It is evident, therefore, that bone resorption may occur in the presence or absence of cells. It is also seen that cells of many types may on occasion cause resorption. It may also be concluded that phagocytosis is not the probable mechanism by which calcium is resorbed, and it is highly suggestive that the mechanism is a chemical one. Thus the activity of cells in the matter of resorption could be explained by their ability to liberate substances in close proximity to matrix (possibly carbon dioxide) so that calcium salts become more soluble in tissue fluid, and less easily held in matrix. Futhermore it appears that bone is well constructed to protect matrix from close association with any cells except those of the osteogenic variety, and that when these differentiate into osteoclasts instead of osteoblasts, some erosion of the matriz will occur. Osteogenic cells usually give rise to osteoblasts but when there is foreign material about, such as dead bone or cartilage, or when calcium resorption is extensive (example—hyperparathyroidism) it appears that a foreign body stimulus is exerted by the breaking down tissue, which results in the differentiation of the osteogenic cells into osteoclasts. Thus, the cells which arise from the stimulus of the breaking down tissue, result in still more destruction. On the other hand, this stimulus, exerted by dead bone or cartilage, will, because of the latter's calcium content, induce osteogenesis, so that the osteoclast picture under a normal calcium metabolism is usually accompanied by a considerable amount of new bone formation.

Summary

The mechanism of calcification of bone matrix is discussed in some detail, together with the manner in which calcium is carried in the blood. The action of the parathyroid hormone and vitamin D on blood calcium levels also received consideration, and evidence concerning the probability of an equilibrium between the non-diffusible calcium of the blood, the diffusible calcium of the blood and the bones is outlined. It is then seen

that shifts in the equilibrium toward the non-diffusible calcium could lead to a loss of calcium from the bones, while a shift in the other direction might lead to precipitations in the tissues. As it appears fairly obvious that the calcium of the bones may be either deposited, and, at least, partially withdrawn, by virtue of the fact that it is in equilibrium with the blood, it is seen that bone resorption is not necessarily the result of cellular activity. The part that cells play in bone resorption is then described and the origin and function of the osteoclast is discussed in detail. The mechanism of creeping replacement is also examined, and it becomes evident that matrix may be resorbed when it is subjected to certain changed environments, and that the normal structure of bone allows its usual separation from other cell types. It is thought that the direct proximity of unfamiliar cells could easily result in matrix erosion, if the normal osteogenic cell lining was not present. It is also pointed out that, once some bone destruction occurs, the stimulus of the breaking down tissue encourages the differentiation of osteogenic cells into osteoclasts. Osteoclasts may, therefore, be considered to be both effect and cause of bone resorption, and care should be used in explaining their significance.

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