THE FORMATION OF TRUE BONE WITH CELLULAR (RED) MARROW IN A SCLEROTIC AORTA.

By C. H. BUNTING, M. D.,
Associate in Pathology.

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)

At a recent post-mortem examination in the Johns Hopkins Hospital one of the most striking lesions found was an extremely sclerotic and calcified aorta. Microscopical sections from the dorsal portion of this aorta showed at one point in the much thickened intima a new formation of true bone. While the finding is not unparalleled, the references in medical literature to similar lesions are so few that the case seems worthy of note, especially as in one particular at least this case differs from those already reported.

The patient was a white male 72 years old, and was admitted to the surgical service for the removal of a hypertrophied prostate, but died before the operation, apparently as the result of a general infection, following an attack of furunculosis, and of complications as indicated in the anatomical diagnosis. The post-mortem examination was made by the author August 12, 1904, three hours after death. It does not seem necessary for the purpose of this paper to give the full post-mortem findings. The Anatomical Diagnosis was as follows:

Hypertrophy (adenomyoma) of the prostate; dilatation of the bladder with hypertrophy of its musculature; chronic diffuse nephritis; aortic sclerosis with calcification; furunculosis; purulent bronchitis; broncho-pneumonia; mural thrombi of aorta; embolism and thrombosis of the right brachial artery, with gangrene of the right extremity, and of the right internal carotid artery, with encephalomalacia of the right cerebral hemisphere; atrophic spleen; surface atrophy of the liver.

The lesions of interest are those of the aorta and its branches. In addition to the sclerosis, the presence of the thrombi in the

aorta seems noteworthy. The larger of these formed a globular mass about 15 millimetres in diameter, attached to the convex side of the ascending portion of the aorta at a point just below the orifice of the innominate artery. It was firmly adherent to the wall of the vessel. This was quite evidently the source of the emboli which were carried to the right brachial and carotid arteries. A smaller, oval, somewhat flattened thrombus mass capped a roughened, projecting, calcified plaque in the upper portion of the thoracic aorta.

The whole aorta showed extreme sclerosis with calcified plaques throughout its extent. The calcification, however, was most marked in the lower dorsal portion and just above its bifurcation the aorta was a rigid tube with walls of bony hardness. It was through this portion that microscopical sections were made after decalcification in 5 per cent. nitric acid.

The sections gave the following picture on microscopical examination:

The adventitia appears normal, showing no increase in the density of the fibrous tissue. At the ends of the section the media is considerably thinner than normal and is about one third as wide as the thickened intima. The inner portion of the media (that next the intima) has a denser appearance than the outer part. Here its fibres stain more deeply with eosin and are in part of the section distinctly hyaline, with but here and there a shrunken pyknotic nucleus. Throughout the media the nuclei seem to be reduced in number. Toward the middle part of the section the media becomes even thinner, the hyaline degeneration of the inner portion is more extensive, and in many places there has been a deposition of calcium salts in the hyaline area. At the thinnest part of the media, its continuity is almost completely interrupted by the passage obliquely through it of vessels of considerable size, surrounded by granulation tissue, which extends inward into the intima. At this point the muscle fibres are extremely atrophied and the nuclei much shrunken.

The intima is thickened about the whole circumference of the aorta. The thickening is irregular with numerous nodules projecting toward the lumen, the largest of which lies over the point in the media where the muscle fibres are most atrophied. This nodule is of the nature of the so-called atheromatous abscess. Toward the lumen of the aorta there is a fairly well-preserved layer of dense connective tissue, poor in cells. This is in large part hyaline and toward the centre of the nodule shows deposits of calcium salts deeply stained by the hæmatoxylin. The main mass of the nodule is made up of a granular débris, in which are calcium salts and numerous slit-like spaces evidently left by the solution of cholesterin crystals. There are also a few round vacuoles, evidently fat spaces. There has been at one point toward the media an attempt at organ-

zation and repair. One finds here fairly well-formed vascular connective tissue, in which are numerous lymphoid cells as well as larger mononuclear cells which contain as a rule coarse granules of a light yellow pigment. There are in this part of the intima masses of deep red (eosin staining) refractile materail, often surrounded by cells, which are evidently deposits of old hyaline fibrin.

At the base of this atheromatous nodule and lying directly adjacent to the calcified media are two masses of true bone, not connected with each other in any of the sections studied, though lying quite close together. The larger mass is ring-shaped and has a definite marrow cavity. The bone gives indication of a definite laminar structure. It contains numerous typical bone-corpuscles and is penetrated at its thickest portion by a capillary vessel surrounded by a layer of cells resembling osteoblasts closely applied to the wall of bone, the whole appearing to represent a Haversian canal. About this mass of bone there is a layer of cells resembling osteoblasts and forming a definite membrane, while at many places in the sections studied are multinuclear cells of the typical osteoblast type. A few of these lie in lacunar depressions in the bone but many more are found in similar depressions in the adjacent calcified media. The marrow cavity of the bone contains in a delicate reticulum numerous fat cells and between these a few cells of the marrow type; large mononuclear cells, among which a few are phagocytic and contain yellow blood pigment; small mononuclear and polymorphonuclear cells, both neutrophilic and eosinophilic. This marrow, however, is not so cellular as the portion lying between this ring of bone and the second bone mass. This second mass consists of two bars, a slender one lying immediately adjacent to the calcified media and a larger one separated from the first by a small amount of myeloid tissue, containing vessels, fat cells, and large multinuclear giant cells, apposed to the media. These masses of bone are also surrounded by a layer of osteoblasts and contain bone corpuscles.

The tissue lying between these bony masses has the typical appearance of the cellular red marrow of cancellous bone. It is quite richly vascularized by delicately walled capillaries. There are scattered fat cells in the area, and all the types of cells usually found in red marrow, with a tendency to the grouping normally found there. Under the low power one is struck first by the numerous giant cells of the megalokaryocyte type with large twisted polymorphous nuclei. Under higher power one is able to demonstrate mononuclear cells of the myelocyte type, many of which contain eosinophile granules, polymorphonuclear cells both eosinophilic and neutrophilic, lymphoid cells and cells of the erythrocyte series, non-nucleated red cells, normoblasts with their pyknotic nuclei and hæmoglobin-containing cell bodies with sharp contour, and similar cells but with reticular nuclei.

The picture then in brief is that of an aorta with a media much atrophied and showing hyaline degeneration and in places the deposition of calcium salts. The intima is much thickened, shows atheromatous degeneration, and in places the signs either of trauma and hemorrhage or of an inflammatory process (old hyaline fibrin masses and blood pigment in phagocytes). There

is also invasion of the intima by vessels from the adventitia surrounded by young connective tissue cells and mononuclear cells, possibly an attempt at organization following the hemorrhage or inflammation. Where this tissue comes in touch with the calcified tissue there is the formation of true bone with a cellular marrow as found in cancellous bone.

The cases reported in medical literature of bone formation in sclerotic vessels are not numerous, as has been said in a preceding paragraph. Of those reported but six concern the aorta, the two earliest of which were described by Mönckeberg.[1] Marchand[2] in his article in Eulenburg's *Real-Encyclopädie* described a case in which in the crural artery he found the calcified media invaded by new vessels with the absorption of the calcified material and the deposition of the bone by osteoblasts upon the calcium deposits with the formation of a marrow space around the vessel.

Howse[3] in 1877 described a focus of bone in the axillary artery. The axillary artery of the patient had been ruptured by trauma and at his death some time subsequently the artery was found surrounded at the point of injury by a firm mass of connective tissue, in which there was found microscopically cancellous bone "produced in tissue which represents and springs from an altered condition of the middle coat."

In 1886 Paul[4] demonstrated before the London Pathological Society a specimen from a sclerotic tibial artery in which there was a focus of bone formation in the intima.

In the same year Cohn[5] investigated a number of calcified areas in the aorta, without finding anything "even approaching the picture of a bone corpuscle," but did find in a sclerotic and calcified aortic valve a mass of true bone adjacent to the calcified material; and also three cases in which there was bone formation in the media of the crural artery.

In 1900 Rosenstein[6] reported bone formation in one calcified aortic segment, and both bone and cartilage in another leaflet of the same valve.

Rohmer[7] followed in 1901 with three cases, one with bone in the aortic valve, one in the mitral, and one in which there were found several foci of bone in the intima of the femoral artery.

v. Schrötter[8] in 1901 described bone in an organized thrombus in the anterior tibial artery and also in another case in the intima of the crural artery.

The most extensive search for bone formation in sclerosed vessels has been made by Mönckeberg,[1] who in 1902 reported ten successful cases in the study of about 100 cases. In two cases he found bone both in the intima of the aorta and in the media of the femoral artery, in one in the media of the femoral, in one in both media and intima of the femoral, in one case in the intima of the tibialis posticus, and one in the intima and media of both the tibialis anticus and posticus. In this latter case cartilage was formed. He adds in a note to his article that in addition to these six cases he has been successful in four others in finding bone in medium-sized sclerotic vessels, but does not give any further details.

Thorel[9] has found bone formation in the aorta and in the femoral and tibial arteries.

Poscharissky[10] examined fourteen hearts and found bone in three of them, twice in the mitral valve and once in the calcified annulus of the left venous ostium. Of thirty-one vessels examined, four gave positive results, three of which were aortæ, the fourth being the femoral artery. He records further the finding of vascular granulation tissue in vessels in five autopsies.

In all then, bone formation has been found in the aorta six times, in the intima and media of medium-sized arteries twenty-two times, and in heart valves eight times. This cannot represent the frequency of the occurrence of the condition. Virchow[11] had noted the lesion and in his *Cellularpathologie* stated emphatically that, although most of the so-called bony aortæ and vessels were undoubtedly calcified, the formation of true bone does occur, although he cited no specific case. Orth[12] similarly describes in his text-book the formation of bone in the intima of the aorta, again without reference to any special case. Although the author has made no investigation of the frequency of the occurrence of the lesion, Mönckeberg's figures of ten positive results in a hundred cases of marked sclerosis would indicate that the lesion is regarded as rare chiefly because it is not more often searched for.

For an explanation of the presence of these foci of bone in such unexpected places, one would turn naturally to the Cohnheim doctrine of embryonic displacement of tissue, were not the cases so numerous, and did not the figures just quoted show such a frequency of occurrence of the process in advanced arteriosclerosis.

Another argument against such a theory is the evidence offered by the investigation of Pollack,[13] under Lubarsch's direction, of calcified foci in the lungs, as a result of which, of forty-seven cases studied, free bone was found adjacent to the calcium deposits in forty-three. Poscharissky likewise has conducted an extensive search for bone formation in calcified foci in various organs and records seventeen positive findings in the lungs of twenty-eight cases, two in the tonsils, four positive in the lymph glands, twelve in the dura mater, one in a calcified myoma uteri, two positive and one negative in the liver, one in an adrenal

tumor, and one in a hypernephroma of the kidney, besides those in the heart and vessels quoted above.

The final and convincing evidence against the metastasis or displacement theory is afforded by the experimental work of Barth,[14] of Sacerdotti and Frattin, [15] and of Poscharissky. [10] Barth introduced a portion of incinerated bone into the peritoneal cavity of a cat. Six weeks later it was found penetrated by connective tissue and in several places there was found true bone in contact with the calcium-containing material, lined by osteoblasts arranged partly in a regular row and partly in groups, forming an osteoid tissue which passed over without a sharp line into ordinary connective tissue.

Sacerdotti and Frattin tied off the renal vessels on one side in four rabbits. Three months later the animals were killed and in three of them there was found a formation of true bone adjacent to the calcium deposits in the necrotic tissue, with a growth of vascular granulation tissue into the kidney from the capsule. This experiment was repeated by Poscharissky on five rabbits with successful results in three animals. Similar experiments by him on the spleen and ovary in the rabbit were unsuccessful, obviously because there was no capsule in these organs with independent circulation from which granulation tissue might grow.

It seems clear, then, that one has here a metaplasia of connective tissue cells into osteoblasts, for in vessels and heart valves there can be no possibility of periosteal or perichondral sprouts playing a part, although in pulmonary foci such a possibility is not so easily excluded. As to the stimulus which leads to this metaplasia, there seems to be a uniformity of opinion as might be expected from the similarity of the pictures presented by the different cases. The process was clearly stated by Paul as consisting of three steps:

(1) Calcareous degeneration.

(2) Irritation about these places from fracture or other injury leading to inflammatory proliferation.

(3) Ossification in this young proliferating tissue.

In all the cases the bone has been formed where young con-

nective tissue has come into contact with calcium deposits. The metaplasia is apparently the result of the chemical stimulus of the calcium salts. These two factors are accepted by all, but there still seems some question as to the cause for the invasion of the intima of the sclerotic arteries by the new vessels and connective tissue. Paul suggested trauma of the nature of fracture of the calcified plates. This view is supported by Howse's case of rupture of the axillary artery; by one of Cohn's cases, in which there was a rupture of the calcified media of the crural artery; and possibly by the present case, in which there were signs of hemorrhage and old inflammatory exudate in the intima of the aorta. Poscharissky insists that in addition to the calcium salts there is always a deposit between the granules of a material resembling amyloid.

The question as to the mode of the formation of the bone I shall not discuss at length. There is about an equal division of those who have written on the subject between the two views:

(1) That there is a direct metaplasia of connective tissue into bone after the manner of callus formation, and

(2) That the calcified material is eroded with the formation of vascularized spaces containing young connective tissue cells, some of which take on the function of osteoblasts and lay down bone, some of the osteoblasts becoming included and forming the bone corpuscles.

The different cases give evidence that the bone may be formed in either way. In the present case the bone would seem to have been formed by the second method. There was nothing in the section suggesting the formation first of osteoid tissue, then of bone, but instead one found erosion of calcified material being carried on by osteoclasts. There were marrow spaces containing capillary loops, and osteoblasts arranged in a row or membrane adjacent to the bone. The picture was quite that seen at the epiphyseal line, with, however, the calcified media of the aorta in place of the calcified cartilage of the epiphysis. The determination of the exact mode of formation of the bone does not seem of so much interest as is the bare fact that in a person of advanced years the connective tissue cells under the stimulus of the calcium

salts should retain the power of differentiation into osteoblasts. The patient in this case was 72 years of age. The six of Mönckeberg's cases in which the age was specified were between 62 and 84 years. The greater number of Lubarsch's cases were in patients of advanced age, although there were seven under 45 years and one under 10. Poscharissky obtained only negative results in calcified lung foci in four persons under 28 years.

The present case differs from all others thus far reported in which bone was found in the vessels, in the nature of the marrow found in the intima of the aorta adjacent to the bone. In the previous cases the marrow when present was fatty. In this, there was true cellular marrow indistinguishable in section from the marrow of the ribs or of the cancellous bone. Attention was first attracted to it by the rather numerous giant cells with large twisted nuclei distinctly of the megalokaryocytic type. Closer study showed the presence of myelocytes and polymorphonuclear cells, both eosinophilic and neutrophilic, mononuclear and lymphoid cells, and cells of the erythrogenetic group; nucleated cells of the normoblast type and others showing reticular nuclei, as well as many erythrocytes.

The origin of this marrow and its relation to the surrounding tissue seem of the greatest interest. If one excludes the possibility that the bone-formation represents an embryonic displacement, as I think has been done, that explanation can hardly apply to the marrow, and one is left with but two possible explanations: (1) that one has in this also a direct metaplasia of the granulation tissue cells, or (2) that the tissue is the result of transportation of bone marrow cells by the blood stream and their emigration from the blood vessels. Let us consider the latter proposition first. If we accept this view and regard the marrow tissue as the result of emigration of cells we are forced to accept either one of two further propositions. If one be a believer in the Ehrlich idea that in adult life there is no genetic connection between the various types of leucocytes, the granular and the mononuclear (hyaline) groups, or between them and the erythrocytic group, to maintain the emigration idea in this case he must postulate the emigration of a parent cell for each

one of the several types found. This seems on the face of it improbable, as the myelocytes, nucleated red cells, and giant cells are so infrequently found in the circulation in general, and possess so little migratory power. It would seem that one might disregard this possibility as being too remote. The other possibility which is open, if one accepts the emigration theory, is that there has occurred the outwandering of a cell or of cells of an indifferent type, which are capable of differentiating into the various forms of blood cells. The view that such a cell exists in the marrow seems to be gaining adherents among students of hematology, as the result of the writings of Pappenheim, Wolff, Hirschfeld, Naegeli, and others. This cell is a simple undifferentiated cell with large round vesicular nucleus and scanty protoplasm and has been variously called, the large lymphocyte, the indifferent lymphoid cell, the myeloblast, Troje's *Markzell*, etc. Such a cell is found in the cell groups of the marrow and in the germinative centres of the lymph glands. It is found in the circulatory blood under certain conditions, in greatest numbers in acute lymphocytic leukæmia. By an increase in its protoplasm it becomes the large mononuclear cell always found in the circulation. If one grants that such a cell is capable of differentiation into the various types of blood cells in adult life, as is strenuously denied by the Ehrlich school, and as strenuously maintained by others, there is no great difficulty in explaining the marrow focus found in the aorta in question.

It would seem more rational, probably, to assume that a single process was operative in the formation of both the bone and the marrow, that is that they were both the result of metaplasia of the connective tissue cells. The bone corpuscles and marrow cells have a common embryonic ancestor in the undifferentiated mesoblastic cell, although in adult life they seem to have diverged widely in nature and function. While it seems but a short step from fibroblast to osteoblast, merely a question of the nature of the intercellular substance laid down, it seems a much greater transition from the fibroblast to the polymorphonuclear cell with its specific granulation, its highly developed power of motility,

and its power of phagocytosis. Yet it appears (Jackson) that the cells of the marrow in the fœtus are developed from the cells of the original invading periosteal bud, the osteoblasts. So in this case the same stimulus which led to the metaplasia of connective tissue cells into osteoblasts may have determined their further differentiation into marrow cells, or possibly the stimulus may have been the presence of the newly formed bone.

Although this is the only case recorded in which marrow of this type has been found in the blood vessels, it has been found adjacent to bone foci in other organs. Pollack says that whenever bone is formed in the lung, marrow is also formed, though usually it is of the fatty type but showing a few lymphoid cells and cells of myelocytic type. In two cases he found also nucleated red cells. Poscharissky found marrow in every case, but his result is to be accepted only if one accepts his definition of marrow, for he considers the cellular granulation tissue described in every case marrow, and says that usually in old cases it becomes fatty marrow. He also, however, found in some cases erythroblasts, megalokaryocytes, osteoblasts, and myelocytes. Gierke [16] has also described in a calcified adrenal in a 3½-year-old girl, the formation of bone with a marrow focus containing giant cells, myelocytes, and polymorphonuclear cells and eosinophiles.

The case then here reported is not unparalleled, and it would seem that one must accept the view that the marrow focus was the result of metaplasia of cells of the young granulation tissue in the environment of the newly-formed bone. There is but one point which prevents these cases from being absolutely positive evidence of the formation of blood cells from connective tissue cells in adult life, and that is the presence in young granulation tissue of round cells, mononuclear cells of doubtful antecedents but identical in form and staining characteristics with cells of the blood and marrow. Pappenheim, among others, insists that the whole group of round cells and plasma cells found in granulation tissue are histogenetic (as opposed to hæmatogenetic) in origin, but emphasizes in a recent communication the fact that indifferent lymphoid cells (histogenetic) are found

in every granulation tissue. The extensive and careful researches of Maximow led him to the conclusion that the cells of the mononuclear group found in granulation tissue were emigrated cells from the blood stream, and thus arise either from marrow or lymph-glands, and for this view there seems much evidence. The embryonic type of these cells and their similarity to if not identity with cells of the blood and marrow would suggest that the marrow arose from a metaplasia of them, rather than of the fixed connective tissue cells of adult type, and upon the settling of the question of their origin depends the decision in the case here recorded as to whether the marrow focus is the result of metaplasia of connective tissue cells or of metaplasia of indifferent cells of lymphoid type which have emigrated from the blood stream. However that question may be decided, the cases do show that cells of the connective tissue and blood groups retain into late life embryonic characteristics and are capable of a diverse development under appropriate stimuli, and further that there is a much closer genetic relation between the types of blood cells in adult life than is generally accepted.

I would conclude from the study of this case and the literature bearing on the subject, that:

(1) Metaplastic formation of bone in sclerotic vessels is not a rare phenomenon.

(2) The factors in its formation are extensive sclerosis with the presence of calcium deposits; traumatic or inflammatory disturbance in the calcified area, with penetration of the same by granulation tissue; the formation of bone adjacent to the calcified areas.

(3) The bone may be deposited in two ways: either by the conversion of connective tissue into a callus or osteoid tissue with subsequent conversion into bone and at times with the formation of cartilage; or by the resorption of calcified material by vessels and osteoclasts, the formation of a marrow space, and the deposit of bone in its periphery by an osteoblastic membrane derived by metaplasia from the connective tissue cells accompanying the vessel.

(4) In the present case, in which all of the elements of true

cellular marrow were found, one is not justified in deciding whether they were formed by a further metaplasia of connective tissue cells or by the metaplasia of emigrated cells from the blood stream, capable of differentiation into the various types of marrow cells.

BIBLIOGRAPHY OF METAPLASTIC BONE FORMATION.

1. Mönckeberg.—*Virchow's Archiv*, 1902, clxvii, 191.
2. Marchand.—*Eulenburg's Real-Encyclopädie*, 2d Edition, 1885, i, 693.
3. Howse.—*Trans. of the Path. Soc., London*, 1877, xxviii, 90.
4. Paul.—*Brit. Med. Jour.*, 1886, i, 546.
5. Cohn.—*Virchow's Archiv*, 1886, cvi, 378.
6. Rosenstein.—*Virchow's Archiv*, 1900, clxii, 100.
7. Rohmer.—*Virchow's Archiv*, 1901, clxvi, 13.
8. v. Schrötter.—*Nothnagel's Handbuch*, 1901, xv, Part 2.
9. Thorel.—*Ergebnisse d. allg. Path.*, etc., 1904, ix, 936.
10. Poscharissky.—*Ziegler's Beiträge*, 1905, xxxviii, 135.
11. Virchow.—*Die Cellularpathologie*, 4th Edition, 1871, 542.
12. Orth.—*Lehrbuch d. spec. path. Anat.*, 1887, i, 225.
13. Pollack.—*Virchow's Archiv*, 1901, clxv, 129.
14. Barth.—*Ziegler's Beiträge*, 1895, xvii, 65.
15. Sacerdotti and Frattin.—*Virchow's Archiv*, 1902, clxviii, 431.
16. Gierke.—*Ziegler's Beiträge*, 1905, 7th Supplement, 311.