

HEREDITARY OSTEOPETROSIS OF THE RABBIT

III. PATHOLOGIC OBSERVATIONS; SKELETAL ABNORMALITIES

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PLATES 25 TO 30

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The general manifestations and course of hereditary osteopetrosis of the rabbit have previously been described (1, 2). The disease, which is present at birth, is characterized by tooth abnormalities, dense homogeneous shadows of the skeleton in x-ray photographs, retardation of growth, progressive anemia, deterioration, malnutrition and cachexia, and a rapid course with an invariably fatal outcome, usually at 4 to 5 weeks of age. In general, low serum calcium levels became progressively lower, serum phosphorus levels were low in young cases and became higher in advanced cases, and serum phosphatase values were high. The disease is hereditary and the mode of inheritance is on the basis of a simple recessive unit factor, affected individuals being homozygous for the factor. Rabbits heterozygous for the factor are identified only by appropriate breeding tests.

The pathologic observations of this disease will now be reported. The skeletal abnormalities, with the exception of those of the teeth which will subsequently be described, are reported in the present paper. The associated paper (3) contains the general results of postmortem examinations and of histologic study of organs and tissues other than the bones.

Materials and Methods

A postmortem examination was made on the majority of the 293 osteopetrosis rabbits which constituted the basis of this work. Tissues for microscopic study were obtained from cases killed in most instances with chloroform anesthesia; a few very young animals were decapitated. Control material was similarly obtained from normal litter mates of the same age.

The bones were fixed in Petrunkevitch's cupric-phenol solution, 10 per cent formal-saline, or 80 per cent alcohol. The decalcifying fluids employed were 5 per cent nitric acid, 5 per cent formic acid, 5 per cent trichloroacetic acid, 10 per cent diammonium citrate, or Müller's fluid. Phloxine-methylene blue stain, von Kóssa's silver nitrate stain for calcium salts, Gomori's stain for calcium, and Heidenhain's azocarmine-aniline blue stain for collagen were used.

RESULTS

Before describing the profound pathologic condition which involved the entire skeleton, it will be convenient to begin with some general remarks on the external appearance and the consistency of the bones. The appearance of the sectioned surfaces of the bones and the histologic observations will next be described. Most of this description is centered on the femur and tibia as convenient examples of the skeletal abnormality. For the most part a chronological order has been followed, the observations on specimens from early cases being first presented and then those from older advanced cases.

In appraising the various lesions, skeletal and others (3), it is important to keep in mind the comparative rapidity of the disease process. Pronounced pathologic changes were present at birth; the entire course of the disease rarely exceeded 5 weeks and earlier deaths were not unusual (1).

The periosteal surfaces of the bones appeared normal and there was nothing unusual in the external appearance except size reduction and clubbing of the ends of the larger long bones and the ribs. The changes were most marked in the older cases in which growth retardation was well established. The long bones showed a pronounced shortening and marked stunting of the femur in particular was frequently very striking (Figs. 1, 2, and 12). Expansion of the ends of the femur and humerus and to a somewhat lesser degree of the tibia, radius, and ulna took place, and in well developed examples there was some constriction of the central portion of the shaft. Clubbing of the ends of the ribs was also observed but was less prominent than in the leg bones. These characteristic features were, of course, prominently shown in x-ray photographs (2).

The consistency of the bones was abnormal. In the younger cases up to about 10 days of age, the bones were dense, tough, and more resistant than normal but not noticeably harder. Section with a razor blade gave the impression of cutting through a tough homogeneous substance like an old carrot. With normal bones of the same age, there were distinct differences of consistency, the greatest hardness corresponding to the cortex, and the impression on sectioning the bone might be compared to that felt on cutting through a cucumber.

An idea of the peculiar resistance of young osteopetrosis bone is suggested by the homogeneous appearance of the freshly cut surface. The photograph of a split femur from a 6 day old case, which should be compared with one from a normal litter mate (Fig. 1), shows the peculiar dense condition of the entire bone which suggests solidity.

In older cases beginning at about 2 weeks of age, the bones became harder and eventually quite brittle. In some instances they were harder than corresponding normal bones of the same age, but the difference was not marked or constant. The distinguishing feature in addition to the brittleness was the comparatively homogeneous density felt on section and on direct pressure on the sawn surface with a finger or forceps. Section with a razor blade was difficult and it was found necessary to use a jeweller's wire saw. Even with this instrument, chipping and fragmentation were frequent and in some cases, the bones were inclined to fall to pieces. Normal bones were neither brittle nor fragile and were sawn with comparatively little effort. These differences will be appreciated by comparing the photographs of the sawn tibias from an osteopetrosis rabbit and a normal litter mate aged 29 days (Fig. 2).

The bones of the calvarium of both osteopetrosis and normal young rabbits could easily be cut with scissors. Those from osteopetrosis cases were perhaps tougher and more fibrous and by transmitted light appeared somewhat more opaque. Union of the sutures was de-

layed. In the older cases, the bones of the calvarium were increasingly brittle and when cut, showed a definite tendency to crack.

The peculiar tough dense quality of the bones and the opaque whitish appearance of their cut surfaces were most apparent in the long bones. In the femur, for example, from a 6 day old case (Fig. 1), the entire bone was essentially a tough fibrous bony mass with no marrow cavity and an almost colorless cut surface. The epiphyseal zone had a very faint bluish tint, it was increased in width, and its margins were somewhat uneven. Beyond this zone, in the metaphysis, there was an irregular mass of opaque whitish spongy bone considerably wider than the corresponding area of a normal femur. All the rest of the shaft consisted of a similar fibrous bony substance largely of an opaque white appearance with a few areas of an extremely pale pinkish beige tint.

The typical appearance of a longitudinally sectioned young osteopetrosis bone is also well shown in the photomicrographs of a stained section of a tibia from a 3 day old case taken at a very low and a higher magnification (Figs. 3 and 5); a photograph of a similar preparation from a normal litter mate is included for comparison (Fig. 4). The epiphyseal cartilage of the osteopetrosis tibia is somewhat wider than normal and the columns of cartilage cells and the epiphyseal margin are somewhat irregular. The adjacent area of spongy bone is considerably widened and remnants of cartilage are rather prominent. There is no well defined marrow cavity or marrow. The mid-portion of the shaft is occupied by tissue resembling trabecular spongy bone and by strands of fibrous tissue. The photomicrograph at the higher magnification (Fig. 5) shows that the tissue comprising the diaphysis is essentially the same as that of the metaphysis. Very small collection of hemopoietic tissue were distinguished but nothing approaching the amount in the normal bones is seen (Fig. 4).

The characteristic details of the outer metaphysis just beneath the epiphyseal cartilage in young osteopetrosis bones are shown in the photograph of an area of the proximal end of the tibia of a 3 day old case (Fig. 6). A corresponding area from a normal litter mate is also illustrated (Fig. 7). At the top of the photograph of the osteopetrosis bone (Fig. 6) is a loose connective tissue network; the epiphyseal line is just outside this field. Below the connective tissue is an irregular mass of spongy bone with several irregular projections directed downward toward the diaphysis. The staining reaction of the bone is not uniform; a good deal of it stains quite deeply but some of it, especially the upper portion adjacent to the connective tissue, stains poorly. There are a great many osteocytes. Some of them resemble those in normal bone (Fig. 7) in size, linear distribution, and in staining reaction, but the great majority are abnormal. There is great diversity of size, many being quite irregular and a large number, perhaps a majority, stain very intensely and appear to be pyknotic. A further characteristic feature is the close crowding or massing together of from 2 to 6 osteocytes which are enclosed by a circle of rather dark staining bone. Each osteocyte is surrounded by a clear halo. Along the margins of the bony masses are rows of osteoblasts. In this particular field they do not happen to be as numerous or as plump and cuboidal as those in the normal section shown in Fig. 7. The osteoid borders of the trabeculae can be seen and in the central portion of the trabeculae in the lower part of the photograph, there are small amounts of residual cartilages. Between the trabeculae is a loose reticulum containing large prominent blood vessels, some hemopoietic cells, and a few osteoclasts. As compared with the normal section (Fig. 7), the blood vessels are more dilated, hemopoietic cells are very much less numerous, and there are fewer osteoclasts. Further toward the diaphysis, however, outside of this field, the osteoclasts are more frequent.

The presence of fibrous tissue was a conspicuous feature of osteopetrosis bone. It was distributed in strands between and about the persistent bony trabeculae (Fig. 5) and often too in masses in the metaphysis just beneath the epiphyseal cartilage or in the central portion of the shaft or in both locations. Although these areas were generally larger in advanced

than in early cases, still fairly good sized ones were sometimes found in quite young cases as in the following example.

A centrally located fibrous tissue mass in a case 7 days old is illustrated in the photograph of a stained section of the shaft of a tibia (Fig. 8). Perhaps a third of the shaft was thus involved. At both ends of the photograph appear many spongy bone trabeculae, the staining of which is not uniform. An occasional small area of persistent pale staining cartilage in the bone can be identified. Higher magnifications revealed that the spongy bone contains a large number of small irregular osteocytes. The fibrous tissue is of a rather loose type. There are many fibrous extensions between the trabeculae and others which penetrate the cortex in a great many places. In the central mass one small island of hemopoietic tissue and a few blood vessels are seen. Other small hemopoietic areas are present in other fields but the total amount of this tissue was obviously very much reduced. Osteoclasts are scattered about in the fibrous tissue as well as in the vicinity of the trabeculae and the cortical bone. A few osteoblasts, especially in the cortex, were identified at higher magnifications; they are numerous in the metaphyses outside this particular field. The abnormal cortex has a lattice appearance. It is made up of large strands and masses of bone separated by numerous fibrous tissue extensions which penetrate to the periosteum. In the bone itself there is an excessive number of exceedingly small osteocytes, the Haversian systems are less numerous than normal and do not contain as many lamellae, and there are very few definite Volkmann's canals.

The abnormal appearance of the sectioned bones of somewhat older cases has a close resemblance to that of the early cases as is shown in the photomicrograph of a sectioned femur from a case 11 days of age and from another aged 18 days (Figs. 9 and 10). The outstanding features continue to be the persistence of spongy bone trabeculae, the lattice appearance of the cortex, the presence of fibrous tissue in masses and in diffuse distribution, and the absence of a marrow cavity and of areas of cellular marrow. In the photograph of the proximal third of a tibia from a case 13 days of age (Fig. 11) these abnormalities are shown in greater detail.

The entire area from the epiphyseal cartilage well into the diaphysis is filled with spongy bone. Some hemopoietic cells lie between the trabeculae and are somewhat more numerous in the right hand part of the photomicrograph. Beyond this field in the central portion of the diaphysis are slightly larger accumulations of hemopoietic tissue. Here the trabeculae are prominent. The comparatively scanty fibrous tissue in this particular specimen was generally distributed diffusely between the trabeculae.

Examination at higher magnifications showed that along the margins of the trabeculae were numerous osteoblasts and in the spongy bone were numerous osteocytes; the picture was similar to that illustrated in Fig. 6. The trabeculae contained a fair amount of persistent cartilage and in some areas, chondrocytes in group formation were seen. Osteoclasts were not numerous in any field. The cortical bone had the same lattice appearance as that shown in the 7 day specimen (Fig. 8), the osteocytes were small and very numerous and the Haversian systems and Volkmann's canals were reduced in number.

The characteristic abnormalities of the bone continued to be present in advanced older cases, as is shown in the photomicrographs of a humerus from a case aged 27 days (Figs. 12 to 15). The entire shaft is occupied by spongy bone and fibrous tissue (Fig. 12) with the exception of a small central area of hemopoietic tissue. The fibrous tissue is especially conspicuous in the central third of the shaft but, as is shown in a photomicrograph taken at a higher magnification of a field in this area, numerous spongy trabeculae are present (Fig. 13). The fibrous tissue lies between the trabeculae and in many areas appears quite dense. Numerous osteoblasts are seen, principally in rows along the margins of the trabeculae. Large osteoclasts are situated mostly in the fibrous tissue but some are in close proximity to the bone. At still higher magnifications, as is illustrated in Fig. 14, these several features are very

conspicuous. The osteoclasts in the loose connective tissue are especially prominent. The bone stains variously, much of it is fairly dark but many areas stain very faintly, giving the impression of thinness. The osteocytes are small and numerous. Very deeply stained cartilage remnants persist in many trabeculae. The cortical bone has the characteristic lattice appearance (Fig. 15) which contrasts sharply with the compact bone of a normal litter mate (Fig. 16). The osteocytes in the osteopetrosis bone are fairly numerous, the development of Volkmann's canals is comparatively minor, and the Haversian systems are limited. Along the margins of many of the bony lattice masses and particularly in the subperiosteal area, there are rows of osteoblasts in close approximation. A good many osteoclasts, principally in the connective tissue, can be discerned. The difference in the appearance of the marrow area of the normal section (Fig. 16) and the corresponding area of spongy bone and fibrous tissue of the abnormal section (Fig. 15) is very striking.

The extreme degree to which bone lesions may develop in advanced cases is illustrated by the photomicrographs of a longitudinal section of the proximal half of a femur from a case aged 40 day (Figs. 17 and 18). Such a long period of survival is exceptional. It will be noted that the types of the pronounced changes are the same as those seen in younger cases. The proximal end of this bone as well as the distal end not included in the photomicrograph showed some clubbing. The metaphysis is widened and contains many trabeculae and large irregular masses of spongy bone, while the diaphysis is filled with smaller amounts of spongy bone and large masses of fibrous tissue. In the central portion of the diaphysis are a few hemopoietic accumulations. The cortex has the characteristic lattice appearance.

More details of the pathologic picture are revealed in a photomicrograph of the central area of the metaphysis taken at a higher magnification (Fig. 18). There are numerous very irregular small masses of spongy bone with a definite suggestion of trabecular arrangement, separated and surrounded by fibrous tissue. Some of the bones show osteoid margins or borders. At the left of the photograph the fibrous tissue is quite dense. In the upper right portion of the field there is also an apparently similar dense fibrous tissue but in this case there is actually a combination of rather loose connective tissue, many fibroblasts, and many thin walled vascular channels filled with hemopoietic cells.

The bony masses do not stain uniformly and some pale portions look thin and delicate. A few cartilage remnants are present but they are more numerous in the trabeculae of the diaphysis to the right of this microscopic field. The osteocytes, which are barely discernible at this magnification, are very small and quite numerous. Rows of cuboidal osteoblasts are in close approximation to many bony margins. Osteoclasts are fairly numerous but are more frequent in the diaphysis to the right of this field where they are more often found in the fibrous tissue.

A prominent feature of this photomicrograph (Fig. 18) is the large number of cysts in the fibrous tissue. Cysts occurred occasionally in young cases and fairly frequently in advanced cases, but they were not often as numerous as in this section. They were apparently always empty. Examination at higher magnifications showed an extremely thin, barely discernible lining with widely spaced small thin oval nuclei.

Hemorrhage in the bones was occasionally seen as in the section from a humerus of a case aged 23 days illustrated in Fig. 19. The field shown is situated in the outer portion of the mid-diaphysis. At the top of the photomicrograph is a loose network of connective tissue containing a number of cysts and a few small bony trabeculae. In the center is a sheet-like mass of closely packed red blood cells, in which there are several small and large, apparently intact thin walled vessels filled with red cells. Other vessels are empty. The connective tissue in this area is also a loose network and at the right of the photograph where the hemorrhage is less pronounced there are many fibroblasts with prominent nuclei. At the bottom of the photomicrograph appears the margin of a mass of denser fibrous tissue and beyond toward

the central part of the diaphysis and outside this field, are small, medium, and fairly large masses of spongy bone. In the fibrous tissue meshes of the central area are a good many small collections of hemopoietic cells and a fair number of osteoclasts. Several quite large osteoclasts may be seen in the lower right corner.

The description of the marked pathologic changes of the skeleton in both early and advanced osteopetrosis cases has so far been limited to the femur, the tibia, and the humerus as typical representatives of the abnormal bones. Other bones were no less profoundly affected, as may be seen by the examples of a rib (Fig. 20) and two occipital bones (Figs. 22 and 24)

The condition of the ribs, particularly in older cases, is especially interesting because of their extremely small content of hemopoietic tissue. This feature is well shown in the photograph of a cross-section of a rib from an osteopetrosis rabbit aged 25 days (Fig. 20). A corresponding section from a normal litter mate of the same age is also depicted for comparison (Fig. 21). In the diseased bone there is an enormous amount of persistent spongy bone containing a good deal of residual cartilage. The marrow spaces are quite tiny, the quantity of hemopoietic tissue within them is very small, and the connective tissue reticulum is inconspicuous. Practically all the spaces are lined with osteoblasts in close approximation to the spongy bone. The few osteoclasts are situated principally in the periphery of the bone. The cortex is somewhat irregularly thickened and in places has a lattice appearance. The section of a normal rib (Fig. 21) on the other hand shows very numerous, comparatively large, collections of cellular marrow which fill innumerable spaces formed by very thin lines and twigs of cartilage bordered by narrow bone or osteoid edges. Very few osteoblasts or osteoclasts could be identified in this section.

It may be mentioned at this point that in older advanced cases the amount of hemopoietic tissue in the sternum appeared to be proportionately greater than in other bones, particularly the ribs. In the vertebrae and the carpal and tarsal bones also, somewhat larger amounts were often seen than would be expected from the scanty representation elsewhere, as for example in the femur. But in all these bones the actual amount of red marrow present was very much less than that found in the corresponding normal bones. In early cases the various bones showed little or no difference in the very limited amounts of hemopoietic tissue.

The marked involvement of the calvarium of osteopetrosis rabbits is illustrated by the photomicrographs in Figs. 22 and 24. A section of the occipital bone of a case aged 25 days shown in Fig. 22 should be compared with a corresponding section from a normal litter mate of the same age reproduced in Fig. 23. The outstanding features of the osteopetrosis occipital bone (Fig. 22) are its thinness, its generally spongy character, the presence of a good many cartilage remnants, and the small amount of hemopoietic tissue. A few Haversian systems with 2 or 3 lamellae are present. The many small spaces in the immature bone contain a fairly abundant loose thin fibrous reticulum quite rich in nuclei and scanty numbers of hemopoietic cells. The diploic spaces are small and ill defined and above them (toward the outer table at the top of the photograph) are strands of rather dense connective tissue, more abundant on the left than the right. In the normal specimen (Fig. 23), the compact lamellar character of the bone is well shown and the large diploic spaces containing cellular marrow are quite conspicuous.

Another example of an osteopetrosis calvarium is illustrated by a photomicrograph at a higher magnification of a section of the occipital bone from a case aged 23 days (Fig. 24). Of special interest is the lattice appearance of the bone, particularly the upper or outer portion, brought about by the very large amount of interspersed dense fibrous tissue. In many areas there are large numbers of closely packed fibroblasts. Several small cysts may be seen in the prominent fibrous mass in the lower half of the photograph. Most of the bone is of the spongy type but there are some poorly developed Haversian systems. Small cartilage remnants are present in the bone and a few also in the dense fibrous tissue. Some of the spaces in the bone contain a very cellular reticulum of loose connective tissue and a good

many hemopoietic cells while others contain a more dense fibrous tissue. No diploic spaces are seen in this particular field but there were some small ones elsewhere. The bony margins generally are bordered with rows of osteoblasts and scattered about in the fibrous tissue as well as in the vicinity of some bony trabeculae are fairly numerous osteoclasts.

Finally, it should be mentioned that the lesions of the other bones, including the bones of the face and base of the skull, the maxilla, the mandible, the scapula, the vertebrae, the radius, the ulna, and the pelvic bones, were similar in all respects to those which have been described.

DISCUSSION

The pathologic changes in the skeleton in this hereditary disease of the rabbit, which have just been described, represent it is plainly evident, a profound fundamental disturbance of the bones. The most significant aspect of the abnormality was that normal bone development did not occur. Essential features of the condition as revealed by microscopic examination of sections of the bones, included the persistence of spongy bone, a very scanty distribution of hemopoietic tissue, and the presence of fibrous tissue. The amount of fibrous tissue varied greatly but as a rule it roughly paralleled the stage of the disease, that is, it was most conspicuous in advanced cases.

All the bones were similarly involved. At postmortem examination of early cases, they were very tough and firm and in advanced cases there were also an increased hardness and a rather pronounced brittleness. There was no marrow cavity. The diaphysis was filled with an opaque, whitish, bony, fibrous mass resembling spongy bone which was continuous with the metaphysis. This condition explains the dense homogeneous shadows of the bones in x-ray photographs (2). Here and there on the cut surfaces were red dots or very small irregular reddish areas, which were identified microscopically as hemopoietic tissue. They were larger and more numerous in the older cases but nothing resembling a well defined marrow cavity filled with dark red marrow was ever observed. The epiphyseal cartilages of the long bones were somewhat increased in width but the margins were generally fairly smooth. The cartilage columns, however, showed some irregularities.

It will be helpful at this point to recall that an important characteristic manifestation of the disease was that retardation of skeletal development and growth regularly occurred (1, 2). At birth the bones, as a rule, were slightly smaller than those of normal litter mates of comparable body weight, and with increasing age and disease progression, the difference became much greater. At all stages of the disease it was most prominent in the long bones. In cases about 3 weeks of age and older, the long bones and the femur and tibia in particular, showed an expansion of their growing ends together with some constriction of the central portion of the shaft. Clubbing of rib ends likewise frequently occurred.

The calvarium of new-born cases was very tough and fibrous and the central calcified area of the various bones was smaller than that seen in normal young.

In advanced cases there was a general increased thinness of the calvarium and in addition the bones eventually became very brittle.

Histologically, marked abnormalities were regularly observed. The persistence of spongy bone in the metaphysis and diaphysis was very striking. The number and size of the trabeculae, particularly in the younger cases, were conspicuous and in older advanced cases, the amount of immature bone present was always a prominent feature. The osteocytes were numerous and irregular in size and arrangement. The cortical bone was also abnormal. The osteocytes were small and numerous, the Haversian systems poorly developed, and definite Volkmann's canals few in number. Large numbers of osteoblasts were present in all preparations examined. Osteoclasts also were seen and were easily identified but, as a rule, and particularly in the younger cases, they were not especially numerous.

Another striking abnormality of the bone was the presence of fibrous tissue in all stages of the disease but most conspicuous in the older advanced cases. It was found in the spaces between the unabsorbed bony trabeculae and as small or comparatively large masses in which there were variable amounts of persistent spongy bone. It invaded the immature cortical bone. In appearance this tissue varied from a loose network to a more or less dense mass and frequently it contained large numbers of prominent fibroblasts. Small thin walled empty cysts were a fairly frequent feature, especially in advanced cases and an occasional hemorrhage was seen. Osteoclasts were consistently found in the fibrous tissue and often were more numerous here than in the neighborhood of the bony trabeculae.

The development of a marrow cavity and of a medullary red marrow did not occur. Scattered about between the bony trabeculae and in areas of loose or dense connective tissue were small foci of hemopoietic tissue and thin walled blood vessels. The total content of hemopoietic tissue in all the bones, however, was obviously very much reduced. As has been previously described (2), the disease is characterized by marked hematologic abnormalities, chief among which is an anemia which was frequently severe in the longer lived cases. The state of whatever leucocytic elements there were in the hemopoietic tissue is likewise indicated by the hematologic observations. In advanced cases beginning at about 3 weeks of age, there was a moderate leucocytosis with an increased proportion of granular cells and immature polymorphonuclear leucocytes were observed in practically every case together with a high case incidence of myelocytes.

The question of osteoblast and osteoclast representation is of considerable interest. In every section large numbers of osteoblasts were always present and almost always they were situated in the usual row arrangement close to persistent spongy bone trabeculae. Frequently, small heaps or groups of these cells were seen. Osteoclasts were much less numerous but because of their size

and bizarre shape were quite conspicuous. Some were near to or in contact with persistent spongy bone but many were found in the connective tissue, in which little or no spongy bone remained. The eventual removal or disappearance of the trabeculae was not accompanied or followed by marrow development but by an active proliferation of connective tissue often rich in fibroblasts. Although this feature of sclerosis was increasingly prominent in advanced cases, instances of it occurred in very young cases.

It was pointed out in previous papers (1, 2) that the characteristic manifestations of this hereditary disease of the rabbit so greatly resemble those of juvenile marble bone disease or osteopetrosis of man as to suggest the probable identity of the two conditions. The results of postmortem observation and histologic study of the skeleton are also in general agreement with those reported for the human disease, as described by many authors, among whom Kudrjawt-zewa (4), Zwerg and Laubmann (5), Gerstel (6), Dierickx (7), Zawisch (8), and Pines and Lederer (9), have made particularly detailed studies.

In both the human and the rabbit disease normal bone development does not occur. The persistence of spongy bone, the occurrence of connective tissue infiltration, and the failure of a medullary cavity and marrow to develop are strikingly similar features in both conditions. With respect to the abnormal amounts of connective tissue in the bones, the amount in the rabbit disease appears as a rule to be greater than that in the human disease. It is possible that the difference is related to the very marked rapidity of progression of the rabbit disease. Wherever and whenever there is an opportunity for fibrous tissue proliferation, as in the case of any spongy bone absorption, such proliferation and to an excessive degree apparently immediately took place. The practically constant presence of considerable numbers of osteoclasts in connective tissue areas containing little or no spongy bone suggested such a sequence of events.

It is evident, however, that an essential factor in this process was a failure on the part of the mesenchymal cell toward normal differentiation and development. This failure has already occurred by the time of birth and its continuance during the few weeks of life of the affected rabbit can be assumed on the basis of the marked pathologic condition of the bones invariably found.

CONCLUSIONS

Gross and microscopic observations on the skeleton of rabbits with hereditary osteopetrosis have been described. All the bones were invariably and similarly affected.

The outstanding abnormalities included the persistence of spongy bone and the presence of fibrous tissue together with the failure of development of a marrow cavity and medullary marrow. The amount of hemopoietic tissue, which was present only in comparatively small foci, was greatly reduced.

The character of the lesions and their development as determined by examination of material at various stages of the disease suggested that the essential defect concerned the mesenchymal cell.

The pathologic features observed generally resembled those of human osteopetrosis as did the manifestations of the disease during life (1, 2).

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EXPLANATION OF PLATES

PLATE 25

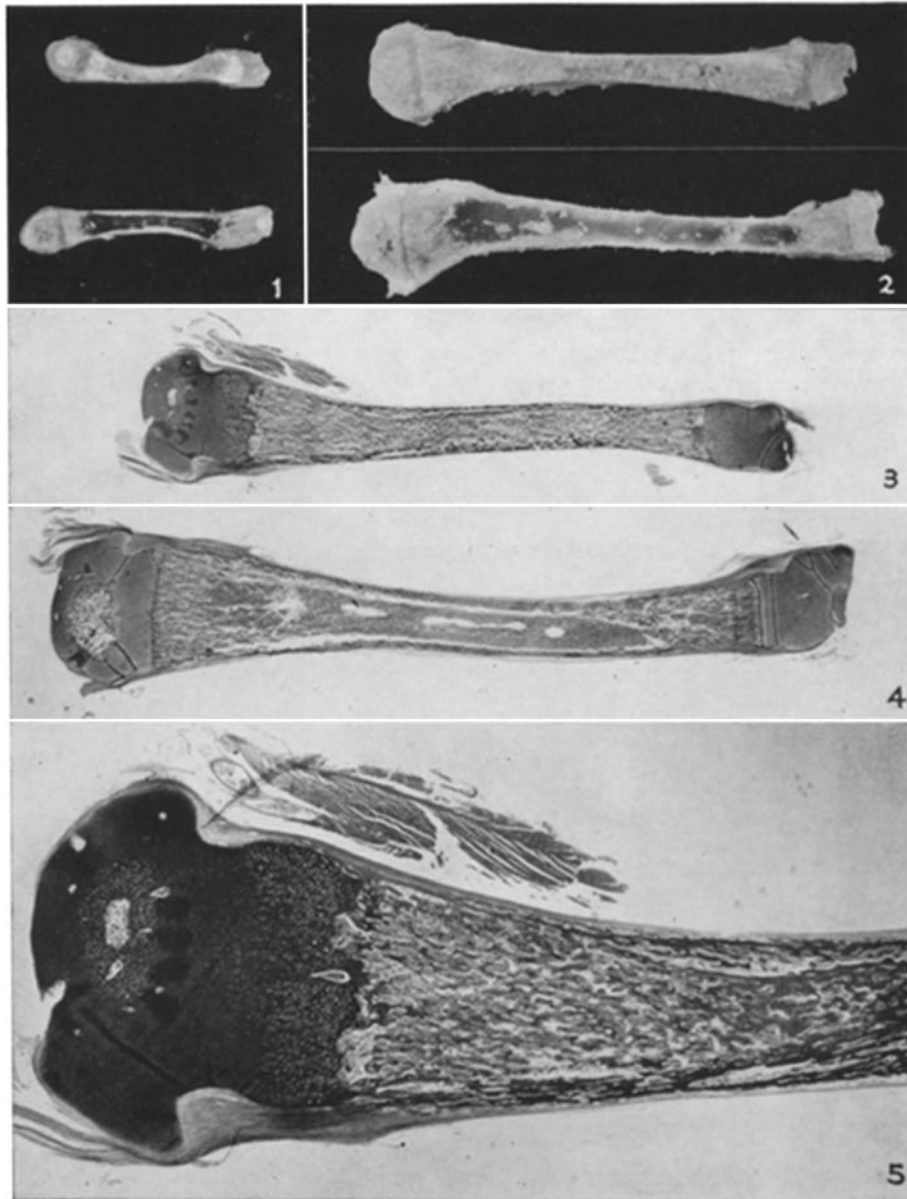
FIG. 1. Photograph of a longitudinally sectioned femur of an osteopetrosis rabbit at the top and of a normal litter mate below, aged 6 days. Note the solid dense appearance of the osteopetrosis bone and the absence of a marrow cavity. $\times 1.38$.

FIG. 2. Photograph of a longitudinally sawn tibia of an osteopetrosis rabbit at the top and of a normal litter mate below, aged 29 days. The osteopetrosis bone has no marrow cavity and the entire shaft is comprised of dense spongy bone and fibrous tissue. $\times 1.26$.

FIG. 3. Photomicrograph of a longitudinal section of a tibia from an osteopetrosis rabbit aged 3 days. This should be compared with the photomicrograph of a similar section from a normal litter mate shown in Fig. 4. Note the spongy bone appearance of the entire shaft and the absence of a marrow cavity and marrow. There is practically no difference in the appearance of the metaphysis and diaphysis. Phloxine-methylene blue stain. $\times 5.4$.

FIG. 4. Photomicrograph of a longitudinal section of a tibia from a normal rabbit, aged 3 days, a litter mate of the osteopetrosis case referred to in Fig. 3. Phloxine-methylene blue stain. $\times 5.4$.

FIG. 5. Photomicrograph at a higher magnification of the proximal half of the tibia shown in Fig. 3. Note the large mass of trabecular bone which extends from the epiphyseal cartilage into the diaphysis. Many trabeculae contain cartilage remnants. There is no marrow cavity and very little hemopoietic tissue. The cortical bone is somewhat widened and has a lattice appearance, particularly in the diaphysis region. The epiphyseal cartilage is somewhat widened, and its lower margin and the rows of cartilage cells show irregularities. Phloxine-methylene blue stain. $\times 14.6$.



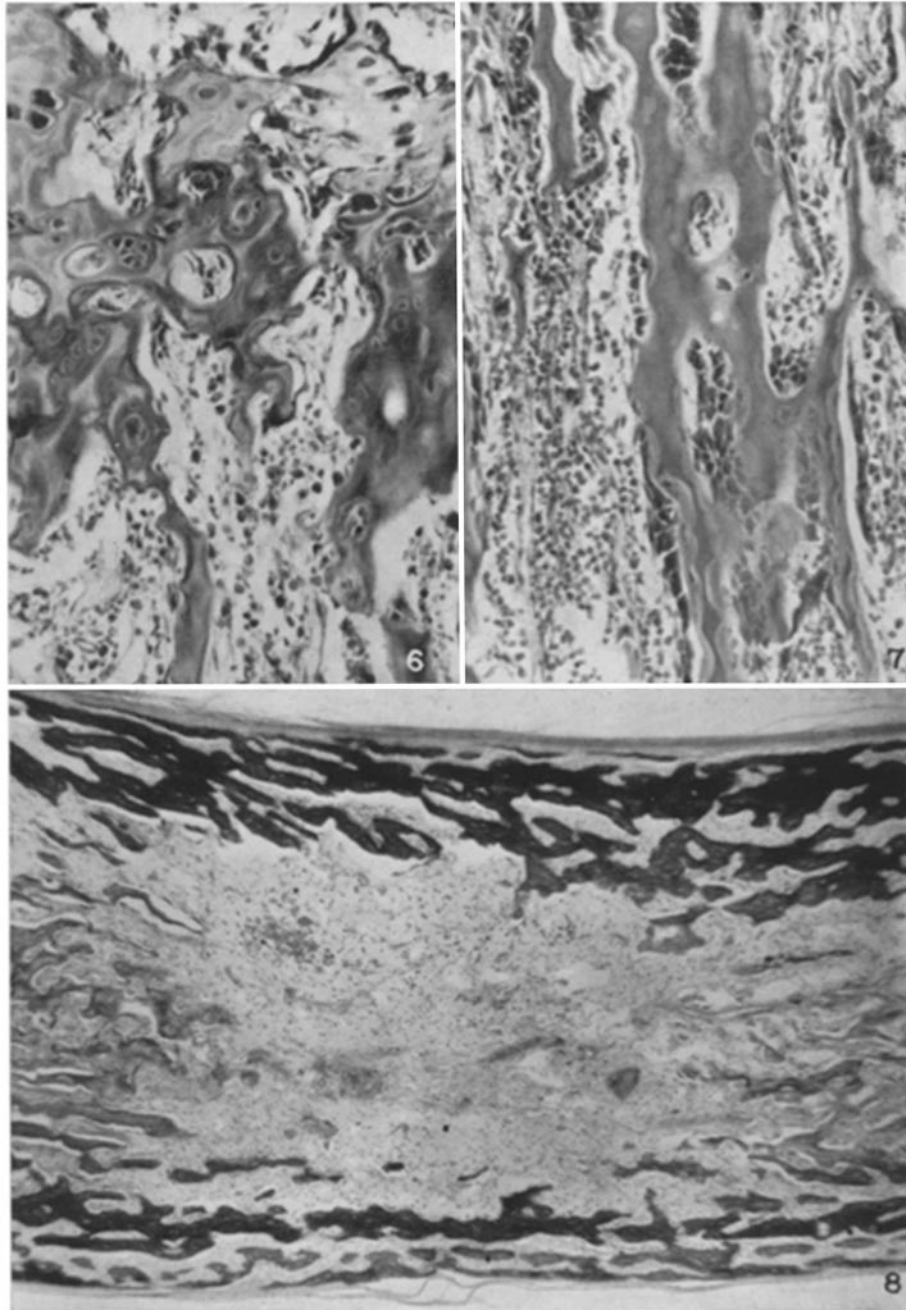
(Pearce: Hereditary osteopetrosis of rabbit. III)

PLATE 26

FIG. 6. Photomicrograph at a high magnification of an area in the outer part of the metaphysis of a tibia from a 3 day old osteopetrosis rabbit. A corresponding section from a normal litter mate is reproduced in Fig. 7. Note the irregular masses of spongy bone containing numerous osteocytes of irregular size, shape, and staining reaction. The staining reaction of the immature bone is also not uniform. Along many of the margins are rows of osteoblasts. A few osteoclasts are present. In the loose connective tissue between the irregular trabeculae are a few hemopoietic cells and dilated thin walled, practically empty blood vessels. Phloxine-methylene blue stain. $\times 220$.

FIG. 7. Photomicrograph of an area in the outer part of the metaphysis of a tibia from a normal rabbit, aged 3 days, a litter mate of the rabbit referred to in Fig. 6. Note the regular arrangement and uniform staining of the bony trabeculae, the bordering rows of osteoblasts, the osteoclasts, and the numerous hemopoietic cells. The blood vessels are not dilated and many are filled with red cells. Phloxine-methylene blue stain. $\times 220$.

FIG. 8. Photomicrograph of the central part of the shaft of a tibia from a 7 day old osteopetrosis rabbit. Note the large amount of fibrous tissue in which there are a small island of hemopoietic cells to the upper left of center, and a scattering of several large osteoclasts. To the right and left are many residual bony trabeculae. The cortex has a marked lattice appearance, the spaces containing fibrous tissue continuous with the central mass; the bone is largely spongy in type with many small osteocytes. Phloxine-methylene blue stain. $\times 53$.



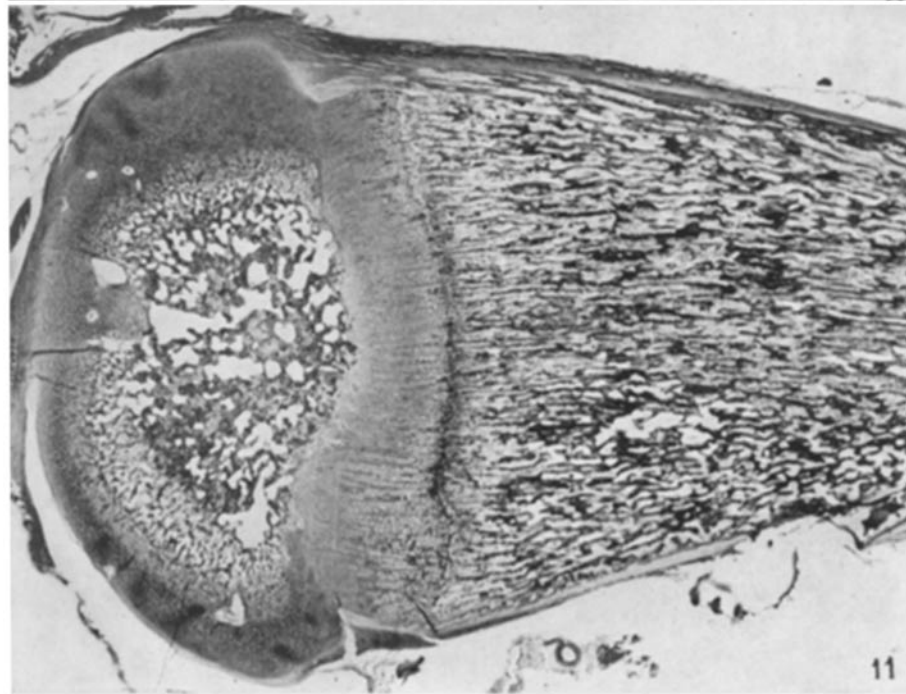
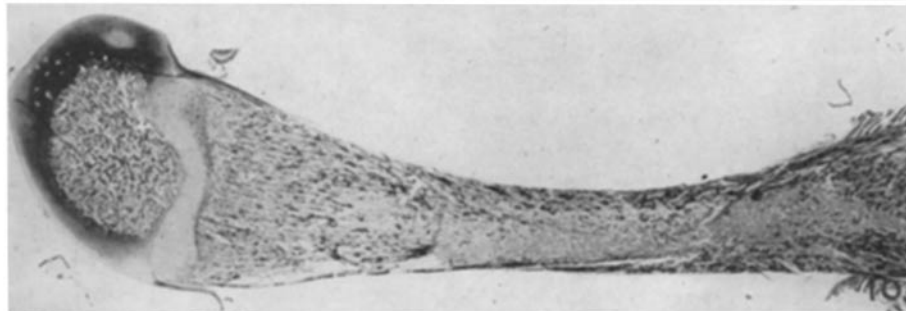
(Pearce: Hereditary osteopetrosis of rabbit. III)

PLATE 27

FIG. 9. Photomicrograph of a longitudinal section of a femur from an 11 day old case. The entire area of the shaft is filled with spongy bone and fibrous tissue. Persistent cartilage remnants are present. The cortex is thickened and has a typical lattice appearance. Phloxine-methylene blue stain. $\times 5.4$.

FIG. 10. Photomicrograph of a longitudinal section of a femur from an 18 day old case. The characteristic abnormalities present in the 3 and 11 day old cases and shown in Figs. 3, 5, and 9 continue to be present in older more advanced cases. Phloxine-methylene blue stain. $\times 5.4$.

FIG. 11. Photomicrograph of the proximal third of a longitudinal section of a tibia from a 13 day old case. In the shaft note the extensive distribution of spongy bone, the considerable amount of persistent cartilage, and the absence of a marrow cavity. Small foci of hemopoietic tissue occur in the loose connective tissue between the bony trabeculae. There is some irregularity of the rows of cells in the epiphyseal cartilage. Phloxine-methylene blue stain. $\times 14.7$.



(Pearce: Hereditary osteopetrosis of rabbit. III)

PLATE 28

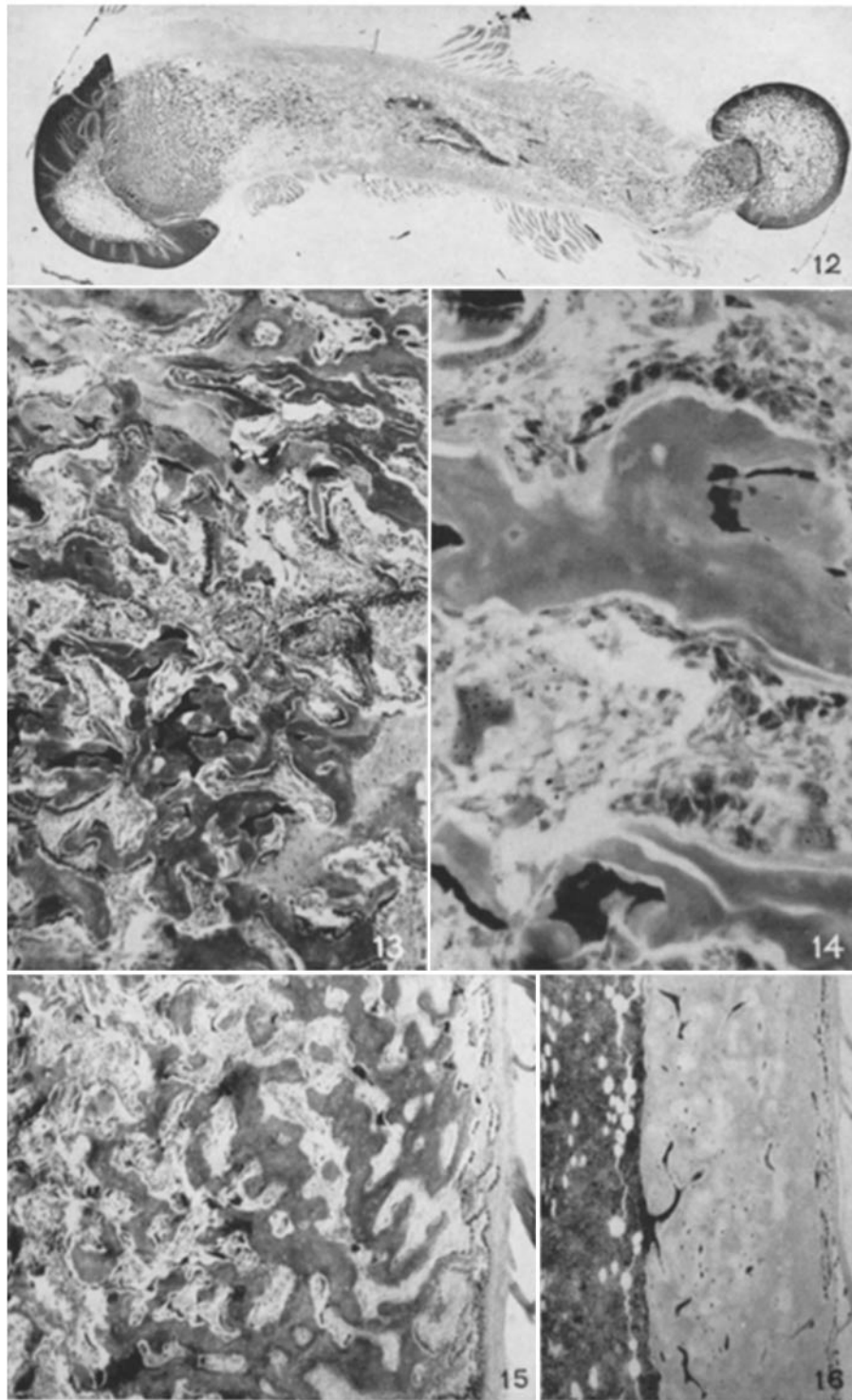
FIG. 12. Photomicrograph of a longitudinal section of a humerus from an osteopetrosis case, aged 27 days. There is a small central area of hemopoietic tissue but all the rest of the shaft is filled with spongy bone and fibrous tissue. The bone is shortened and the ends are expanded. Phloxine-methylene blue stain. $\times 5.3$.

FIG. 13. Photomicrograph at a higher magnification of an area of the section shown in Fig. 12, situated in the center of the diaphysis. Note the numerous irregular trabeculae of spongy bone and the considerable amount of fibrous tissue, some of which is fairly dense. Phloxine-methylene blue stain. $\times 60$.

FIG. 14. Photomicrograph at a higher magnification of an area of Fig. 13 to show the irregular staining reaction of the spongy bone, the persistence of cartilage, the numerous osteoblasts and osteoclasts, and the highly cellular connective tissue. The majority of these cells are rather long fibroblasts. Phloxine-methylene blue stain. $\times 270$.

FIG. 15. Photomicrograph of an area of the cortex in the section of the osteopetrosis humerus shown in Fig. 12. This photograph should be compared with the corresponding one from a normal litter mate (Fig. 16). The cortex is composed almost entirely of spongy bone interlaced with fibrous tissue, which gives it a lattice appearance. There were very little compact bone and an almost complete absence of Haversian systems and Volkmann's canals. Howship's lacunae are numerous, however, and contain large osteoclasts. In the subperiosteal area are numerous osteoblasts. At the left is shown a portion of the center of the diaphysis with residual bony trabeculae, dense fibrous tissue, a few osteoblasts, and numerous osteoclasts. There was no marrow cavity. Phloxine-methylene blue stain. $\times 52$.

FIG. 16. Photomicrograph of an area of the cortex of a longitudinally sectioned humerus from a normal rabbit, aged 27 days, a litter mate of the osteopetrosis rabbit referred to in Figs. 12 to 15. The typical appearance of compact lamellar bone, with its abundant vascular supply, is well shown in the right half of the photograph. At the left is a portion of the marrow cavity containing hemopoietic and fat cells. Phloxine-methylene blue stain. $\times 52$.



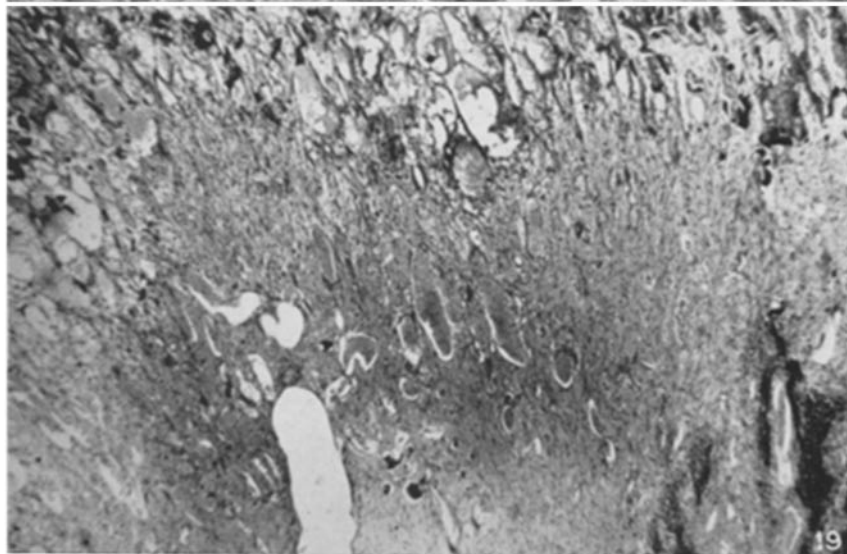
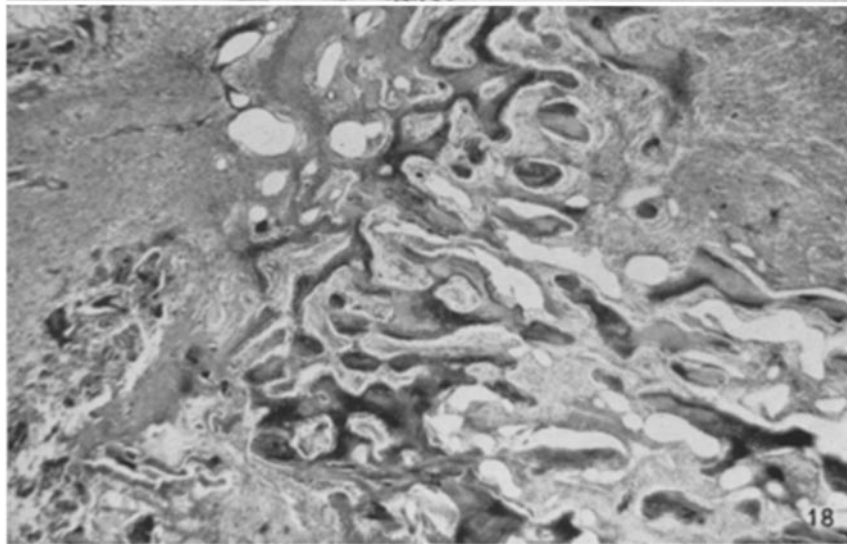
(Pearce: Hereditary osteopetrosis of rabbit. III)

PLATE 29

FIG. 17. Photomicrograph of the proximal half of a longitudinal section of a femur from an osteopetrosis rabbit, aged 40 days. Note especially the large masses of spongy bone in the metaphysis, the large area of homogeneous dense tissue in the upper diaphysis, the presence of spongy trabeculae containing cartilage in the metaphysis and diaphysis, the thickened cortex with its lattice appearance, and the small amount of hemopoietic tissue in the center of the diaphysis. Phloxine-methylene blue stain. $\times 5.2$.

FIG. 18. Photomicrograph at a higher magnification of an area in the inner part of the metaphysis of the section shown in Fig. 17. Note the irregular spongy bone masses and the areas of connective tissue with numerous small cysts. Hemopoietic cells are present in the fibrous tissue meshes in the upper right corner of the photomicrograph. Phloxine-methylene blue stain. $\times 42.5$.

FIG. 19. Photomicrograph of an area in the outer portion of the diaphysis from a longitudinal section of a humerus from an osteopetrosis rabbit, aged 23 days. There are an extensive hemorrhage in a large mass of connective tissue in the central portion of the photomicrograph and several large thin walled vessels filled with red cells. The connective tissue in the upper part of the photomicrograph is a very cellular loose meshed network with many small cysts. In the lower part of the photomicrograph, the connective tissue is quite dense but it is also very cellular and it contains many very large osteoclasts, several of which can be seen in the lower right hand corner. Heidenhain's azocarmine-aniline blue stain. $\times 39.5$.



(Pearce: Hereditary osteopetrosis of rabbit. III)

PLATE 30

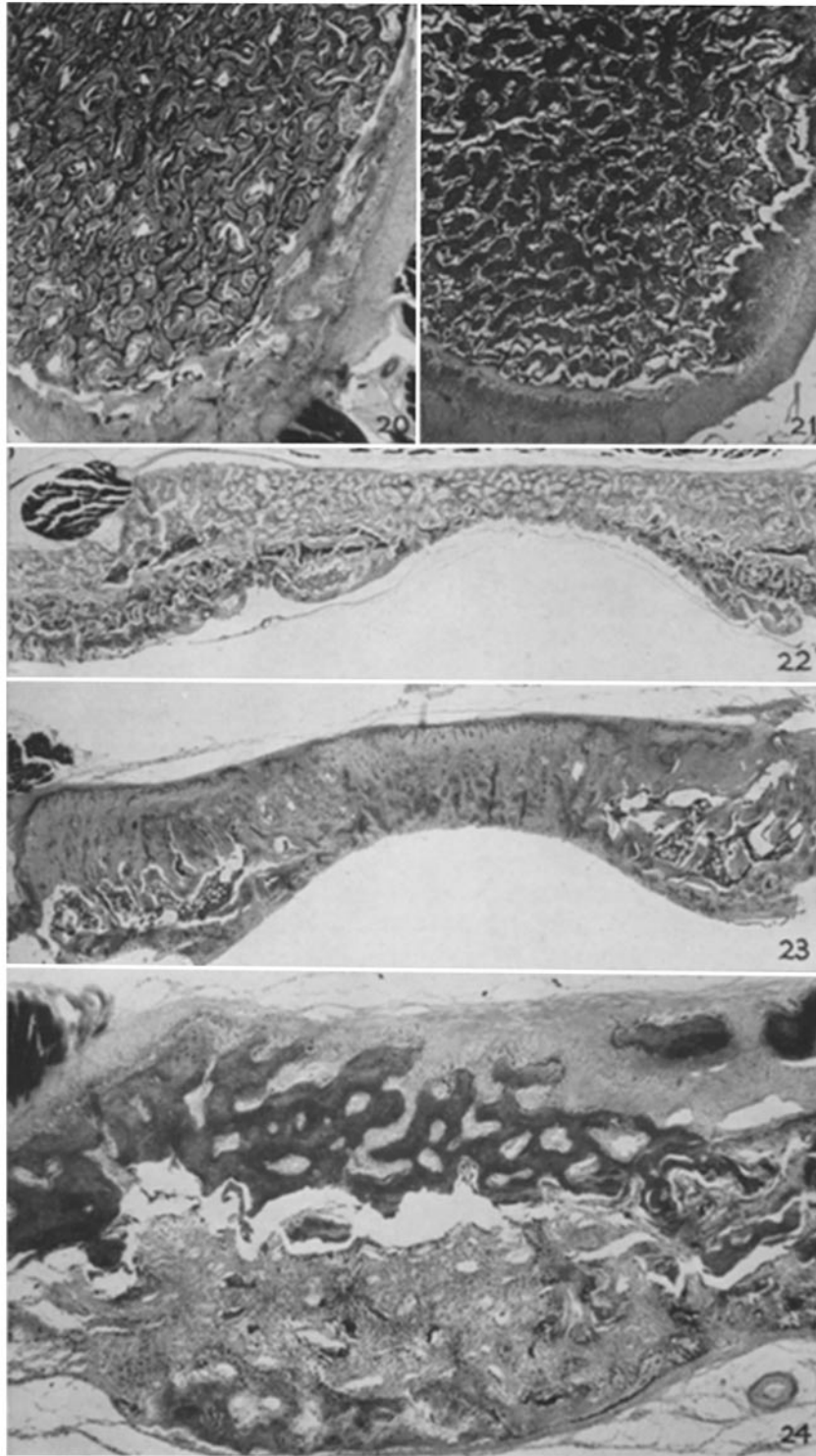
FIG. 20. Photomicrograph of a cross-section of a rib from an osteopetrosis rabbit, aged 25 days, which should be compared with the photomicrograph of a corresponding section from a normal litter mate (Fig. 21). Note that the rib is composed almost entirely of spongy bone with numerous cartilage remnants. The tiny marrow spaces are lined with osteoblasts and contain a loose reticulum and a small amount of hemopoietic tissue. A few osteoclasts are found in the vicinity of the periosteum which has a lattice appearance. Phloxine-methylene blue stain. $\times 46.5$.

FIG. 21. Photomicrograph of a cross-section of a rib from a normal rabbit, aged 25 days, a litter mate of the osteopetrosis rabbit of Fig. 20. Note the numerous large marrow spaces filled with deeply stained cellular marrow. These spaces are enclosed by thin cartilage remnants with faint, scarcely discernible bony borders. Few osteoclasts can be identified. Phloxine-methylene blue stain. $\times 46.5$.

FIG. 22. Photomicrograph of a section of the occipital bone from an osteopetrosis rabbit, aged 25 days. This photomicrograph should be compared with a corresponding one of a normal litter mate (Fig. 23). The bone is predominantly the spongy type with many small spaces containing a very cellular reticulum and some hemopoietic cells. The diploic spaces are small. There are a good many cartilage remnants and a few areas of rather dense connective tissue. Phloxine-methylene blue stain. $\times 13.4$.

FIG. 23. Photomicrograph of the occipital bone of a normal rabbit, aged 25 days, a litter mate of the rabbit of Fig. 22. Note the compact character of the bone and the comparatively large diploic spaces filled with cellular marrow. Phloxine-methylene blue stain. $\times 13.4$.

FIG. 24. Photomicrograph of a section of the occipital bone from an osteopetrosis rabbit, aged 23 days. In the upper part of the photomicrograph there are a large number of spongy bone trabeculae and a large amount of dense fibrous tissue continuous with the thickened periosteum. This tissue is rich in fibroblasts. Some of the spaces in the bone contain a loose connective tissue reticulum and hemopoietic cells. Below is a large area of dense cellular fibrous tissue with some masses of poorly stained spongy bone. Here the thickening of the periosteum is less marked than on the external surface above. Rows of osteoblasts border many of the bony margins and numerous osteoclasts are seen, principally in the fibrous tissue. There are a few small cysts. Phloxine-methylene blue stain. $\times 55$.



(Pearce: Hereditary osteopetrosis of rabbit. III)