STUDIES ON EXPERIMENTAL RICKETS IN RATS *

III. The Behavior and Fate of the Cartilage Remnants in the Rachitic Metaphysis

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Introduction

In describing the bones of rachitic infants practically all writers mention a region just below the proliferative zone of the epiphyseal cartilage which is composed of osteoid tissue and marrow interspersed with islands and projecting masses of cartilage. This is the region which is generally known as the rachitic metaphysis (Text-Fig. 1).

There is no general agreement as to the behavior and fate of these cartilage remnants. It is variously stated in textbooks of pathology that they become changed into osteoid, or that they become encased in osteoid, or that their margins change into osteoid. In none of the papers on experimental rickets has this question received much attention. In a paper on rickets in rats Pappenheimer [1] declines to discuss the "difficult question of the direct metaplasia of cartilage into osteoid tissue." Lobeck [2] considers that in experimental rickets in rats these remnants become incorporated into bone trabeculae, the cartilage cells thereby becoming surrounded by bone and subsequently being transformed into bone cells. Park [3] states that these cartilage masses become changed into "pseudo-osteoid, . . . which greatly resembles the osteoid tissue produced by the osteoblasts."

In 1935 [4] we reported briefly on the question but in a later, more extensive paper [5] omitted reference to the matter. We consider the behavior of these masses of considerable interest, not merely as part of the pathological process, but also as having a bearing on the fundamental relations between bone and cartilage.

* Scientific Paper No. 223. Received for publication July 20, 1939.

This paper presents more mature observations, clearing up some questions and pointing the issue on certain others.

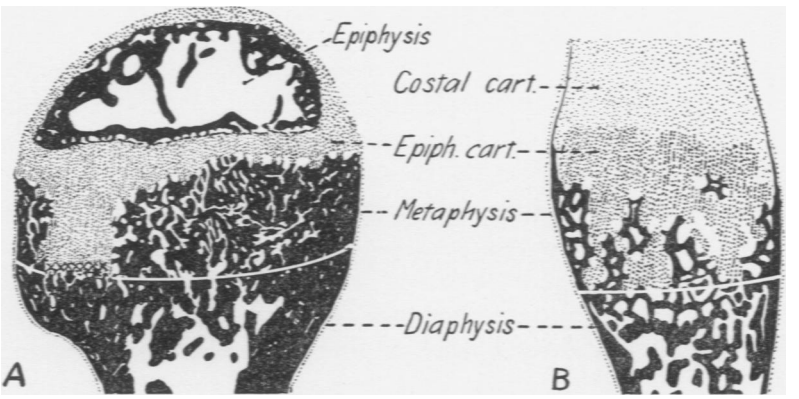
Material and Methods

This paper is based on continued study of the material used in earlier papers on rickets. Use was made of 125 albino rats; 31 with active rickets, 68 in various healing stages, and 26 healthy animals. Rickets was produced by the Steenbock-Black rachitogenic diet with high calcium, low phosphorus and deficient vitamin D. Healing was induced either by irradiated ergosterol (Viosterol) or cod liver oil. In some of the rats healing occurred spontaneously. The rachitogenic feeding was begun at 4 weeks and the administration of the curative agents at 8 or 9 weeks. Both longitudinal and transverse sections were cut at 10μ after decalcification in Müller's fluid. Hematoxylin and eosin stains were used. Our main study was on the head of the tibia, with frequent comparisons with other bones. The behavior of the cartilage remnants in the rachitic bones followed essentially the same course whether in active rickets or in early healing stages. The later healing stages furnished important information about the relation between cartilage and osteoid in the metaphysis of rachitic bones.

The Rachitic Metaphysis. Its Genesis and Nature

It is now generally recognized that the rachitic metaphysis is produced by partial removal of the thickened epiphyseal cartilage by the agency of highly vascular marrow from the diaphysis. The process is excellently described by Park.[3] The cartilage cells and the uncalcified cartilage matrix are destroyed with about equal rapidity. The cells degenerate, in the main by pyknosis (Fig. 11), although a few undergo fragmentation and others fading. The invading narrow tongues bore irregular holes in the cartilage, leaving anastomosing cartilage trabeculae of varying thickness, but all large enough to include entire cells as well as matrix. Before long it is seen that there is considerable osteoid associated with the cartilage remnants while at the same time much of the cartilage seems to have disappeared (Fig. 1). The question under consideration is, *does the cartilage change into osteoid?* The location and general extent of the rachitic metaphysis are shown in Text-Fig. 1.

It should be remembered that the cartilage composing these remnants is not of the active growing type, but rather of the kind that makes up the greater part of the rachitic epiphyseal cartilage. The cells are large and are generally considered to be well on the



TEXT-FIG. 1. Drawing showing general structure of bones with well advanced rickets. The open spaces represent marrow. In both bones the greatly thickened epiphyseal cartilage has been partly replaced by the characteristic structures of the rachitic metaphysis. The trabeculae of the metaphysis (shown in black) are composed at the one extreme of persisting portions of the epiphyseal cartilage, and at the other of well developed osteoid, as illustrated in Figs. 1 to 12. Within the irregular trabeculae there runs, with no great distortion, the parallel trabeculae of the epiphyseal cartilage. The region is wholly uncalcified or very nearly so. The trabeculae of the diaphysis and of the epiphysis are composed of persisting prerachitic bone covered with osteoid.

A = Head of the tibia from a white rat, 12 weeks old, with severe rickets. From a tracing over a photograph. × 10. XXXX indicates the position of cartilage rejuvenation described on page 731.

B = Costochondral junction in well developed rickets. Adaptation of a figure in a current textbook of pathology, directly comparable to the head of the tibia in Text-Fig. 1 A, except that removal of the cartilage has not been carried so far and the metaphysis is consequently less extensive.

way to disintegration. The cytoplasm is highly vacuolated and faintly basophilic; the nucleus is large, about spherical, and has widely separated chromatin granules. The lacunae have become correspondingly enlarged and the intervening matrix in many places is reduced to thin lamellae (Figs. 1, 2, 3, 9 and 11). In severe rickets this cartilage is without calcification. In both the

main cartilage masses and the smaller remnants the cells have retained their arrangement in longitudinal columns of definitely limited length, except where the weakened cartilage has suffered crushing or distortion by mechanical pressure.

These cartilage trabeculae vary in thickness, the thinner ones including but a single layer of cell columns, while the thicker trabeculae and the intersections have four or five. The following description does not deal with the behavior of the much larger cartilage masses, some of which are always present in the rachitic bones (Text-Fig. 1).

The Behavior and Fate of the Cartilage Remnants During Active Rickets

It will be noticed in Figure 1, a typical area from the rachitic metaphysis, that in the upper part of the picture the newer cartilage remnants have the same typically cartilaginous structure as the main cartilage mass from which they spring, whereas those lower down, the progressively modified portions, come to look more and more like osteoid tissue. We will now describe the extent to which cartilage gives place to osteoid and the manner in which this occurs. In this general change we have observed the operation of three general processes — infiltration, envelopment and internal reorganization.

1. *The Infiltration of Cartilage with Osteoid*

It is evident that it is only the extreme tips of the marrow masses that have the power of rapid destruction of cartilage. At a short distance below the front the destructive action is limited to the opening of some of the superficial lacunae on the surfaces of the persisting cartilage trabeculae and the destruction of the contained cells, but the formation of osteoid is going on rapidly. These opened lacunae are promptly invaded by osteoblasts, usually one in each lacuna, which have in this region developed in large numbers in the marrow from the cells surrounding the blood vessels. Within each such lacuna there promptly builds in from the periphery toward the center a layer of osteoid matrix, surrounding the enclosed osteoblast (Fig. 3), the deposition continuing until there remains only a small lacuna occupied by the

new osteoid cell. During the filling of the cartilage lacunae with the osteoid matrix the cytoplasmic processes of the osteoblasts, in the usual manner, become surrounded from apex to base by the new matrix, thus forming the canaliculi extending outward from the reduced lacunae (Fig. 10).

The new matrix thus formed within the opened cartilage lacunae usually stains but faintly with eosin, although in some filled lacunae the new matrix shows a deeply staining, granular zone. Thus the superficial portions of the cartilage remnants become infiltrated with osteoid. In the new tissue formed in this manner the pattern of the persisting cartilage matrix shows clearly within the new osteoid formation (Fig. 3).

This tissue might well be called osteoid filled cartilage. It is quite comparable in its mode of formation to the intrachondrial bone described by Bast [6] in the human otic capsule. We are not sure, however, whether any distinction should be made between endochondrial and intrachondrial ossification, inasmuch as both types involve the laying of bone matrix upon a base of cartilage matrix, the only difference being the extent of the cartilage matrix involved. In both types the cartilage matrix persists visibly within the new formation. The concept is nevertheless a useful one and Bast has made a useful contribution in pointing out the exact nature of the process occurring in the otic capsule.

2. The Envelopment of Cartilage Remnants by Osteoid

When the superficial lacunae have been filled by osteoid, as described above, the osteoblasts continue to build more osteoid on that already deposited in the lacunae. In other areas this enveloping layer of osteoid is laid directly on the surface of cartilage trabeculae whose superficial lacunae have not been opened. In either condition the osteoid thus formed may build up to considerable thickness, thus reducing the bore of the marrow canals. This process is most active in a zone of the metaphysis a short distance from the cartilage removal front.

The tissue thus formed is a typical well developed osteoid. It differs from that formed by infiltration of cartilage in that it contains none of the cartilage matrix, that its cells are placed rather at random instead of with a strong tendency to linear arrange-

ment, and that its matrix stains very deeply with eosin, so that it shows as dark areas in photographs (Figs. 1, 2, 4 and 8).

3. *The Internal Reorganization of Cartilage*

The foregoing two types of tissue are direct products of the marrow, inasmuch as the cells are derived from the marrow and the matrix is formed under the direct influence of these cells. The type now to be described has a quite different origin, being derived from the cartilage.

We have observed that when the cartilage trabeculae are not more than one or two cell columns in thickness all lacunae are usually promptly opened and filled with osteoid as already described, but when the masses are three or more cells in thickness there are some internal cells that remain unchanged for a longer time (Figs. 1, 2 and 3). These deeper cells are shut off from immediate contact with the marrow by the superficial lacunae which have been filled with osteoid and by the enveloping layer of osteoid. But if the cartilage masses are not too large these internal cells are not wholly beyond the influence of the marrow, as indicated by the behavior now to be described.

After considerable study of many sections we have become convinced that these hypertrophied cartilage cells in unopened lacunae undergo a form of rejuvenation together with a transformation in type. Many of them undergo mitotic division, take on the appearance of osteocytes, and there forms about them within the unopened lacunae a matrix that has the appearance of osteoid matrix such as forms in the superficial opened lacunae. Thus there is produced a tissue which is largely indistinguishable from that formed by infiltration under the influence of osteoblasts from the marrow (Fig. 8). Several lines of evidence support this view:

(*a*) *Many Lacunae Do Not Become Opened:* After microscopic study of sections, including a series of complete serials through the metaphysis of rachitic bones, it has become definitely apparent that the lacunae under discussion do not become opened. Cross sections of bones have been particularly convincing, inasmuch as such sections enable one to trace the trabeculae of cartilage and the columns of cartilage cells, and thus to determine with certainty which have been opened and which have not (Fig. 2).

(*b*) *Many of the Cartilage Cells Divide by Mitosis:* Two to four cells are seen in many of the unopened lacunae in these remnants, although only one is present in each lacuna in the general mass of cartilage from which the remnants have come (Figs. 2, 4, 5 and 7). In explanation of this condition mitotic figures of normal appearance are frequently seen in cells which are clearly of the cartilage type. We have observed both first mitoses, making two cells, and second mitoses, making four cells within one lacuna (Fig. 6). The number of dividing cells varies from rat to rat.

(*c*) *The Cartilage Cells Undergo Transformation into Cells Resembling Osteoid Cells:* This transformation is indicated by the presence of wide gradations of cells in these unopened lacunae, from typical cartilage cells at the one extreme, to cells very much like osteoid cells at the other. These intermediate type cells occur mainly in an intermediate position in the metaphysis. Close to the parent cartilage are found cells only of the cartilage type, while toward the end of the shaft they are mainly of the osteoid type (Fig. 1). This distribution of cell types, when observed repeatedly, leads one to the conclusion that the various intermediate forms are indeed actual intermediate stages leading toward a fairly uniform final type.

The transformation involves change in the staining reaction of the cytoplasm from basophil to acidophil, condensation of the cytoplasm, the development of cytoplasmic processes, and the condensation of the nucleus (Figs. 2, 4, 5 and 7). In general these cells, when fully transformed, are smaller than osteoid cells from the marrow and their cytoplasm is scant and likely to be vacuolated. Their cytoplasmic processes are more slender than those of true osteoid cells. These cytoplasmic processes do not develop by growth but rather are strands of cytoplasm which have retained contact with the capsule of the lacuna while the general mass of cytoplasm underwent condensation. Both kinds of cells are variable and frequently they cannot be distinguished unless it can be seen whether the lacuna has or has not been opened. The transformation begins before the first mitotic division and is continued after the divisions have been completed. Some of the cells, while still of large size, show numerous eosinophilic droplets in the cytoplasm, similar to those described by Pappenheimer [1] in

the ribs of rachitic rats. We do not know their significance but apparently only a small proportion of the cells ever have them (Fig. 5). There are other variations in the details of the transformation but the foregoing account gives a general picture of the process.

(*d*) *Matrix Forms About These Cells:* While the cartilage cells are undergoing the changes just described the cartilage matrix surrounding the lacunae in which they lie remains undistorted. The lacunae do, however, grow smaller, the change in size being due to the deposition of new matrix within the cartilage lacunae. It appears first as a thin layer in each lacuna, adherent to the capsule, while the cells are yet large and more like cartilage cells than osteocytes in appearance. As the cells grow smaller the matrix increases proportionally (Figs. 4, 5, 6, 7 and 8). The growing matrix progressively surrounds the cytoplasmic processes from apex to base, thus forming canaliculi extending out from the lacunae in which the new osteoid cells lie. When the cartilage cells have divided, producing more than one cell in a cartilage lacuna, some of the new matrix usually forms also between the cells so that each cell thus comes to occupy a separate lacuna. The new matrix in some lacunae stains but faintly with eosin (Fig. 8), while in others it has a zone of granules which stain strongly with eosin (Fig. 7). This deposit is not a thickening of the capsule surrounding the lacuna. The two are of different nature, as indicated by their microscopic appearance, their staining reaction and their behavior during healing of rickets.

The product of these changes is a tissue which is neither cartilage nor osteoid, but with greater resemblance to the latter. Its general staining reaction is pale (Figs. 1 and 8). The cartilage matrix pattern persists. The cells are usually small and look, after their rejuvenation, as if they were once more on the road to degeneration. It must be recognized, although we have not demonstrated it microscopically, that the cytoplasmic processes and the canaliculi in which they lie terminate for each cell at the inner surface of the capsule of the cartilage lacuna, each cell thus failing to communicate by canaliculi with adjacent cells as do the cells of true osteoid. In this respect the tissue resembles cartilage whose cells lack the communicating channels of bone and osteoid.

This tissue resembles the infiltration type of osteoid in that both have the cartilage matrix within the osteoid matrix, and in the usually pale stain of the new matrix. But we are usually not able to distinguish between the two unless the section be cut in a plane to show whether the lacuna has been opened. The two as a group are, however, easily distinguishable from the enveloping osteoid (Fig. 8).

It should be remarked that neither infiltration osteoid nor enveloping osteoid is formed on the large cartilage masses except under special conditions. The surface of such masses usually remains in direct contact with marrow, as if cartilage removal were more or less actively in progress. It was only the smaller masses, not more than about five cells in thickness, which were under the influence of the marrow in such a way as to become covered or filled with osteoid tissue.

In the preceding paragraphs we have described a rejuvenation of cartilage cells, coupled with a transformation into a form and functional activity like that of osteoblasts. Lest the idea of rejuvenation of cartilage cells in rachitic bones may seem unreasonable, or supported by inadequate evidence, we wish again to call attention to a different type of rejuvenation of cartilage cells (Dodds and Cameron [7]) which we have observed frequently and clearly in 43 of the same rats in which we have observed the changes just described. This rejuvenation was recognized also and clearly illustrated by Harris.[8] This process takes place in the main mass of the thickened cartilage close to the end of the diaphysis, the area being marked XXXX in Text-Fig. 1 A. In these areas the hypertrophied cartilage cells become restored to the appearance of cells in healthy growing cartilage: the nucleus becomes smaller and denser, the cytoplasm grows more compact and more sharply contoured, but no cytoplasmic processes develop. The cells undergo mitotic division, giving rise to typical groups of two or four, as in regular cartilage growth, but quite unlike the special flattened cells in the growth zone of the epiphyseal cartilage. The matrix also becomes adjusted to the expanding groups of cells and the characteristic capsules form about the lacunae. The total picture is quite different from the filling process observed in the remnants in the metaphysis. The fact of rejuvenation of hypertrophied cartilage cells is, however, clearly

demonstrated. Comparison of Figures 8 and 9 shows clearly how different are the results of the two kinds of rejuvenation.

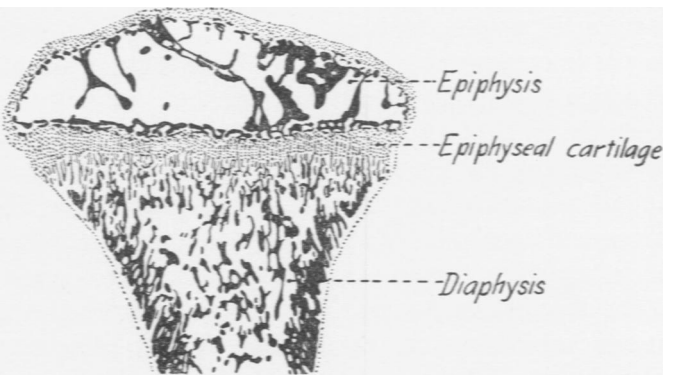
Cartilage Remnants During the Healing Process

Further light is thrown on the fate of the cartilage remnants by study of healing stages, while calcification is taking place and while the orderly bone trabeculae of the normal spongiosa are taking form in the regions formerly occupied by the chondro-osteoid trabeculae of the rachitic metaphysis.

When calcification is resumed three kinds of calcified matrix are seen in the region of the metaphysis. (1) Calcified cartilage matrix, which stains very deeply and uniformly with hematoxylin and which shows as black in photographs (Fig. 12, C). It follows the pattern of the earlier uncalcified cartilage matrix within the osteoid filler except that the capsules immediately surrounding the lacunae remain uncalcified. The general result is that the longitudinal matrix walls between the columns of cells become calcified, while the transverse partitions between adjacent lacunae within each column remain uncalcified. This is similar to the distribution of calcification in the epiphyseal cartilage of normal bones. (2) Calcified osteoid filler of cartilage lacunae. This portion of the matrix, when calcified, shows clearly about each osteoid cell as a zone of deeply stained granules, separated by a paler zone, the uncalcified capsule, from the dark area of the calcified cartilage matrix (Fig. 12, F). This includes both the infiltration osteoid and that formed by internal reorganization of cartilage tissue. (3) Calcified matrix of enveloping osteoid. This kind of osteoid often occurs in large masses. Calcification appears as clouds of deeply staining granules of varying density (Fig. 12, E).

During the early stages of healing the calcified osteoid filler and the calcified enveloping osteoid are removed, in large part at least, leaving the calcified cartilage matrix as a base for bone deposition. We have made no special study of the fate of the osteoid cells when the matrix is removed. They probably degenerate. Nor have we determined conclusively whether this calcified osteoid is wholly removed or if a small portion persists in the new bone trabeculae. As healing progresses the confused pattern of the chondro-osteoid trabeculae which characterizes the rachitic metaphysis gives place to the rather parallel trabeculae

which characterize the regions in normal bones (Text-Fig. 2). This greater regularity has not been produced by the formation of new trabeculae but rather by the removal of the large amount of osteoid, both enveloping and filling, which during rickets has masked the orientation of the cartilage trabeculae. The cartilage matrix has not been changed into osteoid matrix but much of it has persisted with remarkably little distortion to become the dominant structural element in restoring normal configuration of



TEXT-FIG. 2. Head of the tibia from a white rat 12 weeks old. Complete healing of well developed rickets. From a tracing over a photograph. × 10. The somewhat parallel bone trabeculae of the region just below the epiphyseal cartilage have been produced largely from the earlier rachitic metaphysis, the present orientation being determined by the remnants of cartilage matrix which, during rickets, were enclosed in the irregular osteoid masses. This figure serves in most respects also to represent a normal bone which has never shown the rachitic lesion.

trabeculae in the healing bone. After the delay occasioned by the diseased condition the cartilage matrix has performed its normal function in the formation of the early bone trabeculae. This influence of the cartilage matrix is just as effective in the region of the metaphysis, where for a time the cartilage was submerged in osteoid, as it is in regions where the thick cartilage has never taken part in the formation of the metaphysis, except that the distortion due to pressure is somewhat greater in the region of the metaphysis.

We believe that the foregoing paragraphs in large measure answer the question as to the fate of the cartilage remnants in the metaphysis of rachitic rats, although possibly some of the

cells behave in yet other ways than those described. There is every reason to believe that in the main the same conditions will be found to prevail in human rickets when adequate material has been carefully studied.

Discussion

The foregoing account of the behavior of the cartilage remnants in the rachitic metaphysis portrays, in the main, processes which are quite comparable to those familiar changes in the normal development and growth of bones which result in the deposition of osseous tissue on cartilage remnants. The exceptional feature is the formation of osteoid matrix in unopened lacunae containing rejuvenated cells. We do not know of any description of this process, although several investigators have written rather indefinitely about the metaplasia of cartilage into osteoid tissue.

Our present conclusions are in part different from those expressed in our abstract [4] where it was stated: "We have not observed the deposition of osteoid in unopened cartilage lacunae." At that time we believed that the filling of opened lacunae with osteoid under the influence of osteoblasts from the marrow accounted for the entire mass of cartilage remnants, although soon thereafter our observations brought us to our present conclusions.

Concerning the several conflicting opinions as to the fate of these cartilage masses, it now seems clear that they do become incased in osteoid, that their superficial lacunae become filled with osteoid (not changed into osteoid), and that their internal portions become changed into osteoid only in a very restricted sense. Our observations explain the statement of Lobeck [2] that these remnants become incorporated into bone trabeculae (they become incased in osteoid which later becomes calcified), and that the cartilage cells later are transformed into bone cells (they change into temporary osteoid cells). In all probability these osteoid filled areas are "the transitions which undoubtedly occur between atypical cartilage and osteoid" (Pappenheimer [1]). These rejuvenated and transformed cartilage cells may possibly be the "low-grade bone corpuscles" of Harris [8] in the cartilage islands of rachitic bones, although he gives no adequate description.

Unquestionably this tissue is the "pseudo-osteoid" of Park,[3] although neither he nor any other writer has expressed the same

conception of the genesis and nature of the tissue which we have; *i.e.*, the deposition of new osteoid matrix within the framework of the cartilage matrix. His description leaves no doubt that he has observed the transformation stages of cartilage cells but with different conclusions than ours as to their significance. "We used to think," he writes, "that in rickets actual metaplasia took place, *i.e.*, that cartilage cells, having been reduced to the size and appearance of bone corpuscles, became such. Further studies, however, have convinced us that they actually died." He does not mention mitoses of these cells, nor the other evidences of renewed vitality which we have noted. On the other hand, we concede that their rejuvenation is but transient, inasmuch as they are all seemingly destroyed along with the matrix when healing takes place. We believe that Park's term, "pseudo-osteoid," might very properly be applied to the condition we have described.

In view of the persistent belief that at times cartilage undergoes a direct metaplasia into bone or osteoid, a brief discussion of the points involved in such a change may be in order.

It should be clearly recognized that calcification of the cartilage matrix does not in itself constitute transformation into bone. Such calcification leaves still unchanged the typical structure of the cartilage cells; nor does it cause the matrix to assume the structural nature of bone matrix. Calcification is not the same as ossification, although the two terms are still frequently and wrongly used interchangeably. In normal bone development the matrix of the cartilage becomes calcified in advance of, and as a foundation for, bone deposition, but the cartilage matrix does not become bone although it becomes incased in bone.

When osteoid matrix is formed by osteoblasts in opened lacunae of the cartilage of rachitic bones this cannot be called metaplasia. But it unquestionably is metaplasia when cartilage cells in unopened cartilage lacunae assume the form of osteoid cells, and osteoid matrix is formed about them within the lacunae. The cells have assumed the morphological characteristics of osteocytes, and inasmuch as the matrix formed about them resembles osteoid matrix rather than cartilage, it is clear that they are functioning as osteoblasts. Yet the persistence of the original cartilage matrix within the new osteoid matrix is sufficient to cause the tissue as a whole to fall short of complete metaplasia. So far as we know,

no author has heretofore clearly pointed out the persisting identity of the cartilage matrix within the osteoid of the metaphysis, a point we consider of definite significance. It is thus evident that metaplasia of cartilage involves several elements, each of which must be considered separately.

We may now well consider the four different fates which may befall the cartilage in rachitic bones, with definite relation to regional location and probable external influences: (1) complete and prompt destruction; (2) osteoid infiltration; (3) osteoid rejuvenation; and (4) cartilage rejuvenation. Text-Figure 1 shows the regions now to be considered. In this connection there appears to be justification for the belief of Park [3] that "the cartilage cell cannot be exposed to the influences of blood and remain cartilage."

Cartilage is typically an avascular tissue. Prompt destruction of cartilage cells and matrix takes place at the most advanced portions of the marrow tongues where the raw eroded surface of the cartilage is in immediate contact with the marrow. The marrow at the very front is composed almost wholly of endothelial channels containing blood (Fig. 11). Sometimes, but not often, there are a few primitive connective tissue cells in advance of the vessels. Often there are erythrocytes outside the vessels in the recently opened cartilage lacunae. It would seem indeed that, as assumed by several recent writers, the vascular tissue is the active agent of cartilage destruction. It will be recalled in this connection that in the cartilage removal zone of the normal epiphyseal cartilage the blood channels are in intimate contact with the cartilage, but succeed in destroying only the uncalcified portion, *i.e.*, the cells and the transverse walls within cell columns. The calcified portions apparently are destroyed only by the osteoclasts (Dodds [9, 10]).

Connective tissue cells become abundant between the blood vessels and the cartilage masses at a short distance back from the front of the marrow tongues. Some of these cells soon become osteoblasts on the surface of the cartilage. This is the region of "incomplete exposure to blood" (Park [3]). In such regions cartilage removal ceases except for the opening of the more superficial lacunae and the destruction of their cells. Extensive deposition of osteoid occurs both in the opened lacunae and on the general surface of the cartilage masses. Moreover, it is in such regions that there takes place the transformation of the deeper cartilage

cells, and the deposition of osteoid matrix in unopened lacunae. It appears that the osteoid forming influence of the marrow is not limited to the surfaces in immediate contact with osteoblasts, but also extends to the inner cells of the cartilage remnants, causing them to behave like osteoblasts.

Our observations of the transformation of these cartilage masses exemplify the statement of Park [3] that "the closer to blood vessels, the more the condition resembles osteoid and the further away the more numerous are the cartilage cells which still retain normal features."

After considerable osteoid has been formed on the cartilage remnants and the marrow spaces have become smaller the osteoblasts disappear and osteoid formation ceases. By this time the transformation of the deeper parts of the cartilage masses has also become well advanced.

During active rickets the interior of the large masses of cartilage is clearly beyond the range of influence of the marrow, but it is of interest that in such cartilage, near the cartilage-shaft junction, there often takes place the type of cartilage rejuvenation which produces cartilage. This region is not immediately adjacent to any large masses of marrow but it is not far away from smaller marrow channels in the diaphysis whence there undoubtedly diffuse substances into the adjacent cartilage. One would like to know whether the diffusing substances are different in nature from those reaching the cartilage remnants in the metaphysis, or if they are only less abundant so that cartilage instead of osteoid results from the rejuvenation.

The four types of cartilage reaction just enumerated seem to be determined by the marrow, as if there were a definite gradation or zonation of influence. In part this influence must be through the power of the blood to carry nutrient materials to the tissues and to carry away wastes. But other factors must also be involved such as specific action of various cells in the marrow — the vascular elements, the osteoblasts, the osteoclasts, and probably other unidentified cells. The whole complex of processes is highly involved and embraces the entire scope of the differentiation of the mesenchymal derivatives which participate in bone formation.

In this connection one might ask whether the influence of the marrow is first on the changing cartilage cells and through them

on the matrix, or if both cells and matrix show direct influence from the marrow. Obscure as are these points, they are no more so than are the intimate questions of normal ossification, which in considerable measure still await the attention of the anatomist and the biochemist.

SUMMARY

The cartilage remnants in the rachitic metaphysis of experimental rats were observed to behave as follows:

1. Considerable osteoid is deposited on the surfaces of the cartilage remnants — osteoid envelopment of cartilage.

2. Osteoid is deposited in opened lacunae near the surface of these cartilage remnants by the action of osteoblasts which enter the opened lacunae from the adjacent marrow — osteoid infiltration of cartilage.

3. Cartilage cells farther below the surface of the remnants undergo rejuvenation; many of them divide mitotically and assume the form of osteocytes. While this change is going on, uncalcified osteoid matrix is deposited about them in the unopened lacunae — internal osteoid reorganization of cartilage.

4. In the foregoing internal reorganization process the cartilage cells undergo a metaplasia into osteocyte-like form, but the matrix of the cartilage is not changed. It persists, to become calcified during the healing of rickets and to serve as a base for the deposition of bone in the formation of the trabeculae of the restored bone.

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DESCRIPTION OF PLATES

In all photographs the uncalcified cartilage matrix surrounding the cartilage lacunae shows darker than the uncalcified osteoid matrix which has filled the cartilage lacunae. The enveloping eosinophilic osteoid shows as definitely darker than the osteoid which fills the cartilage lacunae. All photographs are from the head of the tibia. All figures except Figure 12 show active rickets.

1 = Cartilage matrix; 2 = osteoid matrix in opened lacuna, infiltration osteoid; 3 = osteoid matrix in unopened lacuna, transformation osteoid; 4 = space between cell and matrix; 5 = osteoblast or osteoid cell in opened lacuna, infiltration osteoid; 6 = enveloping osteoid; 7 = osteoblast; 8 = hypertrophied cartilage cell; 9 = cartilage cell in transformation to osteoid cell form in unopened lacuna; and 10 = cartilage cell which has been rejuvenated as cartilage cell.

p = Cartilage cell undergoing pyknosis (Fig. 11); e = erythrocytes in advance of nucleated marrow cells at invasion front (Fig. 11); C = calcified cartilage matrix (Fig. 12); F = calcified osteoid filler of cartilage lacunae (Fig. 12); E = calcified enveloping osteoid (Fig. 12); and UE = uncalcified enveloping osteoid (Fig. 12).

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FIG. 1. Edge of cartilage and part of metaphysis of a rachitic rat 8 weeks old. Projecting from the general cartilage mass are the persisting trabeculae of the cartilage, composed of the usual hypertrophied cells occupying lacunae separated usually by only thin walls of cartilage matrix. Farther from the cartilage mass these cartilage trabeculae give place to the different forms of osteoid. The true osteoid (enveloping osteoid) shows darkly in the photograph; osteoid filling the cartilage lacunae is usually lighter

in color. Some marrow spaces are empty because portions of the section have dropped out. × 100.

FIG. 2. Portion of a cartilage trabecula from a rachitic rat 8 weeks old showing early phase of transformation. The cartilage matrix is not calcified although it is deeply stained. On the surface is a layer of enveloping osteoid (6) being built up by the action of the osteoblasts (7). A few of the superficial cartilage lacunae have been opened and osteoid deposited (2). Most of the cartilage lacunae have not been opened (8). Some cartilage cells are still unchanged, others are in various early transformation stages, and some have divided. A little osteoid filler has been formed in a few unopened lacunae (not clearly seen in photograph). × 450.

FIG. 3. Margin of cartilage trabecula from a rachitic rat 8 weeks old. The superficial lacunae have been opened and are being filled with osteoid (2). Osteoblasts from the marrow occupy the opened lacunae and also lie on the general surface of the new osteoid (5, 7). A few unchanged cartilage cells remain within the mass (8). × 950.

FIG. 4. Margin of a cartilage remnant from a rachitic rat 8 weeks old showing osteoblasts on the surface (7) and depositing enveloping osteoid (6). This section shows a series of transformation stages of cartilage cells into osteoid cell form in unopened lacunae (U). Some of the cartilage cells have divided. Osteoid filler is present in varying amounts in these lacunae, even where the cartilage cells have changed but little. Other lacunae have been opened and are now occupied by osteoid filler and by osteoid cells from the marrow (O). × 450.

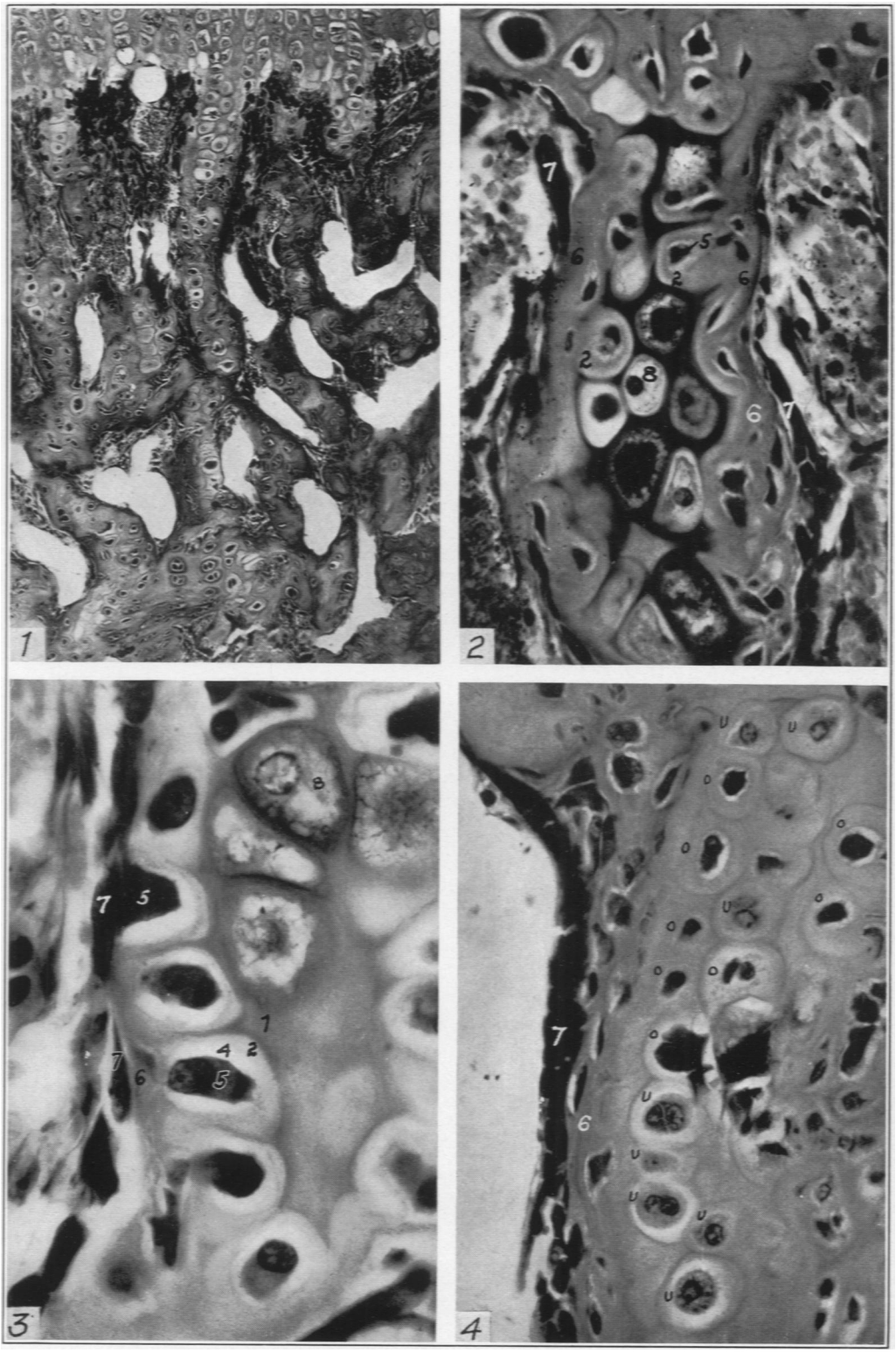

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FIG. 5. Transforming cartilage cells in unopened lacuna from a rachitic rat 8 weeks old. The first mitosis has been completed. These cells show the eosinophilic droplets (dark spots) in the cytoplasm. Some osteoid filler has already been formed about the cells in the lacuna (3). × 950.

FIG. 6. Second mitosis of a cartilage cell in an unopened lacuna from a rachitic rat 8 weeks old. The other cell has completed the second division. A thin layer of osteoid filler is seen in this lacuna (3). × 950.

FIG. 7. Showing transformation of cartilage cells and osteoid filling more advanced than in Figure 4. From a rachitic rat 7 weeks old. Most of the cartilage cells have divided. Considerable osteoid matrix has been formed in the unopened lacunae (3). In some the inner zone of new matrix shows eosinophilic granules (dark color), a condition sometimes seen in the filler of both open and closed cartilage lacunae. One of these lacunae has been opened (2). Enveloping osteoid is also seen in some areas (6). This area is from the lower part of Figure 1. × 450.

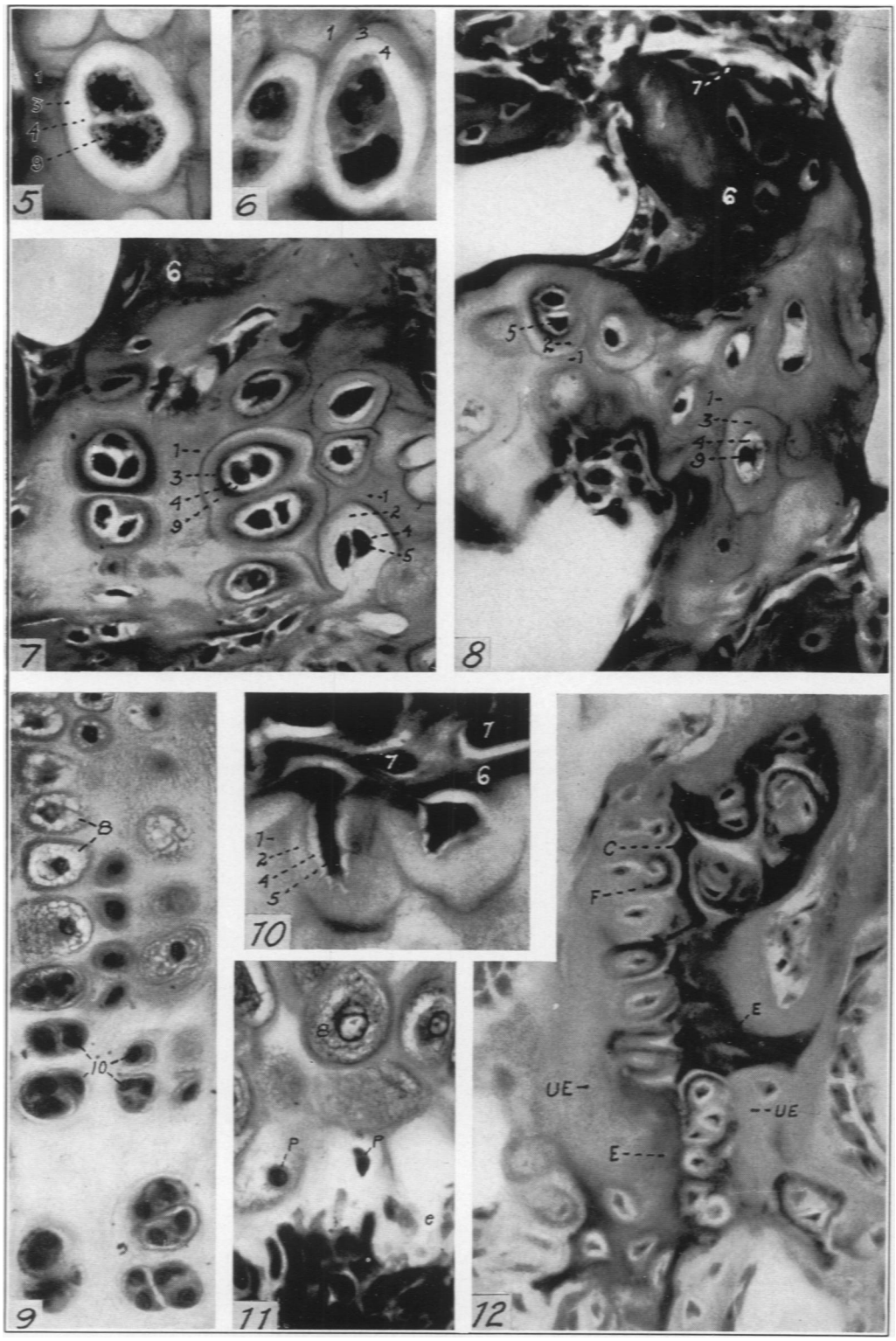
FIG. 8. Portion of a cartilage remnant showing well advanced osteoid filling of an unopened lacunae (light areas) from a rachitic rat 8 weeks old. Shows adjacent enveloping osteoid (6). Osteoblasts (7) are still laying enveloping osteoid in some regions. In the filled area the cartilage matrix pattern (1) shows clearly, as well as the osteoid filler of the lacunae (3). The cells of this area do not appear as vigorous as in the adjacent enveloping osteoid — a common condition. At least one of the lacunae in this field has been filled by infiltration (2). × 450.

FIG. 9. Cartilage rejuvenation in the deeper part of the thick cartilage adjacent to the end of the shaft. From a rachitic rat 10 weeks old. Shows ordinary hypertrophied cartilage cells (8) and others in various stages of rejuvenation (10). Some of the cells have divided once, others twice. The rejuvenated cells appear and act like cartilage cells, producing a tissue which looks like cartilage but not like osteoid. It is very different from the light area in Figure 8. × 450.

FIG. 10. Margin of cartilage remnant from a rachitic rat 8 weeks old. Shows two osteoblasts (5) about which the osteoid matrix is still being deposited in the lacunae (2). These cells show cytoplasmic processes extending into the canaliculi in the newly formed osteoid matrix. Enveloping osteoid (6) is also being deposited by osteoblasts (7) above these cells on the general surface. × 950.

FIG. 11. Edge of cartilage where it is being destroyed by marrow. From a rachitic rat 7 weeks old. Shows the usual hypertrophied cartilage cells (8). Two of these are undergoing pyknosis (p), one in an unopened, the other in an opened lacuna. The marrow shows erythrocytes (e) at the very front followed closely by various nucleated marrow cells. × 450.

FIG. 12. Portion of a trabecula in metaphysis when healing is well under way. From a rachitic rat 9 weeks old. The calcified areas may be differentiated as follows: calcified cartilage matrix, deep and uniform black (C); calcified osteoid filler, dark granular areas surrounding cells (F); and calcified enveloping osteoid, dark granules in varying density (E). Portions are still uncalcified and lightly colored in the photograph (UE). This area suggests Figure 2 and others. × 450.



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