HISTOLOGICAL STUDIES OF THE MEDULLARY BONE
IN THE FEMUR OF DOMESTIC FOWLS

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Several years have elapsed since the author began to carry out histological studies on avian leukosis. During this time, he has performed dissection, on many cases and has become greatly interested in such problems as related to the nature and function of the medullary bone (hereinafter referred to as MB).

Some time ago the author happened to read an article by Seifried and Sassenhoff titled "Osteomyelosklerose". With the subsequent circulation of reports on the histology of "so-called osteomalacia" issued by the Japanese Society of Veterinary Science, he began to pay special attention to the relationship between egg-laying in chickens and changes in bone tissue.

With much interest, the author continued to investigate this phenomenon, preparing many histological sections of chicken bones. Fortunately, he happened to receive a report concerning female pigeons by Bloom, Bloom and McLean. He then began to have a strong doubt about a new theory which had been presented by Seifried Sassenhoff regarding osteomyelosklerosis.

During his investigation of chicken bones, he was perplexed about the fact that the compact substance of the diaphysis never changes, while osteoclasts of the tissue in the cavity of the bone are subject to considerable changes. The facts recorded by Bloom et al. are quite different from those by Seifried and Sassenhoff. (Needless to say, the experimental birds used were different—these used fowls and those pigeons.) Bloom and his co-workers proved from their histological and experimental studies that the substance of the MB was not pathological, but physiological. These findings gave a great enlightenment to the author. Although a cytological investigation of the MB was carried out by Bloom and his co-workers with every precaution, it was impossible to deny that their experiment had only an unsatisfactory histological aspect. Since then the author has been devoting himself to the study of this problem.

Later, the author learned the results reported by Iwanoff and Mladenoff of an investigation of the MB conducted by the Bulgarian school. After that, he became convinced of the reliability of a theory that the MB might be a calcium storage tissue and be highly correlated to egg-laying in fowls.

However, the report published by Iwanoff and Mladenoff (as well as those reports cited in theirs) gave no satisfactory explanation on occurrence of various phases of the MB tissue. It presented the same defect as the report of Bloom et al. It stated the presence of connection between egg-laying and the fact that MB is a calcium storage tissue, but it failed to illustrate the process of occurrence sufficiently. Dorn's report was an answer for, or partial defense of, his master, Seifried, whose opinion had been criticized by Iwanoff and Mladenoff in their report titled "Ist die Osteomyelosklerose

der Hühner eine Krankheit?” However, even Dorn\textsuperscript{2} did not clearly oppose the opinion that the phenomenon observed in the MB is physiological. He mentioned that the MB could be observed in pathological conditions, and illustrated his point by quoting three cases of ovarian tumor. (However, even if the MB is physiological, there is no reason why it should not be present in certain pathological conditions.) In short, it can be said that the physiological significance of the MB has been established, but that morphological investigation is insufficient on the construction and regression of the MB. This is the present state of our knowledge in this field.

In this thesis the author wants to present a summation of the results of his histological studies on the bones of a great number of chickens. He does not at all think that his materials used are sufficient to draw any definite conclusion. He hopes to supplement his findings with future investigation.

**MATERIALS AND METHODS**

The specimens used for research were 276 hens and 34 cocks. The hens ranged from 19 days to nearly 5 years of age, and the cocks from 2 days to about 2½ years. These specimens belonged to the White Leghorn (WL) and Barred Plymouth Rock (BPR) breed and the hybrid White Leghorn×Barred Plymouth Rock (WL×BPR). Some of them had been used especially for other experiments, others were healthy and others were killed. Originally, all of them had been collected for studies on avian leukosis.

Both hens and cocks were subjected to postmortem examination. The entire skeleton was collected from them. Specimens were fixed in formalin. At this time, only the femurs were selected and made to cross and longitudinal sections. After electric decalcification, the bone tissue blocks were embedded in celloidin. The section were stained with hematoxylin and eosin.

**RESULTS**

I. Histology of the Femur

1. Figs. 1～20 show femurs without MB

The MB is part of a particular osseous system. This is proved by the following description of histological changes which occurred during a period before the formation of MB.

(1) Periosteal osteogenesis (Figs. 1 and 2): This began with hyperplasia of the osteoplastic fibrous tissue in the periosteal area. Concrete examples proved that this varied in severity, and that it was not related to the age of the chicken. The fibrous, immature bone tissue began to be formed in the bone marrow cavity. At the same time, it grew out toward the surface of the bone. Then a primitive canal system was formed. The canal system, which was located in the surface layer, formed a coarse network. The inside of the network was lined with an osteoblastic layer which gave aid to the formation of osteoid tissue. In this way, the first stage of the compact substance was completed, and the second stage of the compact substance began to appear on the outer surface bound by a “Kittlinie”, or a cementing line (Figs. 1 and 2).

(2) Endosteal osteogenesis (Figs. 3 to 6): Endosteal steogenesis had less relations to the formation of the compact substance. As shown in Fig. 3, the osseous tissue was not well developed. It was an intermittent development in the inner layer of the compact substance on the side toward the bone marrow. As seen in Figs. 3 and 5, it
was a thin layer of osteoid tissue between the osteoblastic layer and the compact substance. In addition, on the bone-marrow side of the osteoblastic layer, an active proliferation was seen in a tissue (osteoblastic reticular tissue) composed of pale spindle-shaped cells (Fig. 3). With the lapse of time, this tissue grew into a compact substance, which was divided into sections by "Kittlinie", as is shown in Fig. 6.

(3) Formation of trabeculae in the diaphysis (Figs. 7 to 9): As stated in paragraph (2), formation of trabeculae took place in the diaphysis. This was originated from the primary compact substance (Figs. 7 and 9). Soon it began to develop into the secondary compact substance as well (Fig. 8). There could be observed the same histological phenomenon as that exhibited in the development of immature bone in infantile mammals.

(4) Formation of trabeculae in the epiphysis (Figs. 10 to 12): Either in the epiphysis proximalis or in the epiphysis distalis, there occurred an active and vigorous formation of cartilaginous trabeculae. Endosteal osteogenesis took place in that portion of the epiphysis adjoining to the diaphysis (Fig. 11). The epiphyseal cartilaginous tissue formed trabeculae on the bone-marrow side of the compact substance which was very hyperplastic. In the portion surrounding the trabeculae, the cartilaginous trabeculae were metamorphosed to osseous trabeculae as a result of osteoclastic and osteoblastic resorption and removal.

(5) Formation of trabeculae in the epiphysis—basic columns of trabeculae—(Figs. 13 to 17): The author was interested in histological changes to form what he called basic columns of trabeculae. During the preparation of many sections, he observed a mass of chondroblastic tissue which, in median longitudinal sections, appeared at the center of the epiphysis (Fig. 13). This columnar tissue projected toward the diaphysis. Cartilaginous tissue was noticed around the columnar tissue. Cartilaginous trabeculae were formed beyond that tissue. The cartilaginous tissue became osseous trabeculae. These changes are clearly shown in Figs. 13 and 14. By the time when these cartilaginous trabeculae were metamorphosed completely into osseous ones, the basic columns had entirely disappeared, as is clearly seen in Figs. 16 and 17.

(6) Abnormal bone (Figs. 18 to 20): Fig. 18 was taken from a section of abnormal bone in which the first stage of the compact substance was completed in only one area of the diaphysis. In some areas of the completed compact substance, formation of trabeculae was still going on. In most areas, however, there was a histological evidence that the compact substance was incomplete, being still in the process of histogenesis of the canal system. Fig. 19 shows the histology of an abnormal or excessive formation of the compact substance in the diaphysis. The author does not think that this should be regarded as an eosinophilic osseous mass. He deals with this in sections 3 and 4. In any case, he could not find any evidence of MB in this specimen. The lump of bone shown in Fig. 20 is, without doubt, that of abnormal, incomplete, periosteal bone.

2. Primary stage of the newly-formed medullary bone (Figs. 21 to 26)

It was difficult to discriminate the histological evidence of the primary, newly-formed MB from that of MB in a regressive state. This difficulty arose, of course, from the smallness of the MB both in the case of primary, developing MB and in that of retrogressive MB. (The completely developed and the retrogressive MB will be discussed in sections 3 and 4.)

Minute description of the residual MB will be given in another section. Consequently, it is sufficient to discuss only about the primary, newly-formed MB in this section.

The histological picture presented in Fig. 24 is the residue or relics of the MB. It
is quite natural that one must refer to the endosteal osteogenesis mentioned in section 1, paragraph (2). In interpreting Fig. 24 histologically, it must be taken into consideration that the specimen is a completed bone, 226 days old, and accordingly that there is no further endosteal modification. One must therefore acknowledge a systematic distribution of the MB growing on the inside wall of the bone-marrow cavity in the compact substance of the diaphysis. The newly-formed MB was always lined with a layer of pale spindle-shaped cells (osteoblastic reticular tissue), and there were no osteoclasts any longer (Figs. 23 and 25). Histological examination revealed an unsystematic distribution of cells in the retrogressive MB. One could observe primary newly-formed MB in the trabeculae (Figs. 22 and 26). The author points out that the mass of MB shown in Fig. 21 is somewhat irregular. He thinks it necessary, however, to be taken into consideration that the complicated shape may have resulted from the arrangement of trabeculae, as well as from the direction of cutting of the section.

3. Completion of medullary bone (Figs. 27 to 40)

There were 2 or 3 histological changes noticed in the completed MB. The MB varied in quality, but the most characteristic quality of it consisted in the network pattern described in the following paragraph (1).

(1) Characteristic network pattern of medullary bone (Figs. 27 to 34): In the process of periosteal osteogenesis, a canal system was developed from the network formed by the osseous tissue. In this way, the initial compact substance was formed. The network, as such, disappeared. In contrast to this, it was observed in the case of the MB that the characteristic structure of the network consisting of osseous tissue remained intact. Completely developed MB encircling the bone marrow, lined the inner surface of the compact substance. It was normally as thick as the compact substance, but sometimes 1.5 to 2 times as thick. MB was formed around the trabeculae which were projected toward the bone marrow from the compact substance (Fig. 29). Thus, when the MB became vigorously hyperplastic, the whole bone-marrow cavity was filled with it (Fig. 33). The MB had such structure that it did not strongly adhere to the compact substance. There was a difference in quality between these osseous substances. When one half of the femur at the diaphysis was pulled strongly, it was possible to remove a rod-shaped plug from the hollow of the bone in the same manner as one draws a sword from its sheath.

When one carefully observes the characteristic network of the completed MB at its cut surface, stained colorfully, the MB presents such pattern as observed on a topographical map. All the patterns are not the same, but usually look like the surface layer of an onion. It is also common to observe linear structures stained deeply with hematoxylin between the layers of the MB. It is quite usual to observe this histological picture clearly at the point where the MB network touches the bone marrow. As might be expected from the histogenesis of MB, the MB forming a network resembled mosaic. In histological sections stained with hematoxylin and eosin, the color of the MB varied with specimens, ranging from a bright violet to a slightly eosin-stained shade. In those specimens exhibiting affinity to eosin rather than to hematoxylin, the structure of the MB tissue was revealed very distinctly. On the outer surface (i.e., the surface in contact with the bone marrow) of the MB, a slight proliferation of pale spindle-shaped cells (osteoblastic and osteoclastic reticular tissue) and the presence of a few osteoclasts were visible, although they were not important.

(2) Formation of fissures in the MB (Fig. 35): The characteristic network of the completed MB was shown in paragraph (1). When many sections were examined, it was noticed that the fissures in the MB tissue resembled topographical lines in a map.
(Fig. 35). When this happened, the network patterns of the MB in some way lost their freshness in the stained sections. Judging from the histological findings of the characteristic network patterns of the completed MB, and from those of fissures which were evident in the histogenesis of the MB, it can be said that the MB differs clearly from the compact substance. The author is in the opinion that morphologically, the completed MB is different from bone tissue. It is natural to assume that the fissure formation may be connected with some artefact of the specimen. It must be admitted, however, that the fissure in the MB is also related to the very nature of the MB.

(3) Tendency of the MB to become eosinophilic (Figs. 36 to 38): It is regarded as one of the properties of the MB that no network trabeculae take eosin. In some specimens, however, the characteristic network patterns were stained with eosin faintly and the network resembled the compact substance (Fig. 37). At the same time, the outer edge of the trabeculae was distinguished distinctly. No pale spindle-shaped cells are recognized in Fig. 56. There was negligible osteoclastic activity. If one supports the theory that the completed MB is in a functional state, one must admit that at this stage its function is almost nil. This is called the tendency of the MB to become eosinophilic.

(4) Eosinophilic osseous mass (Figs. 39 and 40): This is a nodular mass of osseous tissue of the completed MB which is in the bone-marrow cavity. It became eosinophilic and attached itself to the trabeculae. It is possible to regard it as the type of abnormal bone which was mentioned in paragraph (6) of section 1. (In that case no MB was formed as yet.) The author, however, rather inclined to consider that it is a completed MB in such a dormant state as mentioned in paragraph (3) of section 3. He thinks of this because, in the same histological section a network of trabeculae can be observed in the MB simultaneously with an eosinophilic mass. Among the trabeculae of this network, there were some which showed the phenomenon of retrogression, which is to be explained in the following section (Fig. 39).

4. Retrogression of medullary bone (Figs. 41 to 85)

Retrogression was exhibited by as many as 71.2% of the specimens with MB.

(1) Osteoclastic reticular tissue (Figs. 41 to 51): There was an accumulation of pale spindle-shaped cells on the periphery of the MB. It is referred to as osteoclastic reticular tissue. The morphology of these cells may be clearly seen in the photomicrographs of high-power magnification of Figs. 42, 45, 47, 51, etc. The cells assumed, in general, a roundish spindle shape. In those cases where they appeared in such an arrangement that of the teeth of a comb, individual cells are very often polygonal. In the osteoblastic reticular tissue, the cells had a strong tendency to form a regular, even osteoblastic layer (Figs. 26 and 40). In contrast to this, in the osteoclastic reticular tissue, the arrangement of cells was rather disorderly and the individual cells were rich in cytoplasm. These cells had a clear area in such part of the cytoplasm opposite the nucleus. Furthermore, in many cells, the cytoplasm was apt to be stained deeply with a basic dye (Figs. 47 and 49). It was often noticed, however, that it was quite difficult to distinguish the osteoblastic cells from the osteoclastic ones.

In all the histological sections that contained the entire MB, there were many in which one part of the MB consisted of osteoclastic reticular tissue and the rest of it of completely developed MB tissue (Figs. 41 and 42). Fig. 43 is a section showing vigorous hyperplasia of MB tissue. Because of this change, one is apt to overlook the hyperplasia of the osteoclastic reticular tissue which, in such cases, has already been in an advanced stage. Hence, efforts should be made to discover some of the characteristic features of the osteoclastic reticular tissue which distinguish this tissue from the MB.
tissue. These characteristic features are as follows:

(a) The peripheral portion was faint in the network trabeculae of the MB. This probably indicates that degeneration of the MB began in its peripheral portion. (b) In contrast to the primary stage of the MB, the histological structure and osseous character of the regressive MB was already completed. They had been influenced by the activity of the osteoclastic reticular tissue. Consequently, the histological changes may be summarized as follows: The author's observation revealed that osteoclasts developed simultaneously with the osteoclastic reticular tissue. The characteristic network pattern which lends support to the author's observation is shown in Figs. 41 to 44, 46, 47, 50, etc. The fissure formation is shown in Fig. 47. The accompanying osteoclasts are present in Figs. 45, 47 to 51, etc. The tendency of the eosinophilic bone of the MB to change is seen in Figs. 50 and 51. The thinning and fragmentation or the residue of the MB is exhibited in Figs. 44, 45, and 47 to 51.

(2) Osteoclasia (Figs. 52 to 58): The appearance of osteoclasts was a characteristic feature of the regressed stage of the MB. These cells developed together with the osteoclastic reticular tissue described in the previous paragraph. Some osteoclasts were small, shrunken, and inactive (Figs. 55 and 57), and others large and vigorous (Figs. 53, 56 and 58). The appearance of these osteoclasts may vary in the same specimen. In general, it may be said that the larger osteoclasts were present in a regressive area where there was hyperplasia of the osteoclastic reticular tissue. In the same specimen, the smaller osteoclasts were generally scattered throughout the trabecular network, to which they were closely attached. They showed a tendency to become fragile and fragmentary. Furthermore, regression of MB may be very clearly seen around those areas of MB which face the bone marrow and in those areas facing the compact substance, in which the presence of osteoclasts is also especially prominent (Figs. 53 and 57). Many osteoclasts may also be seen in those areas of MB which are in contact with the trabeculae (Fig. 58).

(3) Thinning and fragmentation of the MB (Figs. 59 to 66): The network trabeculae of the completed MB were doomed to become fragile and fragmentary because of the proliferation of osteoclastic reticular tissue and the progressive activity of the osteoclasts. In those cases where the development of the MB was sufficient to fill the bone-marrow cavity (Fig. 59), or in those cases where there were only fragments of MB in the bone-marrow cavity (Figs. 62, 64, 65 and 66), the fragmentation of the MB was easily distinguished from the primary stage of the MB. However, when the network of trabeculae underwent no fragmentation and was metely in the process of thinning (Figs. 60, 61 and 63), it was rather difficult to do so. In these cases, if careful investigation is made on the activity of the osteoclastic reticular tissues and the osteoclasts in those portions of the MB which surround the trabeculae (see paragraphs (1) and (2) above), it will be rather easy to determine whether the MB is in a regressive or progressive state.

As stated in the section on osteoclasia, the thinning and fragmentation is generally quite obvious in those areas which face the bone marrow on the inside and the compact substance on the outside. This thinning and fragmentation is seen throughout the trabecular network (Fig. 59, etc.). It varies, however, in degree according to the area of the MB, as is illustrated in Fig. 59. In this figure, the deeply-stained nodules are thicker than the other areas. In addition, the diminished MB sometimes retains its characteristic network pattern, and often exhibits a tendency to become eosinophilic (Fig. 66). There are some pictures suggestive of fissure formation (Fig. 65). In some cases, the MB tends to regress in structure and, at the same time, to change, in pro-
properties as previously explained.

(4) Residue of the MB (Figs. 67 to 74): The residue results from the thinning and fragmentation. With a magnifier, scarcely any of the MB fragments is visible (Fig. 67). Microscopic examination discloses the presence of fragments of MB surrounded by markedly proliferative osteoclasts (Figs. 68, 71, 73 and 74) and a general tendency toward eosinophilia. It should be emphasized that the MB in the residual state closely resembles that in the primary stage in structure. Furthermore, it will be useful to mention that while fragments of residual MB may be observed, in the free state, in the bone-marrow cavity, there are no fragments of newly-formed MB in the primary stage (Figs. 69 to 73). On the other hand, as stated in paragraphs (2) and (3), retrogression can be recognized in the MB at such area of contact with the compact substances as shown in Fig. 66.

(5) First stage in the retrogression of the MB (Figs. 75 to 79): As previously explained, when the retrogression of MB is increasing in severity in the network of trabeculae, it is possible histologically to indicate those portions where the thinning and fragmentation of MB is most apparent.

After his investigation of a great many histological sections, the author was able to draw a definite conclusion about this retrogression phenomenon. For this reason, he has added this section to this thesis.

First, in Figs. 75 to 77, one can observe a layer of MB which is in the compact substance at the diaphysis. In the middle of this layer the characteristic network pattern is clearly recognized, but on the periphery of the layer, i.e., on the surfaces which face the compact substance and the bone marrow, it is possible to find the thinning and fragmentation. At the same time, there is a tendency for this layer to become eosinophilic. There is a dividing line in the MB between the advanced stage of retrogression and the incipient stage, especially in Figs. 76 and 77. In Figs. 78 and 79, if one focuses attention to the structure of the MB, one can recognize what seems to be separate layers of MB.

(6) Eosinophilic osseous mass (Figs. 80 to 85): It is possible to assume that the substance shown in Figs. 80 and 81 is such a type of abnormal bone as described in section 1. This assumption, however, would be incorrect, since this mass consists of retrogressive MB (Figs. 82, 83, 84, etc.). In contact with the nodular eosinophilic osseous mass, there is a network pattern of trabeculae showing retrogression. Therefore, it is not proper to consider that these nodular formations are of abnormal bone. The author thinks it more likely that they have been produced by retrogression in the newly-formed secondary MB, which will be dealt with in section 5.

Since the appearance of such a sparse, eosinophilic, osseous mass as this in the bone-marrow cavity is a rare phenomenon, the mass could be regarded as abnormal bone. It will be more correct, however, to call it a sort of relics of the MB rather than abnormal bone.

5. Newly-formed secondary MB (Figs. 86 to 90)

As previously stated, it is not seldom that the MB quite differs in characteristic according to portions even in the same section (See MB in the primary stage of retrogression). It was confirmed that in the primary stage, retrogression occurred in those areas of the MB which were adjacent to the bone marrow.

The histological changes which are to be described in this section present contrasting phenomena to those just mentioned above. In this case, the MB in contact with the compact substance showed the activity of the osteoclastic reticular tissue and a tendency to become eosinophilic. The MB filled the bone-marrow cavity completely
(Fig. 86) and exhibited a characteristic network pattern clearly (See section 3 on the completion of the MB). It has thus becomes obvious that the MB was reformed at different intervals. This reformed MB should be classified as newly-formed secondary MB. This phenomenon is shown in Figs. 87 to 90. It was usually rather localized and confined to some narrow areas. In Fig. 86, however, an example is shown for the newly-formed MB occupying almost the entire bone-marrow cavity.

In classifying this newly-formed secondary MB, unless it is listed under any independent or new category, it may be placed in the same category as the completed or retrogressive MB.

II. Relations between Development of the Medullary Bone and Various Factors

1. Sex: The specimens used for research were 276 hens and 34 cocks. Medullary bone was observed in none of the cocks, but in 212 of the hens. This is a firm and clear evidence that the presence of the MB is sex-related in fowls.

2. Age (Chart 1): The author had to be satisfied with the number of specimens available, since he was not able to collect an equal number of specimens for each age group. However, as the results of observation were summarized on a percentage basis, the number of specimens in any particular age group made no difference in the conclusions reached.

There was no MB in any of the materials collected from chickens 50, 100, or 150 days old, but in those collected from only one or two of the chickens 250 and 300 days old.

Later, MB in the primary stage was found in the specimens from 3 birds less than 200 days old and those from 8 bird less than 250 days old, but never in those from chickens 250 days or more of age.

Completely-formed MB was present in 7.2% of the birds less than 200 days old, 29.2% of those less than 250 days old, and 18.3% of those less than 300 days old. Among the birds of the groups more than 300 days old, there were many with completely-formed MB.

Therefore, it may be evident that the MB is formed completely in hens at the age of roughly 200 days, and that it is consequently found in specimens collected from hens more than 200 days old.

On the other hand, it was observed that retrogression did not begin in the MB until a hen was at least 200 days old. Many of such cases were found among birds more than 200 and less than 250 days old. Retrogression of the MB was revealed in as much as 80.5% of the birds of the groups less than 300 days old. It was observed in specimens from the hens of all age groups more than 300 days old in which it was possible to find specimens of completely-formed MB. In other words, the retrogression of the MB did not begin before 200 days of age, and was present in all age groups older than that.

3. Weight of the ovary (Chart 2): The MB can be found in hens with ovaries weighing less than 5 g. On the other hand, all the specimens in which there was no completed MB had come from birds of those groups which had ovaries weighing less than 5 g.

This fact naturally agrees, to a great extent, with the age of the hens. For instance, as is shown in text-figure 1, any specimens less than 50, 100 or 150 days old never have MB. The author found, however, some hens in which the ovaries weighed less than 5 g when they reached the egg-laying stage. He also noticed that there was no completed
Chart 1. Relationship between Medullary Bone (MB) of Femur and Age in Days in Domestic Fowls.

MB in this group.

Newly-formed primary MB was present in 7.7% of the birds with ovaries weighing less than 5 g, and 6.7% of those with ovaries weighing 10 g. No newly-formed MB was observed in none of the birds with ovaries more than 10 g in weight.

The observations above (Chart 1) were correlated to the age of the hens. As a result, it was found that the newly-formed MB had begun to develop before the first egg was laid.

Only completed or regressive MB could be seen in the birds with their ovarian weights ranging from 10 to 15 g, as well as in those with ovaries more than 15 g in weight.

It was noticed in the birds the ovaries of which weighed less than 5 g that the MB, if present, was a completed one in 20.1% of them and a regressive one in 32.7%. These birds were all more than 150 days old. The circumstances under which egg-laying was performed were, for the most part, not clearly noted in those hens used at this point. Judging from the ovarian weights of the hens, it is quite likely that the hens were not in the egg-laying period, but in some other period preceding to it or in the resting period of the reproductive cycle.

4. Diseases (Chart 3): The author made a general survey, through patho-anatomical diagnosis, on the 271 hens used in his research. He found that only 31 birds (11.4%) of a total of were 271 were healthy. This result was quite natural since the majority of the birds used in the present experiments had been slaughtered or had died, and had also been used in some other experiments. Patho-anatomical diagnosis
Chart 2. Relationship between Medullary Bone (MB) of Femur and Ovarian Weight in Domestic Fowls

Total number of cases: 239.

- Lacking in MB: 41 cases.
- Newly formed MB: 10 cases.
- Completely formed MB: 46 cases.
- Regressive MB: 148 cases.

The numeral "5" on the horizontal stem indicates specimens with an ovarian weight of 5 g or less, and "10" an ovarian weight exceeding 5 g but not more than 10 g. The other numerals should be understood in the same manner.

disclosed that a great many experimental birds were victims of avian leukemia (35.4% of the total birds, or 40.0% of the diseased ones).

The relationship between the presence of MB and health conditions was studied. Of 50 birds less than 150 days old, only 4.0% were healthy ones. Of 48 birds more than 200 but less than 250 days old, 25.0% were healthy ones. (Two of them, however, had no MB.) Of 82 birds more than 250 but less than 300 days old, 15.9% were healthy ones. (These birds included one which had no MB.)

The birds used for the present experiments included both healthy and diseased ones. Besides, the presence of MB was verified both in the healthy and in the diseased birds. Therefore, it is evident that the presence of MB is independent of, and unrelated to, any disease which may affect hens.

DISCUSSION

Previous studies of the MB: The presence of MB in female pigeons were reported by Bloom, Bloom and McLean, and that in chickens by Seifried and Sassenhoff, Iwanoff and Mladenoff, and Dorn.
The conclusions reached by Bloom and his co-workers\(^1\) may be summarized as follows: In pigeons, the MB undergoes a series of striking transformations during the egg-laying cycle. All of them are apparently correlated with the need for storage and transportation of calcium for the calcification of the egg shell. The stages of transformation are composed of: (1) that of intense formation of MB during the preovulatory period, when the follicle is developing in the ovary, (2) a period of intense breaking down of MB during the calcification of the shell of the first egg, or a period continuing for a short time after the first egg is laid, (3) a period of returning to bone formation within a few hours after the production of the first egg, or a period continuing until shortly after the arrival of the second egg at the shell gland, and (4) a period of destruction of bone, beginning while the second egg is in the shell gland, and continuing on a diminishing scale after the production of the second egg and until the disappearance of all the MB.

During these rapid and widespread changes in the bone marrow, it is easy to follow the transformations of the cells involved. In the preovulatory bone-forming period, reticular cells became osteoblasts, which in turn became osteocytes. In the bone-destroying phase, after ovulation of the first egg, the osteoblasts and liberated osteocytes became osteoclasts. The latter turned into osteoblasts during the second, short-lasting period of bone formation following ovulation of the second egg. In the post-ovulation period, osteoblasts and osteoclasts became reticular cells again.

When the experimental pigeons were maintained on an adequate intake of calcium and phosphorus, the newly formed bone matrix became calcified. During the extensive dissolution of bone, the bone matrix and bone salt were dissolved together. Those
osteoclasts which appeared in the resorption of bone contained no bone salt demonstrable by the von Kössa method.

Furthermore, Bloom and his co-workers discussed the relations of hormones and serum calcium to the phenomena described in their paper. They concluded that the MB undoubtedly functioned as a storehouse for calcium. There were great differences in the circumstances of ovulation between pigeons and chickens. In general, the egg-laying circumstances were not clearly noted in the hens used by the author. Therefore, it was impossible to compare the findings of the author with those of Bloom et al. Regarding the problem on the relationship between the reticular cell and the osteoblast, Bloom et al. gave the following description:

\[
\text{Reticular cells} \rightarrow \text{Osteoblasts} \rightarrow \text{Osteocyttes} \\
\text{Osteoclasts}
\]

On the basis of the results of his investigation, the author thinks it more correct to express the relationship as follows:

\[
\text{Reticular cells} \rightarrow \text{Osteoblasts} \rightarrow \text{Osteocyttes} \\
\text{Osteoclasts}
\]

As explained in the paragraph concerning the retrogression of the MB, it is occasionally difficult to discriminate osteoblastic cells from osteoclastic ones. It is evident, however, that reticular cells, in general, may become either osteoblasts or osteoclasts according to physiological demands. Consequently, the author cannot admit that there is a transformation of osteoblasts into osteoclasts or vice versa. At this point he differs in opinion from Bloom and his co-workers. At present, immature tissue or immature osteoclasts in mammals are under much discussion. It will be very interesting in the future to make a detailed cytological investigation of MB.

Seifried and Sassenhoff regarded the presence of MB a pathological, rather than a physiological, condition which they referred to as "Osteomyelosklerose." The specimen they used was a two-year-old hen affected with severe anemia. Later, they subjected the bones of 200 chickens to macroscopic investigation. Of these specimens, ten were examined histologically, but those investigators carried out no intensive histological study on the MB. From the beginning they stressed the similarity between the appearance of MB and what they called human "osteosklerotische Krankheiten." They consider the appearance of MB as a hitherto unreported disease of the osseous tissue system in domestic animals. They called this "disease" "Osteomyelosklerose des Hühnens," because it was followed by some morbid changes in the blood and hematopoietic organs. The author is not in a position to discuss this complication which Seifried and his co-workers noticed in the circulatory system. As the author stated in section II, paragraph 4, many of the experimental birds he used were affected with avian leukosis and other pathological condition, because of the circumstances under which he had obtained them. It was clear, however, that the MB had no particular relation to any disease. On the contrary, the author became firmly convinced by the histogenetic evidence that the MB was a substance belonging to the physiological make-up of chickens.

Let us now turn our attention to the investigation by the Bulgarian school consisting
of Iwanoff and his co-workers, and the papers of Sjölte which Iwanoff and his co-workers\textsuperscript{3} cited in their report. (The author was unable to read these papers in their original form and had to rely on the abstracts presented in the paper by Iwanoff and Mladenoff\textsuperscript{3},)

According to Iwanoff and his co-workers\textsuperscript{3}, the histology of the MB was first studied by Bloom and his co-workers\textsuperscript{3}. After that, it was reported by Dimitroff and Theisz. It is said that these two emphasized the relationship between the MB and the process of egg-laying. Szép also published a paper on this subject.

Mladenoff's work\textsuperscript{3} is primarily with statistics. He pointed out that no MB was present in chickens less than 3 months old, but that MB was usually found in adult chickens. His investigation showed that MB was present in 75.0\% of adult chickens, in 4.1\% of young chickens, and even in cocks.

Seifried's group\textsuperscript{6} mentioned that MB was found at all age levels after sexual maturity had been reached. Iwanoff and Mladenoff\textsuperscript{3} primarily discussed these findings. MB may be observed also in wild pigeons and other wild birds. Furthermore, Iwanoff agreed with Seifried's group which asserted that there was no particular relationship between MB and erythrocyte count. This group acknowledged a fact that very active compensatory hematoxysis took place in the liver and the spleen. Furthermore, it should be noted that interesting results were obtained by Iwanoff's group from its investigation on serum calcium. The content of serum calcium doubled in the period of sexual maturity (16\textasciitilde22 mg\%). It increased to 28, 30, or sometimes 40 mg\% as soon as the egg-laying activity began. Then, in the broad period, it decreased to 14\textasciitilde15 mg\%. In addition to this fact, Iwanoff's group also reported that the egg-shell contained 90\% calcium carbonate (2 g of pure calcium). Hence, at the calcium increase-decrease rate given above it requires 15 hours to produce an egg. To sum up, Iwanoff and Mladenoff\textsuperscript{3} insisted that the formation of MB took place in accordance with some physiological law related to ovarian activity.

Iwanoff and Mladenoff\textsuperscript{3}, and Konstatinoff investigated 118 and 428 male wild birds, respectively. Iwanoff and Mladenoff\textsuperscript{3} examined 198 young wild birds, but they could find any trace of MB in none of them. Furthermore, they observed that MB was present in female wild birds only during a comparatively short period of time every year. Confronted with a fact that MB was also present in a cock (a fact stated by Mladenoff\textsuperscript{3}, they stressed a need for careful study of the sexual gland to distinguish the male gland from the female. Taking the above-mentioned facts in to consideration, Iwanoff and Mladenoff\textsuperscript{3} proposed that the occurrence of MB be called "Vogel-Osteothesaurismose." (It is understood that French scholars use the term "os folliculinique" for MB.) Furthermore, Iwanoff's group\textsuperscript{3} mentioned in the final part of their paper that by reading Sjölte's report they had been introduced to the work of Kyès and Potter, and Bloom et al.\textsuperscript{1} for the first time. The work of Bloom et al. was cited at the beginning of this section. It is Kyès and Potter's conclusion that the formation of MB is the "Ossifikation" of the bone-marrow cavity which is related to physiological hyperplasia of the ovarian follicle.

Iwanoff and Mladenoff\textsuperscript{3} drew conclusions in their report that the "Osteomyelosklerose" described by Seifried and Sassenhoff\textsuperscript{4} was never a disease, and that these investigators\textsuperscript{3} had not been familiar with the reports published by Sjölte.

The author wants to discuss Iwanoff's report in the light of knowledge obtained from his own experiments. Before doing this, he will try to summarize Dorn's thought.

Dorn's work on "der sogenannte Follikulinknochen beim Huhn" was performed at the laboratory of Professor H. Sedlmeier, successor to Seifried. In his paper, Dorn
simply gave the data obtained from his own experiments which had been carried out to clarify the relationship between ovarian hormones and the MB and the histogenesis of the MB. He did not endeavor to make discussion to prove SEIFRIED's theory. He stated only in general that the MB was a physiological product intimately related to the activity of the ovarian hormones. He pointed out, however, that active formation of MB was observed in three of the chickens which had been proved to have ovarian tumors, even though none of the three chickens were capable of laying.

In considering the observation mentioned above, one must not think that DORN\textsuperscript{33} regarded the occurrence of MB as a pathological condition. He simply stated that MB might be produced in hens in which some disease had made the production of eggs impossible, as well as in chickens which had revealed a physiological necessity for the production of eggs.

Thus, it may be said that IWANOFF and DORN agreed in drawing a conclusion that the MB is the product of a particular bone phenomenon natural to chickens. Few authors, however, have thoroughly investigated the histogenesis of MB and consequently disregarded the complexity of changes involved in the formation and retrogression of MB. The present author is of the opinion that unless the process of formation and retrogression of MB is studied separately from the histological angle, there may still be room for somebody to say that DORN still maintains his theory that MB is a pathological substance.

No doubt, there exist specimens, such as those observed by DORN\textsuperscript{33} which seem to indicate that the presence of MB is a pathological condition. It should be remembered, however, that the mineral metabolism in chickens is not entirely dependent upon ovarian hormones.

DORN gave too brief a description of the three specimens he had observed. Therefore, it is better for the author to refrain from making any comment on these special cases. The author would like to assert, however, that the histological changes which he has classified in this investigation will be valuable in promoting the present knowledge on MB. In the future, a more thorough investigation will be performed on the relationship between the capacity of egg-laying and the process of formation and retrogression of MB.

**SUMMARY AND CONCLUSIONS**

This research concerned changes involved in the formation and retrogression of medullary bone (MB), which is a tissue in the bone-marrow cavity of fowls.

A total of 276 hens and 34 cocks were used. The hens ranged from 19 to 1,744 days of age and the cocks from 2 to 857 days.

The results of histological observations are summarized as follows:

1. Changes in the formation and retrogression of MB were complicated, because the phenomenon of egg-laying is complicated.

2. Particular attention was paid to patho-anatomical changes in the entire body and to anatomical changes in the ovary. An important relationship was recognized between such factors as sex, age, anatomical changes in the ovary, and patho-anatomical changes in the entire body and the formation and retrogression of MB.

3. The author compiled a complete series of histological slides showing the process of formation and retrogression of MB in the femur. These specimens ranged from newly-formed to completely-formed MB. This series also included a complete series of specimens showing the retrogression of MB, as well as a series of specimens showing "newly-formed secondary" MB.
4. In the course of this research, the author endeavored to make a complete survey of all the histological findings with regard to abnormal bone, transitional inclination of eosinophilic bone, formation of "eosinophilic osseous mass" (so called tentatively by the author) and other special products, in addition to the survey mentioned in the preceding paragraph 3.

The histological examination of MB gave a firm and clear evidence that MB is a bone tissue which has a direct and important relation to egg-laying in fowls.

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This report is dedicated to Dr. S. Yamagiwa, the president of Obihiro Zootechnical University (1962—), Professor Emeritus in Hokkaido University, Ex-professor in the Department of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University.

REFERENCES

鶏大腿骨の Medullary Bone に関する組織学的研究

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（昭和 41 年 3 月 1 日受付）

鳥類における骨髄腔内組織である Medullary bone の消長に伴う形態学的変化を追及した。検査に用いられた材料は、雌鶏 276 例および雄鶏 34 例である。鶏においては、産卵事情が複雑であるので、Medullary bone の消長像もまた単純なものではない。用いられた諸材料に関しては、全身剖検所見および卵巣の解剖学的所見に、できる限りの注意を払った。これら Medullary bone の消長と諸因子との関係を検討する上に、重要であることを知った。

大脛骨所見に関しては、Medullary bone 第一次

新生像が発現され、完熟像、退縮像を経て、Medullary bone 第二次新生像に至るまでの諸組織像を分析抽出した。その間、異常骨、Medullary bone のエオジン酸および E 骨塊（著者仮称）の他、特微ある組織所見の記載を試みた。著者は、Medullary bone に関する組織学的研究により、一抹の不安をも感じることなく、Medullary bone が鶏類産卵に関連を有する特微組織であることを、確信することができた。

EXPLANATION of PLATES

All specimens are from the femur. All figures were taken from histological sections stained with hematoxylin and eosin. For details see the text.

Abbreviations: WL=White Leghorn, BPR=Barred Plymouth Rock.

PLATES I~IV (Figs. 1~20): No medullary bone (MB).
Figs. 1~2: Periosteal osteogenesis.
Fig. 1. Cross-section through diaphysis. "Kittlinie" divides the compact substance into two layers. Canal system of the inner layer has nearly been completed. In the outer layer, apposition of osteoid tissue is still going on (×50). (Female, No. 14, Pr. 1~973, 49 days of age, slaughtered, WL, 22/9/56.)

Fig. 2. Cross-section through diaphysis. Canal system has been completed. Osteogenesis, however, is still going on around the area indicated. Both new and old layers are divided by Kittlinie (↑) (from upper left to lower right) (×185). (Female, No. 41, Pr. 1~934, 108 days of age, slaughtered, WL×BPR, 21/7/56.)

Figs. 3~6: Endosteal osteogenesis.
Fig. 3. Cross-section through diaphysis. The periosteal osteogenesis of compact substance is already finished. There is active formation of bone trabeculae. Strong proliferation of endosteal osteogenetic tissue (↑) is shown in an area (×185). (Female, No. 21, Pr. 1~1068, 71 days of age, slaughtered, WL, 20/6/57.)

Fig. 4. Cross-section through diaphysis. Compact substance is nearly completed. Note periosteal osteogenesis in the upper left (↑) (×4.1). (Female, No. 38, Pr. 1~1054, 100 days of age, slaughtered, BPR, 29/5/57.) Compare with Fig. 5.

Fig. 5. Bone marrow segment from the right-side portion of Fig. 4 lined with an endosteal osteoblastic layer (↑). Note osteoid tissue stained in various colors parallel to and on the right of the layer (×365).

Fig. 6. Cross-section through diaphysis. Formation of compact substance is complete. Two layers of osseous tissue separated by Kittlinie (↑) are shown on the upper surface of the compact substance. They line the surface toward the bone marrow. Completed bone tissue (×365). (Female, No. 60, Pr. 1~981, 183 days of age, slaughtered, WL×BPR, 18/7/57.)
Figs. 7~9: Formation of bone trabeculae in diaphysis.
Fig. 7. Cross-section through diaphysis. Compact substance is divided by Kittlinie (↑) into two layers. Note trabeculae in the inner layer (×4.1). (Female, No. 37, Pr. 1-1079, 99 days of age, slaughtered, WL×BPR, 18/7/57.)
Fig. 8. Cross-section through diaphysis. Note formation of bone trabeculae in both layers of compact substance (×50). (Female, No. 46, Pr. 1-1066, 120 days of age, slaughtered, BPR, 11/6/57.)
Fig. 9. Cross-section through diaphysis. In the outer layer of compact substance apposition of periosteal osseous tissue is still going on. In the inner layer formation of bone trabeculae is also in process. No Kittlinie is seen between the outer and inner layers (×50). (Male, No. 18, Pr. 1-1056, cockerel, slaughtered, WL, 29/5/57.)

Figs. 10~12: Bone trabeculae in epiphysis.
Fig. 10. Longitudinal section through epiphysis distalis. Hyperplasia of cartilaginous bone trabeculae. Osteoblastic layer surrounding bone trabeculae and osteoclasts are present (×50). (Female, No. 6, Pr. 1-949, 24 days of age, natural death, WL, 28/8/56.)
Fig. 11. Longitudinal section through epiphysis proximalis. Note the relatively thick coating of endosteal osseous tissue (↑) on cartilaginous trabeculae and the osteoblastic layer (×50). (Female, No. 14, Pr. 1-973, 49 days of age, slaughtered, WL, 22/9/56.)
Fig. 12. Longitudinal section through epiphysis distalis. Cartilaginous trabeculae were already transformed into osseous trabeculae (↑). The column of cartilage tissue has been exhausted (×185). (Female, No. 40, Pr. 1-1083, 105 days of age, natural death, WL×BPR, 25/7/57.)

Figs. 13~17: Bone trabeculae in epiphysis. Basic columns of bone trabeculae.
Fig. 13. Longitudinal section through epiphysis distalis. A basic column (↑) of bone trabeculae (so called tentatively by the author) is seen in the central portion of the bone-marrow cavity. Cartilaginous trabeculae were mostly transformed into osseous trabeculae (×4.1). (Male, No. 22, Pr. 1-915, 121 days of age, natural death, hybrid of Barred Plymouth Rock male and Mikawa female (P. 1 generation), 3/4/56.)
Fig. 14. Longitudinal section through epiphysis distalis. Part of a basic column of trabeculae (left). Cartilaginous trabeculae are changing into osseous tissue. Osseous tissue surrounds the column (×185). (Female, No. 43, Pr. 1-936, 108 days of age, slaughtered, WL×BPR, 21/7/56.)
Fig. 15. Longitudinal section through epiphysis distalis. Relics of a basic column of trabeculae (left). Cartilaginous trabeculae surrounding the column has changed into osseous tissue (dark section on the right (×185). (Female, No. 45, Pr. 1-516, 120 days of age, slaughtered, Nagoya breed, 28/6/54.)
Fig. 16. Longitudinal section through epiphysis distalis. Basic column of bone trabeculae (left) almost exhausted. Cartilaginous trabeculae are changing into osseous tissue around the column (×50). (Female, No. 47, Pr. 1-1099, 121 days of age, slaughtered, BPR, 28/8/57.)
Fig. 17. Longitudinal section through epiphysis distalis. Basic column of bone trabeculae totally exhausted. Only bone tissue remains (×50). (Female, No. 49, Pr. 1-1102, 123 days of age, natural death, BPR, 29/8/57.)

Figs. 18~20: Abnormal bone.
Fig. 18. Cross section through diaphysis. Incomplete formation of bone trabeculae in the compact substance (upper right, ↑). Very primitive canal system is seen (×4.1) (Female, No. 34, Pr. 1-1069, 90 days of age, natural death, hybrid of Rhode Island Red male and Nagoya female (P. 1 generation), 20/6/57.)
Fig. 19. Longitudinal section through diaphysis. Abnormal hyperplasia of compact substance (top) and developing bone trabeculae (×4.1). (Female, No. 42, Pr. 1-935, 108 days of age, slaughtered, WL×BPR, 21/7/56.)
Fig. 20. Longitudinal section through epiphysis. Hyperplasia of abnormal periosteal bone tissue (×50). (Female, No. 60, Pr. 1-981, 183 days of age, slaughtered, WL×BPR, 2/10/56.)

PLATES IV~V (Figs. 21~26): Primary stage of newly-formed MB.
Fig. 21. Longitudinal section through diaphysis. Thin bone-tissue network (center) indicates the
primary stage of newly-formed MB (†). It is distinguished from thick bone trabeculae by the difference in eosin staining and by the absence of osteoblastic reticular tissue (×50). (Female, No. 63, Pr. 1–1115, 185 days of age, slaughtered, WL×BPR, 12/10/57.)

Fig. 22. Longitudinal section through diaphysis. MB (†) is covered with osteoblastic reticular tissue (×50). (Female, No. 79, Pr. 1–787, 225 days of age, slaughtered, WL×BPR, 14/12/55.)

Fig. 23. Longitudinal section through epiphysis. MB (†) (×50). (Female, No. 82, Pr. 1–793, 225 days of age, slaughtered, WL×BPR, 14/12/55.)

Fig. 24. Longitudinal section through diaphysis. MB (†). Osteoblastic reticular tissue somewhat obscure (×50). (Female, No. 32, Pr. 1–794, 226 days of age, slaughtered, WL×BPR, 15/12/55.)

Fig. 25. Longitudinal section through diaphysis. MB is thick on the side facing the bone-marrow cavity (†). It is covered with osteoblastic reticular tissue (×50). (Female, No. 84, Pr. 1–795, 226 days of age, slaughtered, WL×BPR, 15/12/55.)

Fig. 26. Cross-section through diaphysis. MB (†) (×185). (Female, No. 87, Pr. 1–798, 226 days of age, slaughtered, WL×BPR, 15/2/55.)

PLATES V~VII (Figs. 27~40): Completion of medullary bone.

Figs. 27~34: Network pattern of MB.

Fig. 27. Cross-section through diaphysis. MB is nearly as wide as the compact substance (×4.1). (Female, No. 123, Pr. 1–833, 253 days of age, slaughtered, WL×BPR, 2/1/56.)

Fig. 28. Portion of Fig. 27 (×365). The MB is presented as coarse network. Its periphery is generally sharp. Moderate development of osteoblastic reticular tissue. Compare with Fig. 27.

Fig. 29. Cross-section through diaphysis (×4.1). (Female, No. 183, Pr. 1–890, 282 days of age, slaughtered, WL×BPR, 9/11/56.) Compare with Fig. 30.

Fig. 30. Portion of Fig. 29 (×365). Coarse MB network with sharp periphery. No osteoblastic reticular tissue is seen. Compare with Fig. 29.

Fig. 31. Cross-section through diaphysis. Surface of MB facing bone marrow is uneven (×4.1). (Female, No. 224, Pr. 1–1052, 421 days of age, natural death, WL, 28/5/57.) Compare with Fig. 32.

Fig. 32. Portion of Fig. 31 (×365). Characteristic network is very coarse with sharp periphery. Compare with Fig. 31.

Fig. 33. Cross-section through diaphysis. Bone-marrow cavity is filled with MB. Note a fissure (×4.1). (Female, No. 268, Pr. 1–1128, 1699 days of age, slaughtered, WL×BPR, 21/12/57.)

Fig. 34. Cross-section through diaphysis. MB with its periphery sharply defined presents a characteristic network pattern (×185). (Female, No. 116, Pr. 1–826, 253 days of age, slaughtered, LW×BPR, 11/1/56.)

Fig. 35: Formation of MB fissure.

Fig. 35. Cross-section through diaphysis. Coarse network pattern is seen. Fissure appears after formation of the network (×185). (Female, No. 209, Pr. 1–623, 365 days of age, slaughtered, WL, 5/2/55.)

Figs. 36~38: Medullary bone showing a tendency to become eosinophilic.

Fig. 36. Cross-section through diaphysis. Network pattern is still visible only with fine strands (×4.1). (Female, No. 115, Pr. 1–998, 253 days of age, slaughtered, BPR, 11/12/56.) Compare with Fig. 37.

Fig. 37. Portion of Fig. 36 (×365). No characteristic network pattern is visible any longer. MB resembles compact substance. Compare with Fig. 36.

Fig. 38. Cross-section through diaphysis (×185). (Female, No. 107, Pr. 1–1104, 240 days of age, natural death, WL, 19/9/57.)

Figs. 39~40: Eosinophilic osseous mass.

Fig. 39. Longitudinal section through diaphysis proximalis. MB possesses a characteristic pattern. It is newly-formed secondary MB attached indirectly to the eosinophilic osseous mass rather than directly to the trabeculae (×50). (Female, No. 68, Pr. 1–977, 210 days of age, slaughtered, WL, 28/9/56.)

Fig. 40. Longitudinal section through diaphysis. Osseous mass of MB has become eosinophilic in
the bone-marrow cavity. This may probably be newly-formed primary MB (×50). (Female, No. 76, Pr. 1-782, 225 days of age, WL×BPR, 14/12/55.)

PLATES XII~XV (Figs. 41~83): Retrospection of medullary bone.

Figs. 41~51: Osteoclastic reticular tissue.

Fig. 41. Cross-section, through diaphysis. Osteoclastic activity (×4.1). (Female, No. 69, Pr. 1-978, 210 days of age, slaughtered, 29/9/56.) Compare with Fig. 42.

Fig. 42. Portion near the center of Fig. 41 (×365). Osteoclastic reticular tissue (↑) is around completed MB. Compare with Fig. 41.

Fig. 43. Magnified section of MB which has nearly filled the bone-marrow cavity. This section is from the surface facing the bone-marrow cavity. Proliferation has occurred in osteoclastic reticular tissue and osteoclasts (×185). (Female, No. 242, Pr. 1-1006, 657 days of age, slaughtered, WL×BPR, 22/1/57.)

Fig. 44. Cross-section through diaphysis (×4.1). (Female, No. 137, Pr. 1-847, 254 days of age, slaughtered, WL×BPR, 12/1/56.) Compare with Fig. 45.

Fig. 45. Portion of Fig. 44 (×365). MB network pattern and osteoclastic reticulata tissue (↑) are present. Compare with Fig. 44.

Fig. 46. Cross-section through diaphysis (×4.1). (Female, No. 162, Pr. 1-868, 281 days of age, slaughtered, WL×BPR, 8/2/56.) Compare with Fig. 47.

Fig. 47. Portion of Fig. 46 (×365). Proliferation of osteoclastic reticular tissue (↑). Compare with Fig. 46.

Fig. 48. Longitudinal section through diaphysis. Thin, fragile network (×185). (Female, No. 138, Pr. 1-848, 254 days of age, slaughtered, WL×BPR, 12/1/56.)

Fig. 49. Longitudinal section through epiphysis proximalis. Prominent osteoclastic reticular tissue containing many osteoclasts (↑) in it (×365). (Female, No. 138, Pr. 1-848, 254 days of age, slaughtered, WL×BPR, 12/1/56.)

Fig. 50. Cross-section through diaphysis. MB is 1.5 times as wide as the compact substance. It has become eosinophilic. Active osteoclastic reticular tissue is present on the surface facing the bone-marrow cavity. Osteoclasts are much reduced in activity. MB network has a blurred periphery (×50). (Female, No. 184, Pr. 1-891, 282 days of age, slaughtered, WL×BPR, 9/2/56.) Compare with Fig. 51.

Fig. 51. Part of Fig. 50 (×365). MB has become eosinophilic. Osteoclastic reticular tissue (↑) has clearly defined cells. Compare with Fig. 50.

Figs. 52~58: Osteoclasia.

Fig. 52. Cross-section through diaphysis (×4.1). (Female, No. 117, Pr. 1-827, 253 days of age, slaughtered, WL×BPR, 11/1/56.) Compare with Fig. 53.

Fig. 53. Part of Fig. 52 (×365). Many osteoclasts (↑) on the periphery of network. Compare with Fig. 52.

Fig. 54. Cross-section through diaphysis. Thin, fragile network (×4.1). (Female, No. 144, Pr. 1-854, 254 days of age, slaughtered, WL×BPR, 12/1/56.) Compare with Figs. 55 and 56.

Fig. 55. Part of Fig. 54 (×365). Many osteoclasts (↑) on the periphery of network. MB has become eosinophilic. Compare with Figs. 54 and 56.

Fig. 56. Part of Fig. 54 (×365). Osteoclastic reticular tissue and osteoclasts (↑). Compare with Figs. 54 and 55.

Fig. 57. Cross-section through diaphysis. Note linear arrangement of osteoclasts on the surface facing the bone-marrow cavity (↑) (×185). (Female, No. 121, Pr. 831, 253 days of age, slaughtered, WL×BPR, 11/1/56.)

Fig. 58. Longitudinal section through epiphysis distalis. Only small fragment of residual MB remains at the femoral epiphysis. Note osteoclasts (↑) and osteoclastic reticular tissue on the periphery of MB (center). Network is very distinct (×365). (Female, No. 111, Pr. 1-993, 248 days of age, slaughtered, WL×BPR, 6/12/56.)

Figs. 59~66: Thinning and fragmentation of medullary bone.

Fig. 59. Longitudinal section through diaphysis proximalis. Thinned and broken MB has almost completely filled the bone-marrow cavity. Osteoclasts are attached to each fragment of
MB. Dark, circular portion (↑) indicates MB which has not yet undergone thinning or fragmentation (×4.1). (Female, No. 237, Pr. 1–749, 515 days of age, natural death, WL, 7/9/55.)

Fig. 60. Cross-section through diaphysis (×4.1). (Female, No. 139, Pr. 1–849, 254 days of age, slaughtered, WL×BPR, 12/1/56.) Compare with Fig. 61.

Fig. 61. Portion of Fig. 60 (×365). Thin, fragile network. Many osteoclasts are attached to the periphery of network. Strong eosinophilic tendency. Compare with Fig. 60.

Fig. 62. Cross-section through diaphysis. MB is thinned and broken into fragments. Osteoclasts are seen on the periphery (×185). (Female, No. 234, Pr. 1–1074, 462 days of age, natural death, BPR, 8/7/57.)

Fig. 63. Cross-section through diaphysis. Thin, fragile network surrounded by osteoclastic reticular tissue and many osteoclasts (↓) (×185). (Female, No. 171, Pr. 1–877, 281 days of age, slaughtered, WL×BPR, 8/2/56.)

Fig. 64. Cross-section through diaphysis. Remarkably fragile network with many osteoclasts (↑) (×365). (Female, No. 198, Pr. 1–904, 324 days of age, natural death, WL, 23/2/56.)

Fig. 65. Longitudinal section through diaphysis. Many large osteoclasts (↑). Note fissure formation (×365). (Female, No. 207, Pr. 1–914, 361 days of age, slaughtered, BPR, 31/3/56.)

Fig. 66. Cross-section through diaphysis. MB network has been loosened and coarsened by thinning and fragmentation. MB has become eosinophilic (×365). (Female, No. 110, Pr. 1–992, 247 days of age, slaughtered, WL×BPR, 5/12/56.)

Figs. 67–74: Relics of medullary bone.

Fig. 67. Cross-section through diaphysis. Note small lumps of residual MB on the surface facing the bone-marrow cavity, compact substance, and periphery of trabeculae (×4.1). (Female, No. 104, Pr. 1–982, 229 days of age, slaughtered, WL, 6/10/56.) Compare with Fig. 68.

Fig. 68. Portion of Fig. 67 (×365). Residual MB tissue has become eosinophilic. Many osteoclasts are attached to it (↑). Compare with Fig. 67.

Fig. 69. Longitudinal section through diaphysis. Thin, thread-like substance is seen on the periphery of the compact substance. Bone trabeculae are residual. MB has become eosinophilic. Note osteoclasts (↑) (×50). (Female, No. 85, Pr. 1–796, 226 days of age, slaughtered, WL×BPR, 15/12/55.)

Fig. 70. Cross-section through diaphysis. Residual MB tissue (↑) is present on the periphery of the compact substance. It is floating in the bone-marrow cavity (×50). (Female, No. 258, Pr. 1–1127, 990 days of age, slaughtered, BPR, 21/12/57.) Compare with Fig. 71.

Fig. 71. Portion of Fig. 70 (×365). Note osteoclasts in osteoclastic reticular tissue on the periphery of small fragments of MB (↑). Compare with Fig. 70.

Fig. 72. Longitudinal section through diaphysis. Fragments of MB (↑) (×50). (Female, No. 105, Pr. 1–1124, 238 days of age, slaughtered, BPR, 21/12/57.)

Fig. 73. Longitudinal section through diaphysis. Fine fragments of MB (↑) with one or two osteoclasts (×185). (Female, No. 88, Pr. 1–799, 226 days of age, natural death, WL×BPR, 15/12/55.)

Fig. 74. Cross-section through diaphysis. Residual fragments of MB and osteoclasts (↑). Note a long, narrow giant osteoclast (×365). (Female, No. 229, Pr. 1–1065, 432 days of age, slaughtered, WL, 8/6/57.)

Figs. 75–79: First stage of retrogression of medullary bone.

Fig. 75. Cross-section through diaphysis. Some portions of the compact substance (extreme left) and MB in the first retrogressive stage (extreme right) are seen (×185). (Female, No. 93, Pr. 1–804, 226 days of age, slaughtered, WL×BPR, 15/12/55.)

Fig. 76. Cross-section through diaphysis. A portion which is attached to the compact substance indicates the first stage of retrogression (×185). (Female, No. 205, Pr. 1–1014, 344 days of age, natural death, WL×BPR, 12/3/57.)

Fig. 77. Longitudinal section through diaphysis. First stage of retrogression shown on the inner and outer surfaces (×50). (Female, No. 257, Pr. 1–1126, 990 days of age, slaughtered, BPR, 21/12/57.)

Fig. 78. Longitudinal section through diaphysis. Inner surface (facing the bone marrow) shows the
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first stage of retrogression (×50). (Female, No. 81, Pr. 1–792, 225 days of age, slaughtered, WL×BPR, 14/12/55.)

Fig. 79. Cross-section through diaphysis (×50). (Female, No. 86, Pr. 1–797, 226 days of age, slaughtered, WL×BPR, 15/12/55.)

Figs. 80–85: Eosinophilic osseous mass.

Fig. 80. Longitudinal section through diaphysis. Eosinophilic osseous masses of MB (†) (×4.1). (Female, No. 135, Pr. 1–945, 254 days of age, slaughtered, WL×BPR, 12/1/56.)

Fig. 81. Longitudinal section through epiphysis distalis (×4.1). (Female, No. 148, Pr. 1–858, 254 days of age, slaughtered, WL×BPR, 12/1/56.)

Fig. 82. Longitudinal section through epiphysis distalis. Note eosinophilic osseous masses (center) (×50). (Female, No. 167, Pr. 1–873, 281 days of age, slaughtered, WL×BPR, 8/2/56.)

Fig. 83. Longitudinal section through diaphysis. Note eosinophilic masses (center) (×50). (Female, No. 188, Pr. 1–895, 282 days of age, slaughtered, WL×BPR, 9/2/56.)

Fig. 84. Longitudinal section through epiphysis distalis. Residual MB around an eosinophilic mass (×50). (Female, No. 229, Pr. 1–1065, 432 days of age, slaughtered, WL, 8/6/57.)

Fig. 85. Longitudinal section through epiphysis proximalis. Eosinophilic MB in the bone-marrow cavity (×185). (Female, No. 238, Pr. 1–357, 520 days of age, slaughtered, BPR, 18/8/52.)

PLATE XV (Figs. 86–90): Newly-formed secondary medullary bone.

Fig. 86. Longitudinal section through diaphysis. MB has filled nearly all the bone-marrow cavity. Areas facing the bone-marrow cavity exhibit a strikingly characteristic network pattern (newly-formed secondary MB) (×4.1). (Female, No. 241, Pr. 1–1000, 624 days of age, natural death, BPR, 20/12/56.)

Fig. 87. Longitudinal section through diaphysis. Residual MB and newly-formed secondary MB (†) (×50). (Female, No. 64, Pr. 1–1116, 185 days of age, slaughtered, WL×BPR, 12/10/57.) Compare with Fig. 88.

Fig. 88. Longitudinal section through epiphysis. Newly-formed secondary MB. Apposition of osteoid tissue and osteoblastic layer. Some portion of this specimen which is not shown in this figure contained a trace of MB relics. In other parts, MB has been changed into eosinophilic osseous mass (×185). (Female, No. 64, Pr. 1–1116, 185 days of age, slaughtered, WL×BPR, 12/10/57.) Compare with Fig. 87.

Fig. 89. Longitudinal section through epiphysis. Eosinophilic MB (dark) is attached to trabeculae (broad white strip). MB has been reformed on the outer edge of this eosinophilic bone (narrow, white fringe) (×50). (Female, No. 174, Pr. 1–880, 281 days of age, slaughtered, WL×BPR, 8/2/56.) Compare with Fig. 90.

Fig. 90. Portion of Fig. 89 (×365). Bone trabeculae (left) and MB (center). Compare with Fig. 89.