BONE MARROW AS A PRACTICAL CLINICAL DIAGNOSTIC AGENT IN CANINE PRACTICE

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BONE MARROW AS A PRACTICAL CLINICAL DIAGNOSTIC AGENT IN CANINE PRACTICE

By

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A THESIS

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ABSTRACT

The principal problem in this investigation was to find a site in the body of the dog which could furnish marrow substance of good quality and in adequate amount to make bone marrow biopsy a practical clinical procedure which could be used as an aid in diagnosis.

Reports of marrow biopsies in dogs were very scant. The procedures described were copied from the human field: puncture of sternum, iliac crest and dorsal processes of vertebrae. The author presented reasons why these sites were unacceptable. The small size of the bones, together with their anatomical position--being relatively deeply situated and well covered--made them extremely difficult to approach and to use as a practical clinical procedure.

In the study of the topography of whole bone marrow sections another problem was encountered. After fixation of a marrow pencil the myeloid substance of the dog becomes very crumbly. To obtain a marrow specimen which would furnish entire cross and longitudinal sections it was decided to process the whole marrow pencil intact up to and including the paraffin bath.

The modus operandi of obtaining marrow from the bone by suction also had to be modified from the procedure used in human medicine. Here again size and shape of the instruments were not directly applicable to use in canine practice. A biopsy cannula was designed and made to fit the specific requirements demanded by the smallness of the marrow cavity. The author introduced a curved biopsy cannula which could be entered into the lumen of the bone for a limited distance.

Femoral puncture has been proposed as a preferred practical clinical procedure for doing bone marrow biopsies on the dog. It involves a minor operation capable of being performed either under local or general anesthesia. The surgery is absolutely safe: there is no danger of penetrating a body cavity. The proximal portion of the femur is a reliable source of good quality marrow inasmuch as it responds very early to any stimuli in the adult.

The locus of the biopsy is easily accessible and causes a minimum of discomfort to the patient.

Normal bone marrow has been described grossly and microscopically. A scheme outlining the gross and microscopic appraisal of myeloid tissue has been presented.

Frank Thorp

It has been shown that qualitative bone marrow findings were often invaluable when correlated with those of peripheral blood; in fact, bone marrow findings may furnish the only clew on which to base a diagnosis.

The photomicrographs were taken by the author of specimens which were used as a part of this investigation.

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INTRODUCTION

A correct diagnosis is the most important aspect of the practice of veterinary medicine. Obviously it would be only a remote coincidence if the therapy prescribed were to help the sick patient to recover if the diagnosis had been incorrect. Twenty years in the practice of veterinary medicine have shown that far too many mistakes are being made in individual private practice. However, the records of large clinics, human as well as veterinary, reveal that they too have erred in their diagnoses altogether too frequently. The inference of the foregoing is: a diagnosis is less likely to be incorrect when four to eight doctors examine a patient than when the entire examination is carried out by one man.

Any clinical procedure therefore which will aid in arriving at more correct diagnoses and/or point to the origin and nature of the patient's ailment at the earliest possible moment is a contribution to, and a step forward in the practice of veterinary medicine.

It was thought that examination of bone marrow could be used clinically to this end. It is not intended here to give the impression that the study of bone marrow is a new idea in veterinary medicine;

instead, the object was to find a practical method of bone marrow biopsy which could be used as a routine clinical procedure.

The literature contained very little information on practical clinical marrow biopsy procedures as applied to small animal practice. Indeed, nearly all of the material reported on has been from dogs which had been used in some other experimental work in the human field and not from apparently healthy animals. The questions asked were:

1. What is the pattern of bone marrow of healthy dogs?

2. What is the differential picture of a bone marrow biopsy smear of presumably normal dogs?

3. Where can biopsy material be obtained if this is to be a routine clinical procedure?

4. Where is the site most representative of bone marrow activity in health as well as in disease?

5. What are some of the diseases in which bone marrow may give a clue or even be the only tissue to give an answer?

6. Are bone marrow biopsies dangerous to the patient if properly executed?

7. In which branch of veterinary medicine does bone marrow examination belong?

The answers to the above questions will be discussed in the following sections, except the last question, which properly should be discussed here.

The science dealing with the study of blood is known as hematology. The thought occurred that the study of myeloid tissue, which is bone marrow, might appropriately be called myelology. These two sciences have much in common and may therefore be studied by the same group of medical men.

Karl Rohr, in his book "Das Menschliche Knochenmark," expresses the opinion that the pathologic anatomist might think it arrogant for the clinician to have made the study of marrow-pathology the latter's problem. Contrary to this belief, it may be stated that the study of bone marrow as viewed here properly belongs in clinical pathology.

Hematology is as old as medicine itself. In fact, no authentic record has been found which would claim when blood was first used as a diagnostic agent. On the other hand, the study of bone marrow as a diagnostic agent is relatively recent. What has been the reason for the delay? It was shown by Neumann (1869) that bone marrow is the sole source of red blood cells of the normal adult individual. This pronouncement has never been successfully

challenged. It has also been shown by numerous other investigators that granulocytes are produced exclusively by bone marrow under normal conditions. Bone marrow is also capable of giving rise to lymphocytes and cells of the reticulo-endothelial system. Thus it may be said that bone marrow is the most important hemopoietic organ of the normal adult.

Evidently the reason for the delay of the use of bone marrow as a diagnostic agent lay in that no practical way had been found as yet to use it clinically. The development of marrow biopsy will be presented in the historical review of this thesis.

Bone marrow biopsies have furnished not only information about the progress of disease, its prognosis and response to treatment, but also have given some important clues of other functions. Prior to this time marrow functions were based on deductions made from peripheral blood pictures, embryological and experimental research conducted on laboratory animals.

In comparing blood and bone marrow it will become apparent that blood is merely a vehicle for products of metabolism and some formed elements, and does not produce anything, while bone marrow is an active manufacturing center. One must not, however, minimize the importance of blood. One only needs to recall the words of the

famous German poet, Johann Wolfgang von Goethe, who said, "Blut ist ein besonderer Saft."

No satisfactory substitute for blood has been found as yet. To assure the greatest success in arriving at a correct diagnosis the clinical pathologist should always be mindful not to divorce pathologic anatomy, hematology, and bone marrow study, but evaluate the findings comparatively.

Cytogenesis will be discussed only briefly because the viewpoints of investigators are still too divergent. Furthermore, it is questionable whether the mode of genesis of the cells per se is as important from the standpoint of diagnosis of disease as it is academically.

HISTORICAL REVIEW

During the first part of the nineteenth century students and research workers in the field of biological sciences thought that the usefulness of bone marrow appeared to be ended with the part it played in the development and growth of the skeleton, except for a few minor functions such as to reduce the weight of bones, decrease their brittleness, and act as a shock-absorber or cushion for intramedullary vessels.

That vital functions of bone marrow began to be appreciated and can be realized from the writing of Koelliker in Germany and Beale and Wharton Jones in England about the year 1846 as quoted by Neumann (1869).

Neumann (1868, 1869) of Germany was the first to show that bone marrow was the only place in the body of normal adult animals where red blood cells were produced. He also recognized the nucleated, granular variety of blood cells originating in this newly discovered hemopoietic organ. Almost simultaneously and entirely independently came the announcement of bone marrow as a hemopoietic center by Bizzozero of Italy (1868). His findings were almost identical with those of Neumann.

The introduction of special staining methods in hematology by Ehrlich about 1882 gave that science the impetus it needed to come into its own. The newly discovered staining methods were applied to bone marrow studies, and much work was done in various parts of Europe.

Pianese (1903) punctured the lower femur to prove a diagnosis of a parasitic disease. The same year Wolff (1903) in Germany recommended tibial puncture of experimental animals. Ghedini (1908) described in detail trephining of the proximal third of the tibia for bone marrow. Twenty-six cases convinced him of the diagnostic value of this new procedure and showed the difference between the composition of blood and bone marrow.

This work received very little attention outside of Italy. Three reasons were given for this disinterest:

1. A knowledge of dependable differential blood morphology was still lacking.

2. Quality of tibial marrow was very variable and uncertain.

3. Trephination was too much of a surgical procedure.

About fifteen years elapsed before any important biopsy procedure was reported. Seyfarth (1923) was the first one to do sternal and costal trephination on living human patients. Since this was a

surgical procedure like that of Ghedini's it was not generally accepted. However, Rohr (1949) stated it was used by Schilling in Germany, Dameshek, Custer, and Peabody in the United States of America.

It was Arinkin (1929) who introduced a simple and practical method for doing marrow biopsies on living persons.

Many technical improvements of biopsy instruments, methods, and sites followed in the next few years.

The first reports of marrow biopsies in the United States of America appeared about 1933 (Rohr, 1949).

The use of bone marrow biopsy is now a firmly established, standard procedure in most of the modern hospitals and clinics of the world.

MATERIAL AND METHODS

Distribution of Bone Marrow

The preliminary work involved the investigation of the normal distribution of myeloid tissue in the skeleton of the dog. For this purpose use was made of necropsy material as it came to the post-mortem room. Dogs of small to medium size were selected because the purpose of this work was to find a practical biopsy procedure capable of being used in routine small animal practice.

It was learned that thoracic and lumbar vertebrae, sternum, iliac crest, ribs, and diploes contained only red marrow. The long bones contained a widely variable mixture of red and yellow marrow. The proximal end of the tibia was rich in red marrow in dogs less than six to nine months old. After one year of age one would find a considerable admixture of yellow and red marrow. The femur and humerus have an ample supply of red marrow in the proximal portions and it persists in adequate quantity even in advancing age. As the animals advance in age a peculiar distribution of red marrow was noted: red marrow was more noticeable at the periphery of the marrow pencil throughout the length of these bones. However,

there was a greater density of red marrow at the proximal end of the femur and humerus.

The marrow cavity of the ribs was so small that it was not considered further as a biopsy site.

Vertebrae, sternum, and tuber coxae do not have a marrow cavity per se. The interior of these bones is entirely cancellous. The minute spaces between the relatively thick cancelli are filled with red marrow.

The Normal Bone Marrow Picture

Ten dogs were used to obtain entire cross and longitudinal sections of bone marrow in order to establish the normal topography and differential picture of myeloid substance. The ages of the dogs varied from seven months to twelve years. There were five males and five females in this group. The breeds included some pure-breds as well as mixed breeds. Sizes of dogs varied from small to medium. All animals were in good physical condition; there was no history of any illness for the past several weeks, and up to several years in some instances. See Table I.

A thorough physical examination was given each animal at the time of acquisition. The dogs were then held for a ten-day observation

TABLE I

SIGNALMENT OF DOGS USED

Case	Age	Sex	Breed	Weight
I	12 years	female	Fox Terrier	15 lbs.
II	7 months	female	German Shepherd (mixed)	25 lbs.
III	4 years	female	Cocker Spaniel	40 lbs.
IV	9 months	male	Terrier (mixed)	18 lbs.
V	$2\frac{1}{2}$ years	female	Collie	43 lbs.
VI	1 year	male	Collie & Shepherd	40 lbs.
VII	1 year	male	Collie & Shepherd	52 lbs.
VIII	6 years	male	Terrier (mixed)	40 lbs.
IX	7 months	male	Terrier (mixed)	18 lbs.
X	11 years	female	Terrier (mixed)	12 lbs.

period. Following this a final checkup was made which usually required several days. This included the following:

Fecal examination for parasites

Urine analysis

Hemoglobin determination

Red blood cell count

White blood cell count

Differential blood cell count

Nonprotein nitrogen determination

Creatinine determination

Blood sugar determination

The results are tabulated in Table II.

Bone marrow biopsy for differential count was performed under complete nembutal anesthesia. The animals were then sacrificed while still under complete anesthesia by inhalation of chloroform. Whole bone marrow substance was obtained immediately following death and fixed in a solution consisting of 15 per cent formalin, 1 per cent acetic acid, and 5 per cent zinc chloride. After twenty-four hours in the above solution the specimen was dehydrated and embedded in tissue matt.[1] The mounted sections were stained with eosin and hematoxylin.

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[1] Fisher Scientific Company, New York, New York.

TABLE II

CLINICAL TESTS OF TEN DOGS

Test	Case Number			
	I	II	III	IV
Blood count:				
Differential:[1]				
Neutrophils	80.0	59.0	61.0	79.0
Lymphocytes	14.0	33.0	34.0	17.0
Monocytes	4.0	2.0	2.0	4.0
Eosinophils	2.0	6.0	3.0	0.0
Basophils	0.0	0.0	0.0	0.0
W.B.C.[2]	15.6	13.05	16.05	11.80
R.B.C.[3]	5.40	5.30	4.70	5.90
Hemoglobin[4]	107.0	87.7	87.7	83.0
Blood chemistry:				
NPN[5]	42.7	37.5	34.5	41.2
Creatinine[6]	1.20	0.70	0.60	0.70
Sugar[7]	67.0	65.0	63.0	72.0

[1] Numbers indicate per cent of total number of cells counted.

[2] Numbers indicate thousands/mm^3 White Blood Cells.

[3] Numbers indicate millions/mm^3 Red Blood Cells.

[4] Numbers indicate per cent of normal. Hemoglobin of 14.5 mg per 100 ml of blood = 100 per cent of normal.

[5] Numbers indicate mg % Nonprotein Nitrogen.

[6] Numbers indicate mg %

[7] Numbers indicate mg %

TABLE II (Continued)

Case Number						Average
V	VI	VII	VIII	IX	X	
59.0	84.0	50.0	76.0	82.0	79.0	70.9
13.0	10.0	25.0	15.0	10.0	19.0	19.0
8.0	2.0	10.0	5.0	5.0	2.0	4.40
20.0	4.0	15.0	4.0	3.0	0.0	5.7
0.0	0.0	0.0	0.0	0.0	0.0	0.0
9.55	9.15	6.70	9.40	13.5	7.25	11.20
5.40	5.60	5.30	5.90	5.30	5.46	5.42
76.0	97.0	91.0	103.0	90.7	97.0	92.01
39.0	33.7	39.0	45.0	42.7	47.2	40.3
0.70	1.50	1.50	1.60	0.70	1.10	1.03
67.0	74.0	60.0	70.0	80.0	72.0	69.0

Technic for Fixing Bone Marrow

In the study of bone marrow of the dog as a hemopoietic organ the conclusion was reached that the humerus and the femur play an important role. Sternum, dorsal processes of thoracic and lumbar vertebrae, and the iliac crest do not appear a satisfactory source for obtaining bone marrow as a biopsy procedure. In the experimental laboratory one usually finds dogs of medium size or often of a large medium size. However, in the practice of small animal medicine the small breeds numerically predominate. It is very difficult, if not impossible, to do satisfactory and practical bone marrow biopsies in the sites last mentioned.

Advances in methods of reduction of fractures of the long bones have given much valuable information as to procedures which may be used in performing biopsies involving bone marrow.

Obviously the tibia of the dog will be brought to one's mind because of its ease of accessibility for bone marrow biopsy. Unfortunately tibial marrow is just as unsatisfactory in the dog as it is in the human being except in the very young or in those cases where there is extraordinary hemopoietic activity. In dogs just past one year old red marrow in the tibia is prominent by its paucity.

If necropsies are performed, bone marrow for imprints, smears, or small sections can be obtained by squeezing marrow from a rib with a suitable plier, and from the femur or humerus with a curette.

To study entire cross sections as well as longitudinal specimens one should follow a different technique. It was learned early that bone marrow of the long bones of dogs cannot be extruded from its bed intact as is possible in some laboratory animals; e.g., the rabbit (Steinberg and Martin, 1946). Many bone papillary projections extend from the wall of the lumen of the marrow cavity. Furthermore, the marrow pencil is delicate and becomes very crumbly after fixation. To obtain entire and intact sections of marrow of any portion of the long bones the author used the following procedure, which is a modification of a method described by Mayer et al. (1945) for staining femoral marrow of rats and guinea pigs:

Cut the long bone transversely at both metaphyses. Hold the diaphysis in a small bench vise with the cut ends against the jaws of the vise. Then proceed to cut a groove or slit lengthwise from one extreme end to within about one-eighth to three-sixteenths inch of the opposite end just through the cortex of the bone. Do the same about one-fourth around the circumference of the bone. Follow this

by carrying out the same procedure on the opposite side of the bone. Now cut transversely through the cortex of the bone at the end and between the two slits on each side. The author used a Dremel Moto-tool No. 2[1] with a steel cutting rotary saw, one inch in diameter and about 0.025 inch thick. It is now possible to lift the portion of bony cortex away from the marrow. Two small periostum-lifters are very useful for this. As the portion of bone to be removed is raised gently, inspect the junction of cortex and marrow, and free any portions of marrow adhering to wall of lumen of bone. The marrow pencil is now held between the two remaining strips of cortex, the latter being held firmly in a normal position by the annular ring of bone allowed to remain (Figures 1 and 2). The entire specimen can now be fixed and embedded in paraffin. In cases where the marrow substance is more than four millimeters in diameter, additional slits may be cut through the cortex of bone to facilitate penetration of fixing and embedding solutions. The remaining portions of bone can now be removed carefully and the marrow will be held intact by the paraffin. This latter step is very important, as otherwise the marrow pencil may fall apart. Portions for embedding in preparation for sectioning can now be selected from the marrow pencil.

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[1] Dremel Manufacturing Co., 14th and Clark Streets, Racine, Wisconsin.

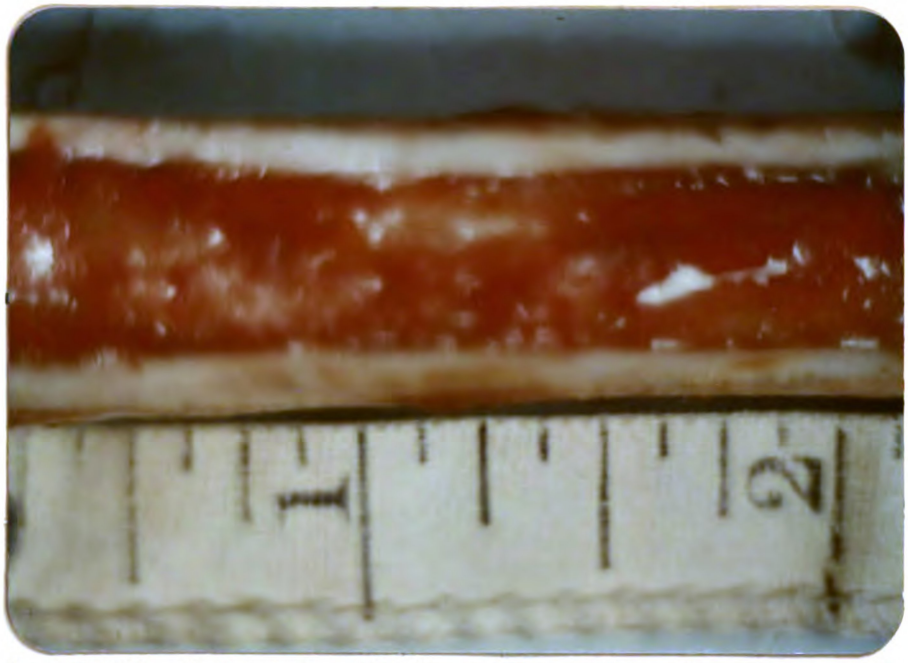

Figure 1. Shaft of femur with a portion of cortex removed showing marrow intact before fixation. The light and dark areas are indicative of the variation of the thickness of the peripheral layer of red marrow. The central portion is all fatty marrow. X $2\frac{1}{2}$. Compare with Figure 2, below.

Figure 2. Shaft of femur with a portion of cortex removed showing marrow intact after fixation. X $2\frac{1}{2}$. Compare with Figure 1, above.

Sections of five microns thickness are preferred for topographical studies as well as the determination of the myeloid-erythroid ratio. The latter will be dealt with in the section under "Discussion."

The regressive staining method with hematoxylin and eosin was used throughout this work on paraffin sections. It was found that more satisfactory differentiated staining for making the myeloid-erythroid ratio count was obtained if the sections were definitely overstained with hematoxylin, and then decolorized to a point where the leukopoietic cells appeared decidedly vesicular. At this stage of decolorization the cells of the erythropoietic system still retain much of the basic stain. It seems that only the chromatin structure of the white cells stains with hematoxylin. Parachromatin, specific granules, and azurophil granules either do not take this basic stain at all or do not retain it. The author noticed that if the staining with eosin was allowed to remain more intense than usual the contrast was enhanced still further. A Whipple disc was used to indicate the area being counted; it also served as a measuring device. The nuclei of the normoblasts in sections measure two to four microns in diameter and stain so intensely that no structure within them can be discerned. Often a small, lighter, bluish staining portion of the cytoplasm was visible.

A New Biopsy Procedure

The human clinical pathologist obtains marrow for biopsy from the sternum, iliac crest, and dorsal processes of vertebrae of adults and older children. In infants the tibia is used also because the marrow of this long bone is still sufficiently active to be of value in obtaining a reasonably reliable myelogram.

Veterinary literature contains very few accounts of biopsy procedures in dogs. Bloom (1945) stated that sternum and iliac crest have been used to obtain marrow by needle-puncture method. Horn et al. (1953) in Germany described sternal puncture in the dog and claimed to have obtained marrow specimens which produced good smears for satisfactory myelograms.

At the outset of this investigation the task of finding the best location for doing bone marrow biopsies was pursued. Since the object of this work was to find a way to use bone marrow as a practical clinical diagnostic agent, dogs of small, medium, and medium large size were examined for favorable biopsy sites. The workers previously mentioned do not state the size of dogs used. In this work no dogs of the so-called large breeds were used because relatively few of these animals are seen in the average small animal practice.

As can be seen from Figures 3, 4, and 5, there is a paucity of marrow in sternal, iliac, and vertebral bones. To this must be added the difficulty of executing punctures of these small and well-covered bones.

The femur has presented the best quality of marrow, and can be made accessible without too great a discomfort to the patient. The most advantageous site on the femur is the lateral surface of the junction of the proximal and middle thirds.

Biopsies have been done during this investigation under local anesthesia without apparent pain to the patient. The only time that any signs of discomfort were noted was when suction was applied to draw marrow substance into the syringe. The latter sensation is experienced by human patients also according to information obtained from human clinicians.

General anesthesia is such a simple and safe procedure now that it is recommended in all cases unless contraindicated. In nervous and apprehensive animals general anesthesia is to be preferred.

Description of the Biopsy Procedure

For local anesthesia, codrenin[1] has been used.

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[1] Parke, Davis & Company, Jos. Campau Ave. at the River, Detroit 32, Michigan.

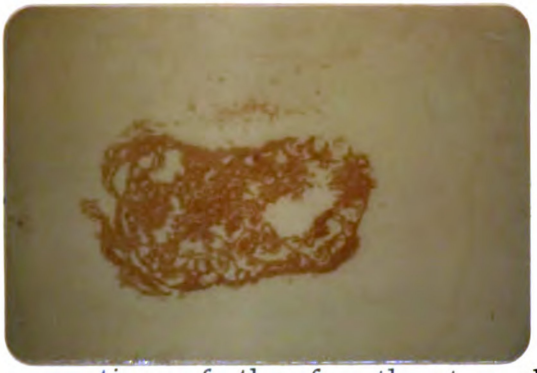

Figure 3. A cross section of the fourth sternal segment from a twenty-pound dog. The entire bone is finely cancellous and well covered. The larger vacuoles are artifacts. X $4\frac{1}{2}$.

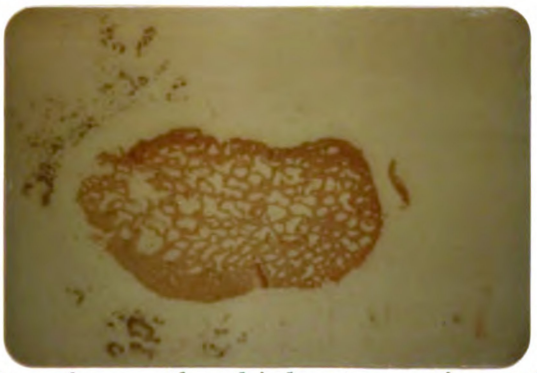

Figure 4. A section from the highest portion of the wing of the ilium (crest) from a twenty-pound dog. The part is entirely cancellous. The cancelli are relatively hard and thick. X $4\frac{1}{2}$.

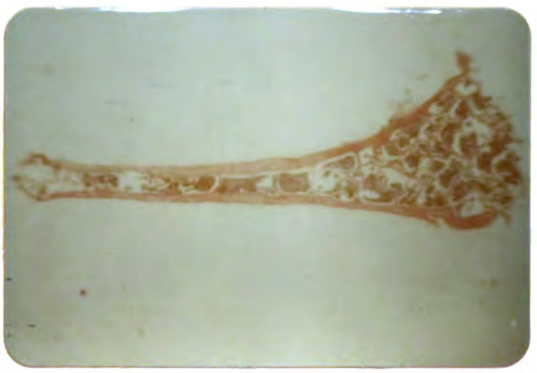

Figure 5. A section of a dorsal process of a thoracic vertebra from a twenty-pound dog. The portion where marrow puncture would have to be made is one millimeter thick. X $4\frac{1}{2}$.

Pentathol sodium or some of the other short-term general anesthetic substances were used by the intravenous route.

The lateral surface of the hind leg was clipped very closely with a No. 40 Oster clipper blade from the knee to just above the trochanter major. The clipped area was then scrubbed with germicidal detergent.[1] The detergent was removed with water and sponge. Ether was applied, followed by 70 per cent alcohol with a final application of tinted phemerol.[2] The field of operation was then draped with a sterile shroud provided with a slit to permit making an incision through the skin over the femur at the junction of its proximal and middle thirds. The skin incision was generally about one and one-half inches long for a medium-size dog. After passing through skin, subcutaneous fascia, and fat, the tensor fascia latae and biceps femoris muscles were severed. The posterior portion of the vastus lateralis was now in plain sight. No incision was made into this muscle. It covers about two-thirds of the anterior portion of the lateral surface of the shaft of the femur. By spreading the previously incised muscles somewhat and then retracting the vastus lateralis

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[1] Ibid.

[2] Ibid.

cephalically the whitish glistening surface of the femur comes into plain view. A specially designed spreader (Figure 6) has given satisfactory results for dilating the opening. The work can be done without an assistant if necessary. A hole one-sixteenth or three thirty-seconds inch in diameter was drilled through the cortex of the bone. A safety stop (Figure 6) prevented the drill from plunging into the marrow substance. At the start of the drilling operation caution must be taken to avoid letting the drill slide off to the sides of the bone into soft tissues. A closely fitting cannula can be inserted into the previously drilled hole, and with the aid of a conventional-type syringe marrow may be aspirated. Inasmuch as the marrow cavity of smaller breeds of dogs is very small, biopsy with the usual straight needle or cannula has not given the desired results in some instances. It has been found that bending a hypodermic needle so that the included angle was about 120 degrees will permit the passage of the biopsy needle through the previously drilled hole. The needle was then thrust into the marrow substance for a distance of about one-fourth inch in a direction longitudinal to the lumen of the bone and the aspiration was made.

If the bending was made over a round shaft about three-eighths inch in diameter the curvature was not too abrupt, and allowed insertion

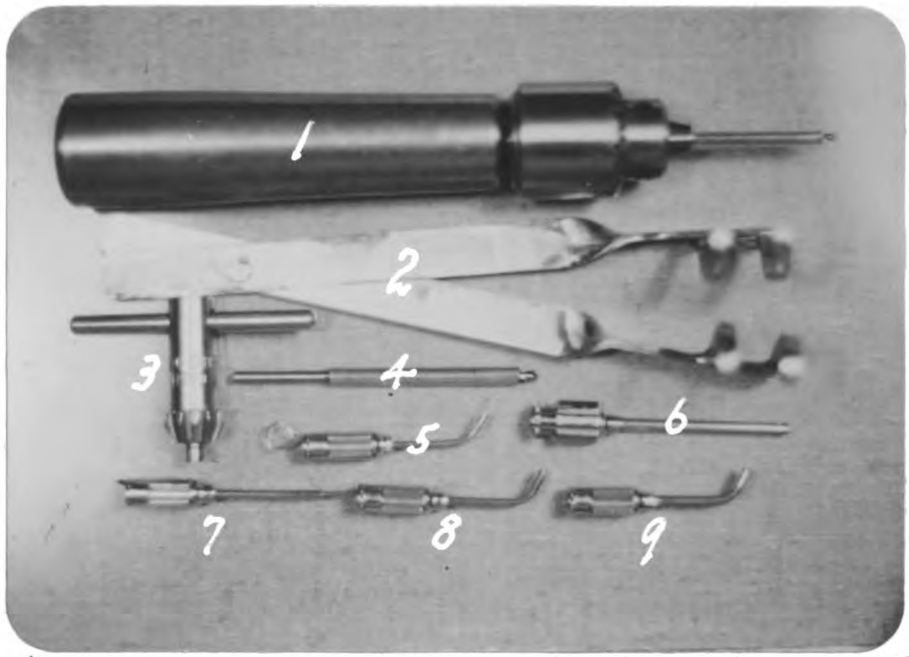

Figure 6. Instruments used in performing femoral marrow biopsies: (1) drill chuck with drill and safety stop in place; (2) retractor; (3) drill chuck wrench; (4) drill with safety stop; (5, 6, 7, 8, 9) aspirating needles. Approx. X 2/3.

and withdrawal of a stilet. The free end of the biopsy needle or cannula should be honed and polished smoothly to prevent tearing the stroma and blood vessels of the bone marrow. To have a little blood mixed with the marrow substance is not altogether undesirable. The blood serum appears to free the cells from the matrix of the myeloid tissue, giving a better smear. If there was no bleeding--and frequently there was not--a drop or two of plasma or serum on a watch glass was mixed with the aspirated marrow (Isaacs, 1937). From this mixture a smear was made exactly as in the case of peripheral blood films. After air drying, the slides were stained with Wright's stain.

The following size aspirating needles should be available: fourteen, sixteen, and eighteen gauge.

All instruments were sterilized by autoclaving.

The wound was closed with interrupted sutures using No. 000 plain catgut to approximate the muscle tissue, whereas cytor[1] was used on the skin.

The most surprising observation following the femoral marrow biopsy as described here was that the animals did not go lame or

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[1] Bauer & Black, Division of The Kendall Company, Chicago, Illinois.

show any signs of discomfort. There was little swelling, and healing was by first intention in all cases.

Other methods of processing biopsy material preparatory to making smears and stains:

The method of adding a little homologous serum to the marrow aspirate as suggested by Isaacs (1937) prompted the author to try commercial homologous serum (canine). The red cells were crenated, and the staining quality was poor. Homologous serum is readily available in veterinary hospitals, and would have simplified the biopsy procedure further. It is presumed that the preservative in the commercial product was responsible for the poor results.

Physiological saline solution was tried as a diluent. This resulted in smears in which the nuclei were much more distinct than in any other method used, but most cytoplasms became very fragile. Nuclear pattern is the most important single factor in the differentiation of blood and marrow cells. Some modification of a saline solution might have some merit.

Addition of anticoagulants to the marrow aspirate also resulted in inferior staining quality of the cells.

The better-stained smears were obtained from direct smears of the orange-red liquid portion of the aspirate before coagulation, or by adding a little autogenous serum or plasma.

It is advisable to make six to eight smears. One has a better choice of selecting a superior quality smear as well as having prepared slides available if special staining procedures should be desired. Marrow smears were made like blood smears, air dried, and stained with Wright's stain as soon as they were dry. Staining time was two minutes with stock solution to cover the slide and four minutes more after addition of an equal volume of distilled water. A new batch of stain should be timed until satisfactory staining quality is obtained. The slides were rinsed with tap water while in a horizontal position and rapidly air dried at room temperature.

ANATOMY AND HISTOLOGY OF BONE MARROW

Early Development

Before proceeding with a more detailed description of the various types of marrow it is well to discuss briefly the three periods through which the development of the hemopoietic organs pass in the ontogenesis of mammals.

The mesoblastic period is initiated by differentiation of the mesenchyme from mesodern in the wall of the yolk sac of the embryo in the first few days of development of the individual. The so-called blood islands spread to other extraembryonic tissues. A primitive blood vascular system is built to supply a rapidly expanding embryo with blood. Beginning about the second month of life in the human embryo the hepatic period begins with formation of blood cells in the liver. The medullary period starts approximately the fifth month of prenatal human life (Neumann, 1869). The common origin of bone and bone marrow lies in the deeper layers of periosteum and perichondrium, respectively (Maximow, 1910).

In postnatal life bone marrow is the only source of blood cells in the mature and normal individual, except such cells as are derived from lymphoid tissue (Neumann, 1869).

It is to be noted that the perivascular mesenchyme is ubiquitous in distribution and has retained its primitive potencies of hemopoiesis (Best and Taylor, 1950).

Of the five kinds of bone marrow which differ macroscopically and microscopically (Jackson, 1904), the two most important varieties are the red and the yellow or fatty bone marrow Only the red marrow plays a role in hemopiesis, producing the red blood cells and leukocytes. In the embryo and newborn, red marrow only is found in the bone cavities. With progressing age red marrow in the long bones is gradually replaced by yellow marrow with its fat cells. In the normal adult, red marrow is found in the vertebrae, the ribs, the sternum, the diploes of the bones of the skull, and in the proximal epiphyses of the femur and humerus. In the dog red marrow is present at the periphery of the marrow pencil of the last-named bones. Bone marrow forms 2 or 3 per cent of the body weight (Wetzel, 1920; Fairman and Whipple, 1933).

The Five Different Types of Marrow

1. Embryonal marrow. This consists of red marrow only but differs from adult red marrow as will be subsequently shown. The latter is also known as lymphoid marrow (Neumann, 1869).

Waldeyer (1865) stated that this primary marrow substance belongs to a group of connective tissues of a more undifferentiated character which may develop in various directions, and may assume a permanent morphology when required. It consists of spindle-shaped and stellate cells which connect freely with each other through long processes. The protoplasm has been changed to a very fine fibrillar intercellular substance which is particularly prominent near embedded blood vessels. The entire structure is permeated with an albuminous and mucous liquid. He claimed to have observed a similar embryonal connective tissue in the deeper layers of primitive periosteum which later changed to bone and bone marrow. This occurs at the onset of bone formation in the embryo; more specifically at the beginning of ossification.

2. Lymphoid or red marrow. The reticular structure is much less prominent while the leukopoietic and erythropoietic cells predominate. Normally myelocytes, metamyelocytes, band cells, and polymorphonuclear cells make up the bulk of the cellular elements, decreasing in number in the order named. Myeloblasts are rare and progranulocytes are present in only moderate numbers. The ratio of erythroid to granulocytic elements is approximately one to three. Another cell type seen regularly in normal red marrow is the megakaryocyte. Some fat cells are always present. The ratio of fat to

lymphoid cells varies greatly in different animals and at different times in the same animal, depending on the physiological activities.

The principal method of replacement of the myeloid elements of the normal blood is by homoplasia. Under unusual conditions more primitive structures may be stimulated to activity. When this occurs heteroplasia aids in hematopoiesis.

3. Fibroid marrow. This type of marrow is characterized by the presence of a fibrillar tissue in the medullary spaces and the larger Haversian canals of the long bones. It is particularly noticeable in the terminal phalanges of man. Generally speaking it resembles ordinary connective tissue.

4. Fatty or yellow marrow. No sharp line of demarcation can be drawn between red and yellow marrow. In this tissue lymphoid marrow cells are prominent by their paucity. The proportion of fat cells to lymphoid cells is reversed from that in red marrow. The amount of yellow marrow in the body varies inversely as the hematopoietic activity. Neumann (1869) observed that the fat cells in the long bones recede from the proximal to the distal end, and from the periphery to the center of the marrow pencil during increased hematopoiesis. This process is reversed during decreasing

hematopoietic activity. This change is known as Neumann's law (Neumann, 1882).

The interstices between the fat cells are taken up by a few lymphoid cells, blood capillaries and reticular cells and fibers. The general appearance of this tissue on casual inspection microscopically reminds one of lung tissue where the respiratory alveoli are occupied by fat cells and the interalveolar spaces are the interstices described above. Macroscopically it is of yellowish color, and greasy to the touch (Figures 7 and 8).

5. Gelatinous marrow. In the long bones of starving animals or some suffering from debilitating diseases gelatinous marrow has a reddish, translucent and jelly-like appearance. The fat cells have atrophied and assume a stellate appearance. The intercellular spaces are now filled with a homogeneous groundsubstance of slightly different shades of red. Fixed preparations bear some resemblance to cartilage. Blood vessels and red marrow cells are extremely rare. Virchow (1859) believed this to be a degenerative process of fatty connective tissue. He called it a mucoid degeneration because he allegedly was able to demonstrate the presence of mucin.

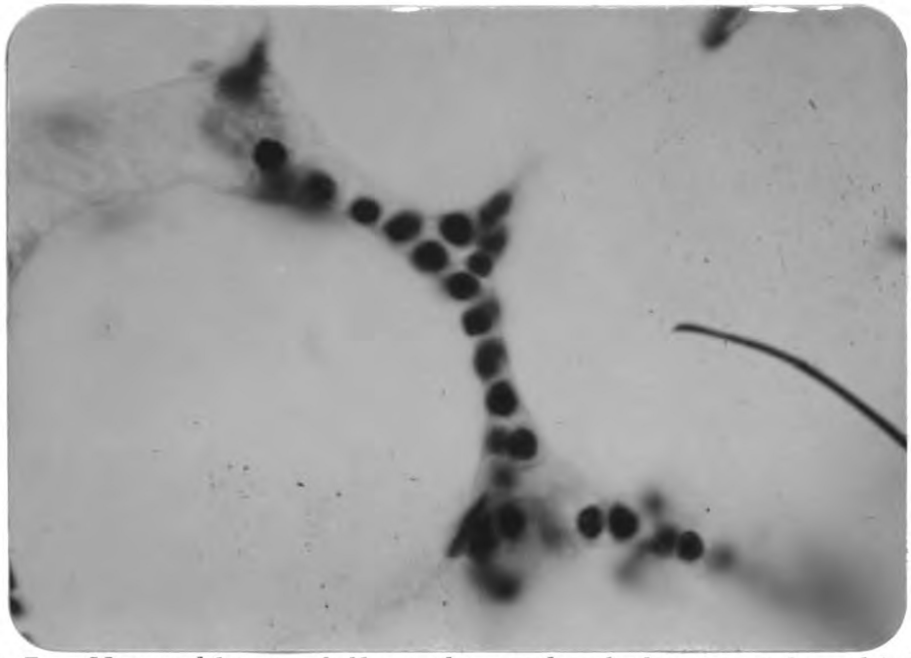

Figure 7. Normoblasts follow the path of the vessels which occupy the interstices between fat cells. See Figure 8 (below), which is a section through a capillary. X 1125.

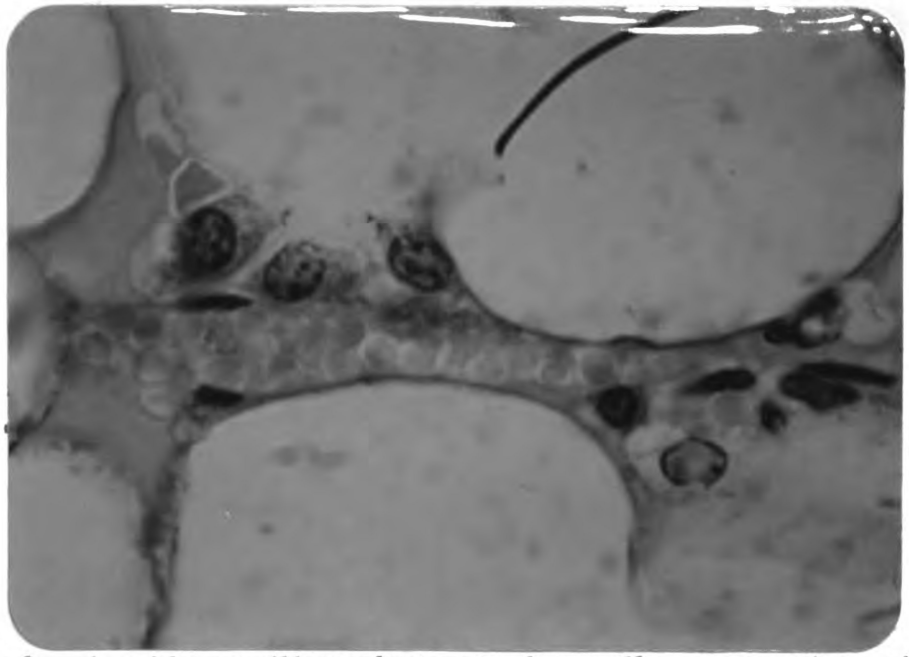

Figure 8. A wide capillary between fat cells connecting sinusoids. Note littoral cells in the capillary wall and myeloid cells outside of vascular channel. X 1125.

The characteristics of the different types of marrow are of great importance from a diagnostic standpoint. It is obvious that embryonal marrow and gelatinous marrow are in no way identical.

Lymphoid marrow differs from the two just mentioned in that it contains much less intercellular ground substance and has a considerably greater number of lymphoid marrow cells. Its red color is due to the many blood vessels present. It must be emphasized that all kinds of transitional forms of the marrow types discussed may occur.

Marrow Stroma and Vascular System

As in lymphatic tissue, the stroma consists of primitive and phagocytic reticular cells. The network of cells and fibers is looser and its meshes are larger than in the lymphatic tissue (Maximow and Bloom, 1949).

The circulation in bone marrow is a closed system (Drinker et al., 1922), and is characterized by the presence of many large vessels, called sinusoids, through the walls of which innumerable cells pass into the blood stream. The sinusoids are lined by flattened, fixed macrophages (littoral cells) like those forming the walls of lymph node sinuses (Figures 9 and 10).

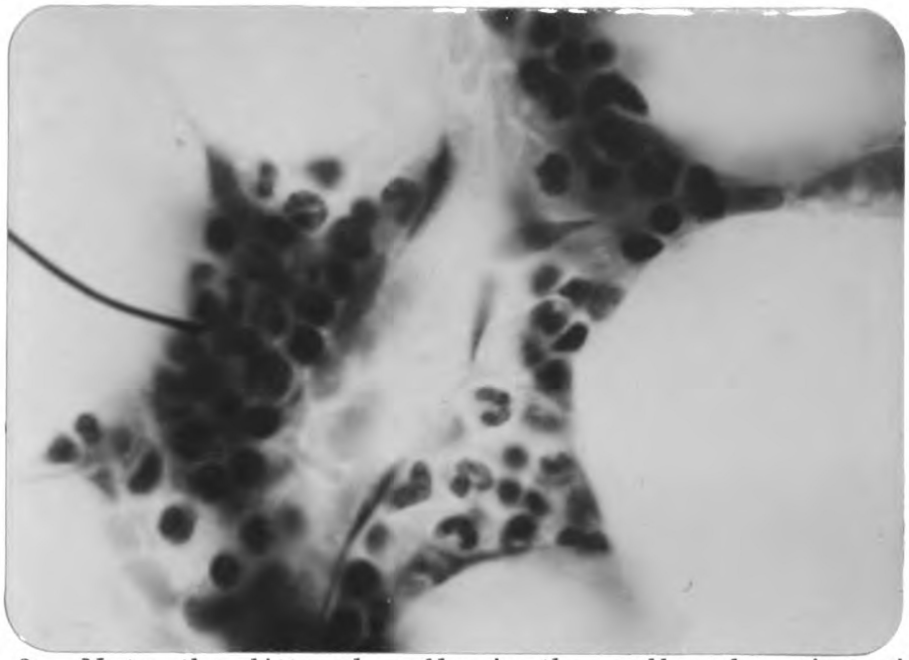

Figure 9. Note the littoral cells in the walls of a sinusoid. Also observe the extravascular position of the newly formed blood cells (left), as well as the intermingling of normoblasts, band cells, and polymorphs (right). X 1125.

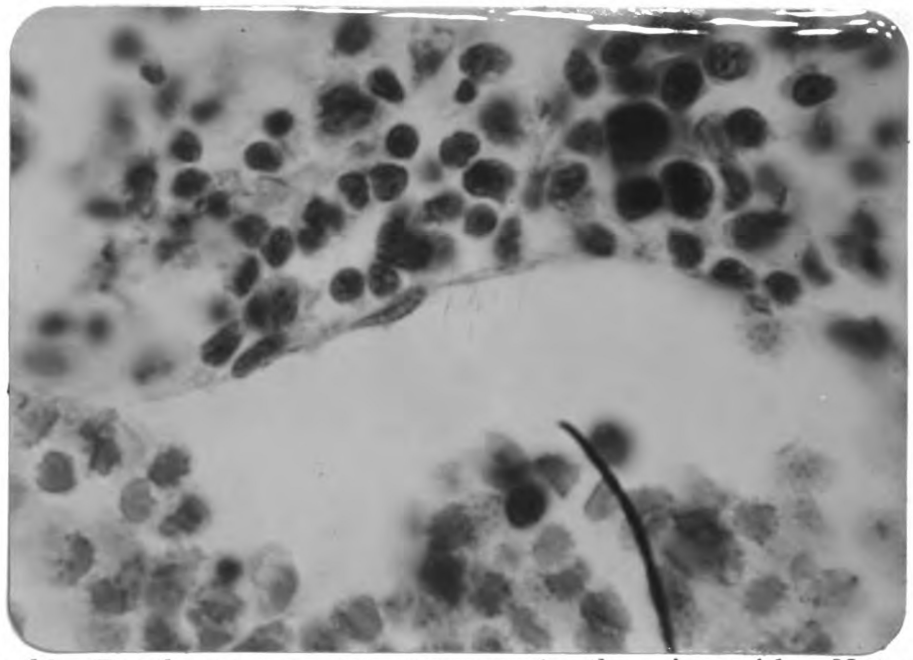

Figure 10. Erythrocytes are present in the sinusoid. Note the wall of the vascular structure with a littoral cell in the wall and the variety of myeloid cells outside the vessel wall above. X 1125.

The medullary artery, after passing obliquely through the nutrient foramen of the bone, usually divides into two main divisions, one running towards each end of the bone, and gradually assumes a more or less central position in the marrow (Figure 11). This central artery gives off smaller branches which pass out radially, occasionally almost at right angles to the parent trunk, sometimes more obliquely. It may also divide into two or more primary branches, especially as it approaches the more cancellated parts towards the end of the bone. Evidently some branches of the medullary artery pass directly into the cortex of the bone. The author has seen cross sections of small arteries in the bone substance and in Haversian canals. Many islands of myeloid tissue within the cortex are not an infrequent occurrence in younger dogs. The intraosseous arteries apparently furnish the blood supply for these little islands of red marrow. No blood vessels were observed in the cellular islands (Figure 12).

The central artery, for the greater part of its course through the marrow, is usually accompanied by a large, very thin-walled, venous channel, which receives innumerable smaller tributaries running more or less radially from the periphery towards the center of the marrow (Figure 13).

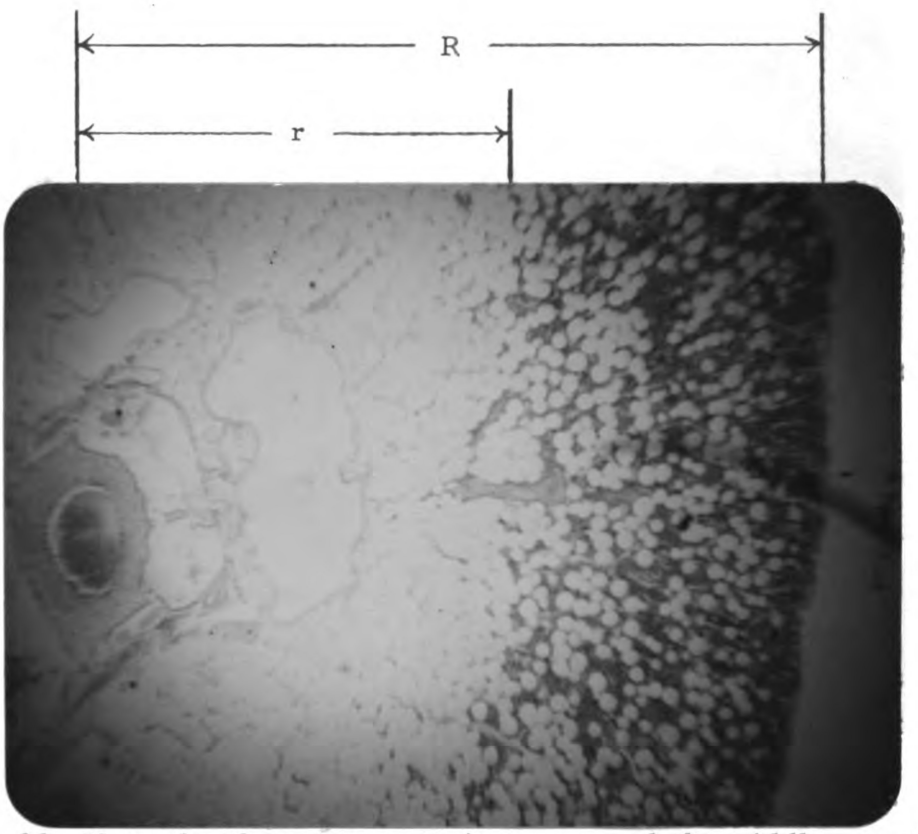

Figure 11. Note the three concentric zones: left middle, central artery; to right, myeloid tissue proper; extreme right, peripheral or endosteal zone. X 40. (see formula, p. 58)

re 12. Cross section of a humerus from a dog one year old. There is relatively little yellow marrow present. Note the many islands of marrow substance in the cortex of the bone. The numerous small arteries within the osseous substance are difficult to discern at this magnification. X 15.

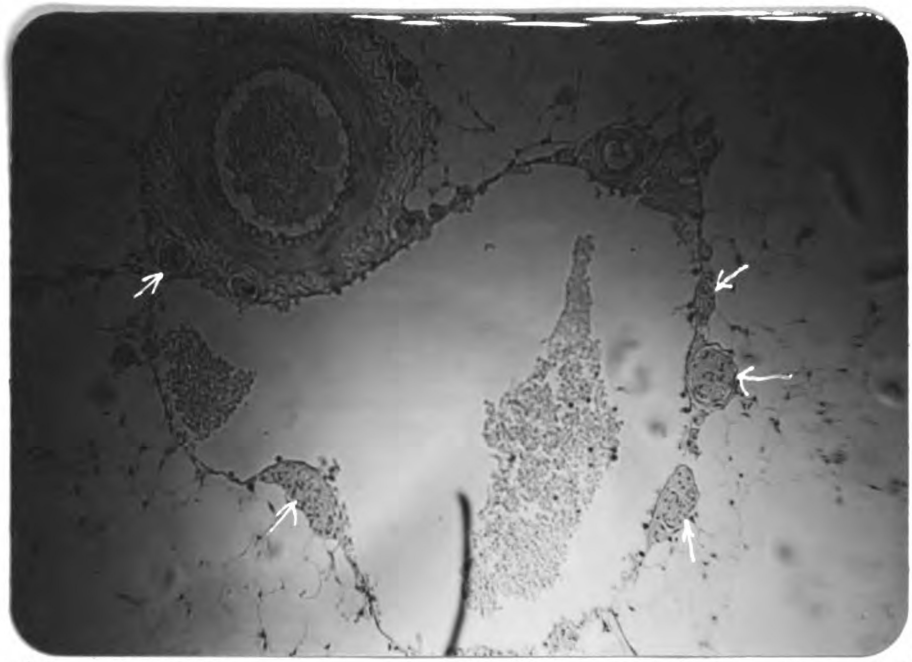

Figure 13. Central artery and vein. Note the large size and the extremely thin wall of the vein. Several nerves (arrows) are seen adjacent to the venous wall and in the periarterial connective tissue. X 125. See close-up of nerve in Figure 13a, below.

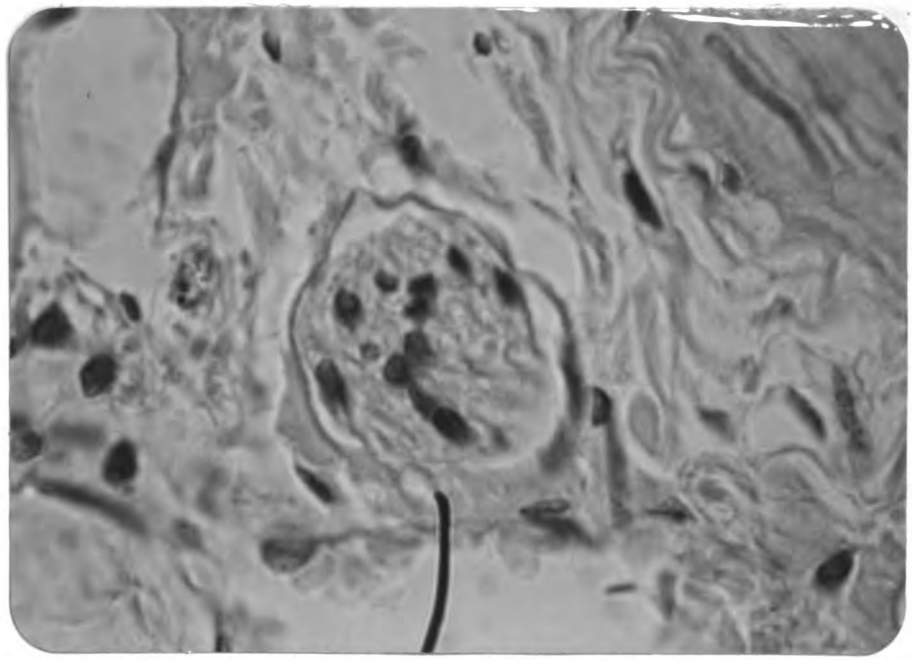

Figure 13a. Close-up of a nerve. Note a portion of arterial wall in upper right-hand corner. X 1125. Compare with Figure 13.

The sinusoids are connected by relatively wide capillaries (Sabin, 1928), making the whole vasuclar structure a closed system as stated above.

In transverse section, the marrow may, for convenience in description, be mapped out into three concentric zones:

1. Central or Vascular Areas, containing the thick-walled medullary or central artery of the marrow, together with the accompanying venous sinus, nerves, and connective tissue. It appears that anatomy and histology of nerve tissue in bone marrow have not received attention by investigators. The photograph presented by the author shows several nerves embedded in perivascular connective tissue. The nerve fibers appear to be of the myelinated type (Figures 13 and 13a). Amyelinated nerve fibers were not seen.

2. Intermediate zone, or marrow tissue proper, lying between the central and peripheral zones, and consisting of a delicate reticulum composed of branching and anastomosing connective tissue cells, and fine connective tissue fibrils, in the meshes of which lie the marrow cells to be described in some detail later. In this layer are also found the fat cells which may constitute a considerable part of the whole (Figures 11 and 14).

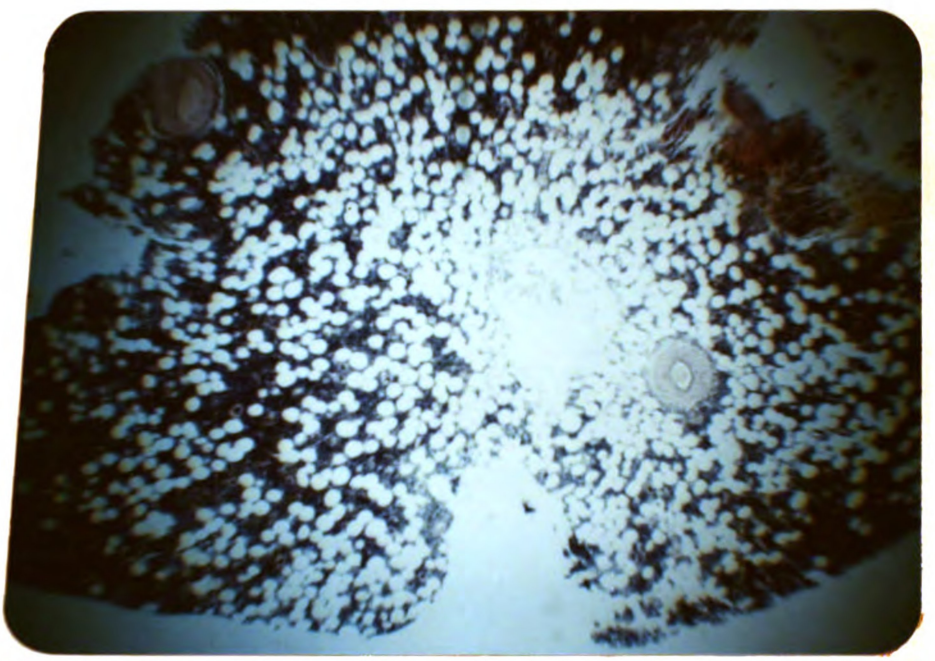

Figure 14. Entire cross section of the marrow pencil from a seven months old dog. Note the spreading of fat cells from the center toward the periphery. There is a very large branch of the central artery near the periphery in the upper left-hand corner. X 40.

3. Peripheral zone, or endosteum, composed of a condensation of the connective tissue framework of the marrow--a delicate, membranous structure to which allusion has already been made in describing the removal of the marrow from the interior of the bone.

The stroma of the myeloid tissue is distinguished by the constant presence of fat cells. These are scattered singly in the red marrow, but in the yellow bone marrow they have crowded out practically all of the other cells. Between them remain, besides the blood vessels and reticular fibers, only scattered fixed macrophages and primitive reticular cells. The latter are probably the main source of the new blood cells when the yellow bone marrow is transformed into red marrow (Figure 14).

The Free Cells

In contrast to the free cells of the lymphoid tissue, those of ıe myeloid tissue are extremely varied in form and are scattered regularly throughout the tissue. The vast majority of them are ng myeloid elements (erythroblasts and myelocytes).

Mature and Immature Elements

Mature myeloid elements. A few mature, nonnucleated erythrocytes and the three types of granular leukocytes as they occur in the circulating blood are found between other cells. Thus, the tissue which produces these elements always contains a ready supply of them, and in case of need can forward large quantities at once into the blood.

Immature myeloid elements. The other free cells in the bone marrow are: (1) hemocytoblasts (free stem cells); (2) erythroblasts (the precursors or the red blood corpuscles); (3) myelocytes (the precursors of the granular leukocytes; (4) megakaryocytes; and (5) some lymphocytes.

(1) Hemocytoblasts. The myeloid tissue of all adult mammals contains ameboid, nongranular, basophil cells of lymphoid nature. They vary in size, the largest measuring fifteen microns, and are scattered singly or in groups of two or four. Their structure corresponds exactly to that of the lymphocytes (Maximow, 1909). These elements are the free stem cells of all the other myeloid elements. From the unitarian point of view, they are morphologically identical with and have the same prospective developmental potencies as the larger lymphocytes.

(2) Erythroblasts. The young forms of the red blood corpuscles are spherical cells with spherical nuclei and are called erythroblasts. In living cells the cytoplasm is homogeneous, and of a yellow color which intensifies as the cells develop into erythrocytes. The round nucleus of the erythroblasts always presents a checkerboard distribution of angular particles of chromatin. The nucleoli gradually involute. The number of mitotic divisions in the cell lineage is not known. The changes in the erythroblasts as they develop into erythrocytes are clearly shown in marrow smears.

The youngest erythroblasts (those closest to the stem cell) are called basophil erythroblasts, because of the intense basophilia of the protoplasm.

The erythroblasts of the next youngest generation have a very small amount of hemoglobin. After fixation and staining with the Romanowsky mixture (eosin-methylene-azure) the cytoplasm varied from a purplish-blue to lilac or gray. These erythroblasts are, therefore, called polychromatophils. This staining reaction is due to the appearance of the pink-staining hemoglobin in the basophil cytoplasm of the erythroblast, which stains blue with eosin-methylene-azure.

The polychromatophil erythroblasts divide mitotically. Some of them remain in the tissue in a resting condition for future use. In the others the amount of hemoglobin increases while the basophilia of the cytoplasm diminishes. In this way normoblasts arise in which the cytoplasm stains a bright pink with the Romanowsky mixture. Normoblasts are smaller than polychromatophil erythroblasts and only slightly larger than mature erythrocytes. The small round nucleus contains a dense accumulation of angular chromatin particles and stains very dark. After an unknown number of mitotic divisions, the normoblasts lose their capacity for proliferation, and the nucleus is condensed to a dark-staining body. Each mature normoblast loses its pycnotic nucleus and is transformed into a red blood corpuscle. Some investigators hold that the nucleus is lost by karyolysis, while others believe this occurs by extrusion.

(3) Myelocytes. Besides the erythroblasts, the young forms of the three types of leukocytes (heterophil or neutrophil, eosinophil, and basophil) are common cell types of the myeloid tissue. The myelocytes of each of the three types are provided with the characteristic granulation and cannot be transformed into myelocytes of another type or into elements of another kind. They have a compact round or oval-shaped nucleus, and proliferate intensely by mitotic

division. Some of the progeny remain unchanged while others undergo progressive maturation. Finally, each cell is transformed individually into a mature polymorphonuclear granular leukocyte.

Myelocytes with heterophil granules (neutrophil myeclocytes of man) are larger than the mature heterophil leukocytes.

The youngest generation is that of the promelocytes. The oval or round nucleus contains a loose chromatin network and several nucleoli. The ameboid cytoplasm is slightly basophil, although it often shows acidophil areas. The specific granules are scarce and usually are confined to the periphery of the cytocentrum and to the acidophil areas in the cell body. In dry smears the promelocytes may contain, in addition to the heterophil granules, azurophil granules.

The promyclocytes often show mitosis. In the following generation, which may be called heterophil myelocytes proper, the protoplasm becomes diffusely acidophil while the specific granules increase in number and fill the whole cell body, except for the cytocentrum. The chromatin network of the nucleus becomes coarser and stains darker, and the nucleoli become indistinct. Mitoses are common. During division the granules are evenly distributed among the daughter cells and continue to increase in numbers as the latter grow.

As the result of an unknown number of mitoses, a generation of heterophil myelocytes appears which has lost its capacity for division. The nucleus in these cells, as soon as it is reconstructed after the last mitosis, shows a beginning polymorphism and has the shape of a kidney or a horseshoe. Such cells are called metamyelocytes and band cells, respectively; each of them maturates without division and is transformed by progressive constriction of the horseshoe-shaped nucleus into a mature heterophil leukocyte.

Myelocytes with eosinophil granules are less numerous than the heterophil myelocytes, but in general undergo the same changes. These appear more numerous in the dog. Among them also different generations can be distinguished. They all have a slightly basophil protoplasm. The eosinophil promyelocytes contain a small number of specific granules which do not all stain alike. The youngest among them show a distinct basophilia and stain bluish with eosin-azure. From these there are all transitions to mature, purely eosinophilic, red granules. Mitoses are common in the eosinophil myelocytes, especially in the large ones. The horseshoe-shaped nucleus of the metamelocytes becomes constricted into two lobes in the mature leukocytes.

Myelocytes with basophil granules are much scarcer than the heterophil myelocytes and are difficult to study because the granules in man are easily soluble in water. This difficulty induced some investigators to declare that cells with basophil granules in the bone marrow are degenerated eosinophil myelocytes. For the most part the basophil myelocytes are small, with a paler nucleus than other types of myelocytes. The protoplasm contains a widely varying number of specific, basophil, metachromatic granules of unequal size. Mitoses have been found, but are very rare.

(4) Megakaryocytes. These giant cells with a polymorphous nucleus are characteristic of the mammalian bone marrow, where they are scattered evenly among the other elements. Some of them have a diameter as large as seventy microns. The form of the cell body is spherical, but its surface is often provided with irregularly shaped processes.

The nucleus is deeply constricted in many places; the lobes bulge at the periphery, while their central parts are all interconnected by short, branched stalks. The interior of the nucleus shows a chromatin network and indistinct nucleoli. In the living cell the abundant cytoplasm is homogeneous. The presumed role of the megakaryocytes is the production of platelets.

In every normal bone marrow many megakaryocytes are found degenerating while there are frequent signs of their formation from hemacytoblasts.

(5) Monocytes. Many hematologists believe that monocytes are formed in the myeloid tissue. Others claim they are formed extramedullarily; e.g., lymphoid tissue. Under normal conditions they are found only in sparing numbers. The general appearance is like those of peripheral blood. Earlier forms are extremely difficult to differentiate from large lymphocytes.

Heteroplastic Hemopoiesis

Under physiologic conditions, the needs of the adult organism for myeloid blood elements are usually supplied by homoplastic hemopoiesis--the production of mature cells by young elements of the same type. But not all of the young forms reach maturity; some of them remain unused in the tissue. Whenever the requirements of the body are increased--in the very young organism or in the adult under pathologic conditions--homoplastic hemopoiesis does not suffice, and new erythroblasts and myelocytes develop from stem cells. This is called heteroplastic hemopoiesis.

When a hemocytoblast (stem cell) divides, one of its various latent potencies suddenly develops and both of the daughter cells which originate from such a mitosis show new properties. Some hemocytoblasts always remain in the tissue as the source of future hemopoietic processes when demand arises. Of the progeny of the hemocytoblasts, some become basophil erythroblasts. These divide mitotically and are transformed in the succeeding generations into polychromatophil erythroblasts. When hemocytoblasts develop into myelocytes the two daughter cells, immediately after the reconstruction of the nuclei, accumulate the characteristic granules in their protoplasm. Transitional cells between the hemocytoblasts and early myelocytes have been described under the name of leukoblasts and more recently as progranulocytes (Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood Forming Organs, 1949).

The New Formation of Free Cells from Fixed Cells

In the embryo as stated before the hemocytoblasts of the bone marrow which produce the young myeloid forms originate from undifferentiated fixed mesenchymal cells. In the adult the same process may occur. It has been explained that some of the cellular elements

of the reticulum always remain undifferentiated. But under physiologic conditions it is rare for primitive reticular cells to become free, basophil hemacytoblasts in the bone marrow. The mitoses of the myelocytes and erythroblasts--and occasionally of hemocytoblasts --usually are sufficient. Pathologic stimuli sometimes facilitate the new formation of hemocytoblasts from the primitive reticular cells. Heteroplastic development of lymphocytes may also occur. The author has not seen lymphoid follicles in the bone marrow of dogs. They are frequently found in man (Rohr, 1949).

How the Myeloid Elements Enter the Blood

Because the myeloid elements arise outside the blood stream, it is obvious that the newly formed, mature myeloid cells must pass through the walls of the blood vessels to enter the circulation. The very thin-walled venous sinusoids make this possible. Through them easily pass not only the ameboid mature granular leucocytes, but also the nonmotile erythrocytes. When these are ready for circulation they slip through the membrane into the blood stream in the lumen of the sinusoid. The mechanism of this phenomenon is probably regulated by changes in the permeability of the vessel walls, in the surface energy or some other specialized mechanism.

The claim that red blood corpuscles in the adult, normal man or other mammals are formed intravascularly is based on unconvincing evidence (Drinker et al., 1922; Sabin, 1928). In the embryonic liver and bone marrow the red blood cells develop extravascularly, while in the yolk-sac they are preponderantly of intravascular origin. The latter statement is not entirely true because blood cells were forming in the blood islands before a vascular structure had been provided.

Functions of the Myeloid Tissue

The main function of the bone marrow seems to be the production of myeloid elements for the blood. The macrophages of the bone marrow also function like the macrophages in other tissues.

The cellular composition of the blood is intimately connected with the condition of the bone marrow. Under physiologic conditions, the relative numbers of the different cells in the bone marrow, as in the blood, vary only a little. But all general pathologic processes immediately affect the composition of the bone marrow. In most general infections, and during suppuration, the heterophil granulocytopoietic apparatus is stimulated and the percentage of myelocytes of this type increases greatly. In certain other conditions, as in

typhoid fever and in a granulocytic angina of man, the heterophil myelocytes decrease in number. Whenever there is an increased need of erythrocytes or where the erythrocytes are destroyed in large quantities, they and the precursors predominate in the myeloid tissue. That temperature plays some role in the control of hemopoiesis is shown by the fact that the fatty marrow of the tail bones of a rat becomes hematopoietic when the temperature of the bone is raised to that of the body, as by placing it in the body (Maximow and Bloom, 1949).

The production of erythrocytes depends in part on an antianemic factor which is stored in the liver. This factor probably is the result of the interaction of a substance in the gastric juice (intrinsic factor of Castle) with some substance in the diet (extrinsic factor). The absence of the antianemic factor results in pernicious anemia in man, which can be treated successfully by the administration of liver, gastric extract, or B_{12} vitamin (Best and Taylor, 1950).

Regarding the Question of Lymphatics of Bone Marrow

The author has not observed lymphatics in the marrow of dogs.

The literature appears to ignore that part of the anatomical structure almost entirely. Drinker et al. (1922) stated that they know of no work upon the lymphatic system of the bone marrow.

Roger and Josue (1899) denied the existence of a lymphatic system in bone marrow. No one has challenged them as far as has been determined.

PRESENTATION AND EVALUATION OF FINDINGS

General Considerations

Up to this time no one had attempted to investigate the bone marrow of the dog in a broader sense and with a view of using the information thus found as a practical clinical diagnostic agent in small animal practice. Only two groups of veterinarians have reported bone marrow biopsies in the dog. Their work was restricted to cytology of marrow smears (Meyer and Bloom, 1943; Bloom and Meyer, 1944; Bloom, 1945; Horn, Jahn, and Wille, 1953).

Other reports of bone marrow studies on dogs came from medical men engaged in research who were interested in the cytology of the myeloid tissue of these animals used in other experimental work (Mulligan, 1941a, 1941b, 1945; Van Loon and Clark, 1943). No reports of the preparation of whole marrow sections were published by the preceding workers, except Mulligan (1941b). The information obtained by their work was of much value. It was insufficient and even unreliable to make diagnoses on the basis of cytological findings from smears alone.

It was exceedingly important that sections of marrow be made, as will be seen from citation of the following:

Block (1950) reported an exhibit at the International Congress of Hematology in Buffalo, New York, 1948, of twelve cases of metastatic carcinoma which were incorrectly diagnosed as acute leukemia on the basis of smears by experienced hematologists at the University of Michigan.

It is not implied that sections should be made in all cases, as will be shown in the discussion to follow later in this thesis.

The diagnostician should be fully conversant with the anatomy and histology of normal bone marrow. With this in mind the author investigated ten carefully selected dogs which were considered normal on the basis of the clinical examinations, as shown in Table I in the chapter on Material and Methods.

It was probably the first time that whole cross sections of myeloid tissue were prepared to study the topography of normal marrow of the dog. The cellular elements of bone marrow vary considerably quantitatively in different animals within the same age group. Furthermore, they may be very similar in widely varying age classes in apparently normal specimens (Figures 11 and 14). This should indicate that not too much emphasis should be placed on

quantitative proportions between fat marrow and cellular marrow. It appeared that the physiological activity of hemopoietic cellular elements was more important. As long as the cellular elements were in proper relative proportions qualitatively even though there was an apparent wide difference quantitatively, the marrow specimen should be pronounced as being normal by the clinician. It was obvious that there would be a quantitative minimum. From the normal specimens examined it may be concluded that a minimum amount of cellular marrow well proportioned qualitatively could be set at approximately one-third of the total cross-sectional area. This applies only to marrow of such bones and portions of such bones as are known to be functionally active and responsive to normal and pathologic physiological demands.

The degree of normalcy of marrow in long bones can be appraised grossly or under very low magnification by applying the following formula:

$$\frac{R^2 - r^2 \times 100}{R^2} = \% \text{ of red marrow}$$

where (r) equals the inner radius and (R) the outer radius of the concentric ring formed by the hematopoietically active myeloid tissue. (See Figure 11, page 38.)

If the numerical value thus obtained is thirty or greater, the marrow tissue may be considered to be within a normal range provided that the active cellular elements are well proportioned.

This was in close agreement with values given for human marrow by Berman and Axelrod (1950).

For the dog the junction of the proximal and middle thirds of the femur was such an area. Custer (1932) and Stasney and Higgins (1937) found that the proximal end of the femur responds to changes in physiological activity very early and quickly. This was in full agreement with observations made during this investigation.

Meyer and Bloom (1943) described the technic for iliac puncture in the dog. They admitted that one might meet with a great deal of uncertainty in the execution of this procedure.

Horn et al. (1953) suggested sternal puncture. They did not state the size of dogs used, nor did they admit any failures. Iliac crest and sternal puncture were rejected in this investigation for the following reason: To make it a practical clinical procedure the marrow puncture should be capable of being performed on dogs of all sizes with absolute safety and certainty.

The practicing veterinarian would soon find out that puncture of iliac crest and sternum would be an impractical task clinically

on the many small breeds of dogs which he is called upon to treat. Furthermore, sternal puncture was not entirely without danger in human practice where the cross section of the sternal marrow space of the average adult measures approximately one-half to one inch. Six deaths following sternal puncture have been reported (Fortner and Moss, 1948).

No clinical procedure to aid in arriving at a correct diagnosis is acceptable in practice if it is not absolutely safe. No owner brings his pet to the veterinarian to have him killed or maimed in order to make a diagnosis. Femoral puncture has been safe, reliable, and without appreciable discomfort to the patient in all cases, as has been described in Material and Methods.

Also, if material obtained by the puncture method was insufficient for preparing sections, should such be desired, more marrow substance could be had by trephination at the same time. The latter will rarely be necessary.

Appraisal of Marrow Structure

Gross evaluation.

(A) Necropsy material. The humerus and femur appear to be first choice in order to judge the quality of marrow. In dogs less

than six months old the marrow was usually almost blood red, soft jelly-like or nearly semiliquid throughout most of the length of the bone. If any variation in the distribution of red and yellow marrow occurred it followed Neumann's law as defined in the section on Anatomy and Histology; i.e., more red at the proximal portion, with increase of fat at the more distal region (Neumann, 1882).

Animals of age from six months to one year more often show a marrow quality as just described, but some exhibit a definite increase of yellow marrow as they approach one year.

After one year of age the transition from red marrow at the proximal end to fatty marrow at the distal portion was more obvious. As the age of the animals advances the central portion of the remaining red marrow becomes fatty, leaving only a concentric ring of red marrow at the periphery of the marrow pencil.

About this time a peculiar distribution of red marrow was observed in several cases. A thin layer of red marrow covered the full length of the entire periphery of the marrow pencil. This condition has not been described as far as was known. Cross sections revealed yellow marrow in the more central area. In these cases the transition from red to yellow marrow was usually very abrupt. A clue to the true condition of the marrow in these cases was afforded

on closer inspection; namely, the color was of a somewhat lighter red. Caution must be exercised not to confuse this condition with the gelatinous-type marrow described in the section on Anatomy and Histology. The latter was a lighter red throughout the marrow pencil and would have to be differentiated microscopically (Figure 1).

(B) Biopsy material obtained by puncture method. The aspirated material was deposited on a watch glass and quickly mixed. Bone marrow substance had a light red color, was shiny, with a fatty surface. If much fatty marrow was present a slightly yellowish sheen could be detected. The entire consistency was that of a thick fluid and sometimes somewhat stringy or like ropy milk.

Microscopic evaluation.

(A) Fixed whole marrow substance obtained at necropsy, trephination, or puncture method. Prepared sections from above material make it possible to distinguish three different marrow structures under low power:

1. Normal bone marrow. Cellular and fatty marrow from the average young adult dog were about equal quantitatively. As stated earlier in this chapter the proportions vary considerably, and favor fatty marrow as age advances.

2. Hypoplastic marrow. This was generally designated as fatty marrow. Its color was characteristically yellowish white. The puncture material has a pronounced fatty sheen when mixed with a little marrow blood. Caution must be exercised not to designate every fatty appearing biopsy a hypoplastic marrow. Peripheral blood and other clinical findings must be evaluated as will be discussed later.
3. Hyperplastic marrow. The characteristic feature of this type of marrow was the paucity or almost complete absence of yellow marrow. It consisted almost entirely of cellular marrow. If erythropoiesis was predominent an intensely red coloration was noticeable macroscopically. If leukopoiesis predominated a grayish red color was observed.

Other conditions which can be recognized by a change in marrow structure due to presence of an abnormal number of a certain cell type were:

1. Hemolytic, toxic, and deficiency anemias (Rohr, 1949; Isaacs, 1937). Determination of the myeloid-erythroid ratio was a simple and rapid method of evaluating normal and abnormal marrows.

2. Leukemias, agranulocytoses of infectious nature, acute inflammation, chronic infections, and sometimes allergic conditions (Custer, 1935).
3. Disturbance of the thrombopoietic system (increase of megakaryocytes).
4. Lymphoid marrow in lymphatic hyperplasia and granulocytopenia (Rohr, 1949).
5. Excessive reticular elements as in agranulocytosis (Custer, 1935) and panmyelophthisis generally.
6. Pure fatty marrow as in aplastic conditions (Rohr, 1949).
7. Tumor cell marrow in carcinomata, sarcomata, myelomata, and leukoses (Rubinstein, 1948; Rohr, 1949).

(B) Direct smears from puncture biopsies. Stained smears should first be looked over under low magnification for density of cellular elements and fat content. This permits a general evaluation of the character of the marrow. A differential count was made under oil immersion. The myelogram will be discussed at the end of the section.

The color reactions in staining of smears were based on the results with Wright's stain. Sections were stained with hematoxylin and eosin as outlined in the section on Material and Methods.

A brief description will be given here covering the principal features of the various cells as they appear in stained smears. For more detailed description reference is made to Albritton (1952), Kohanawa (1928), and Rohr (1949).

Table III contains the more recently recommended nomenclature for cells of blood and blood-forming organs. The table is arranged to list the cells of each series in the order of their development starting with the youngest of each specific cell type. The older names still in use are given in parenthesis in the section "Description of Hemopoietic Cells."

Description of the Hemopoietic Cells of Bone Marrow[1]

Erythrocytic series. There are no cytoplasmic granules present in this series.

1. Rubriblast (pronormoblast, proerythroblast).[2] Size, about fifteen microns. Cytoplasm usually a narrow, deeply basophilic band around a relatively large, round nucleus of delicate chromatin, more

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[1] Nomenclature recommended by the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs (1949).

[2] Names in parenthesis are still commonly used.

TABLE III

NOMENCLATURE RECOMMENDED[1] FOR CELLS OF BLOOD AND BLOOD-FORMING ORGANS

General Cell Type	Term for Specific Cell Type
Erythrocytic series	Rubriblast
	Prorubricyte
	Rubricyte
	Metarubricyte
	Reticulocyte
	Erythrocyte
Lymphocytic series	Lymphoblast
	Prolymphocyte
	Lymphocyte
Monocytic series	Monoblast
	Promonocyte
	Monocyte
Granulocytic series	Myeloblast
	Progranulocyte
	Myelocyte
	Metamyelocyte
	Band cell
	Segmented
Plasmacytic series	Plasmablast
	Proplasmacyte
	Plasmacyte
Thrombocytic series	Megakaryoblast
	Promegakaryocyte
	Megakaryocyte
	Thrombocyte
Not identifiable	Disintegrated cell

[1] By the Committee for Clarification of the Nomenclature of Cells and Diseases of the Blood and Blood-Forming Organs (1949).

dense and less translucent than that of myeloblast. Parachromatin was distinct and pink. Nucleoli not too readily visible.

2. Prorubricyte (basophylic normoblast, basophil erythroblast). Size, about like preceding or a little smaller. Cytoplasm basophilic. Nucleus smaller and more dense than preceding, chromatin may clump more, parachromatin as in preceding, nucleoli usually not visible.

3. Rubricyte (polychromatophilic normoblast, polychromatophilic erythroblast). Size, eight to twelve microns. Increase of hemoglobin renders cytoplasm pastel blue to almost copper red. Nucleus becomes still smaller (pycnotic), chromatin dense, and nuclear membrane coarse, less parachromatin, nucleoli not visible. Note: Most mitotic divisions occurred in this group.

4. Metarubricyte (orthochromatic or acidophilic normoblast). Size, seven to ten microns. Nucleus very small, practically no parachromatin. Cytoplasm stains slightly less red than in mature erythrocyte.

Lymphocytic series.

1. Lymphoblast. Size, ten to eighteen microns. Cytoplasm colorless to dark blue, no specific granules, usually a narrow band. Nucleus round or oval, usually one to two nucleoli, fine chromatin network, parachromatin pastel pink and distinct.

2. Lymphocyte (small and medium). Size, six to fourteen microns. Small amount light blue cytoplasm. May have a few azurophil granules, no specific granules. Nucleus round or oval, slightly indented, chromatin in blocks which were indistinctly separated, appeared blurred. Parachromatin slight and difficult to distinguish from chromatin.

Monocytic series.

1. Monoblast. Size, fourteen to eighteen microns. Cytoplasm may or may not contain fine azurophil granules, moderate amount, stained slightly basophilic, no specific granules. Nucleus may be round, oval, and slightly indented, one to five pale blue nucleoli, chromatin pattern very fine strands, stained faintly, chromatin-parachromatin relation distinct.

2. Monocyte (transitional or blood mononuclear). Size, fourteen to twenty microns. Cytoplasm billowy, grayish blue, muddy blue, Tauben blau, ground glass appearance. No specific granules. Azurophil granules dust-like, hyaloplasm may stain pinkish. Nucleus appears in many shapes. A rough contour, hill and valley effect. Chromatin strands fine, network was lace-like, stained faintly, had marked chromatin-parachromatin distinction. No nucleoli.

Granulocytic series.

1. Myeloblast (hemocytoblast, stem cell). Size, ten to eighteen microns. Cytoplasm was basophilic, not as deep blue as rubriblast, formed a narrow band around nucleus. There were no specific granules. May contain fine purple azurophil granules. The nucleus was round or slightly oval, usually central, had up to five pale blue nucleoli. The chromatin was in fine strands and was knitted into a fishnet-like structure. The interstices between the thin chromatin strands were distinct, often rectangular and regular; parachromatin was scant, pink or bluish and very distinct from chromatin. Sometimes the nucleus had a stippled appearance almost like a red raspberry.

The pattern of the myeloblast of the dog was not as distinct as that of man. Of course not many of them have been viewed inasmuch as they are relatively rare. The author wishes to reserve further comment on morphology of all myeloid cells because additional study of these cells is now in progress.

2. Progranulocyte (leukoblast, promyelocyte). Size, fourteen to twenty-one microns. Cytoplasm is less basophilic and more abundant than that of the myeloblast. No specific granules. May contain some azurophil granules. Nucleus round or oval, central or eccentric.

Nuclear pattern generally like the myeloblast, but less regular and some slight clumping of chromatin at intersection of strands. Usually not more than two but less distinct nucleoli. Good distinction of chromatin-parachromatin.

3. Early myelocyte (promyelocyte). Although this cell does not appear in Table II, it is included in nearly all myelograms. Three types were recognized. Their outstanding characteristic was the presence of specific granules. May contain some azurophil granules. Size, twelve to eighteen microns. The abundant cytoplasm was of varying shades of blue, generally pale blue, a little darker at the periphery. The nucleus was round or oval, central or eccentric. Nucleoli were not clearly visible if at all. The interstices of the chromatin network were irregular; the chromatin strands were becoming coarse. The pink parachromatin was distinct.

(a) Neutrophilic. Specific granules stain weakly pink in the dog, much less distinct than in man. A blurred appearance.

(b) Eosinophilic. Round or spherical large specific granules, like little oranges, uniform size, orange red. Not very numerous.

(c) Basophilic. A few large metachromatic specific granules, uneven in size and distribution, dark color.

4. Late myelocyte. These cells have their full complement of specific granules. They have the appearance of the respective mature forms except the shape of the nucleus, which was still round or oval. The cytoplasm of the neutrophilic myelocytes was colorless or clear, that of the other two may be pale blue. The thickened chromatin strands form a coarse meshwork and stain deeply purple. The small amount of parachromatin was distinct. Most mitoses occurred in this stage.

5. Metamyelocyte (juvenile). Size, ten to sixteen microns. The cytoplasm of the three types was colorless or slightly acidophilic, relatively abundant. Specific granules were more numerous, stain slightly more intensely. The nucleus was reniform, had a thick nuclear membrane, a coarse meshwork of thickened strands of deep-staining chromatin. Parachromatin was scant but distinct from chromatin. Nucleoli were not visible.

6. Band cell (stab, nonsegmented), neutrophilic. Size, ten to fifteen microns. The abundant, colorless or slightly acidophilic cytoplasm had a full complement of pale pinkish specific granules. The nucleus was rod-like, deeply indented, with parallel sides. Thickened chromatin strands form coarse masses with distinct interstices. Scant parachromatin visible between chromatin masses. No

nucleoli visible. Cells with mere indentations from side to side were still counted as band cells.

7. Segmented (polymorphonuclear, filamented). Size, nine to fifteen microns.

(a) Neutrophilic. The abundant cytoplasm was colorless or slightly acidophilic. Specific granules were pale pinkish, tan, buff, or beige, rather indistinct, of irregular size and distributed haphazardly throughout the cytoplasm. The nucleus had a distinctive pattern, a checkerboard appearance. The chromatin formed large, irregular, deeply staining blocks. Parachromatin is thin and of light rust color. The sharp contrast between chromatin and parachromatin gives it a characteristic pattern. Several blocks of chromatin form lobes or bulbs. Normally there may appear from two to not more than five such bulbs in a single cell. The bulbs were connected by a thin thread or filament of chromatin. If the connections between bulbs were more than a thin thread the cells were not counted with segmented cells but were included with band cells. No nucleoli were visible.

(b) Eosinophilic. Size, nine to fifteen microns. There was usually not much visible cytoplasm. In the dog many of them have abundant cytoplasm. It was basophilic and had specific granules scattered throughout. The latter were large, spherical, or irregular and numerous. The specific granules of some of the mature cells stain dark tan, and may be of irregular size. Some of them look like Queen Anne cherries. The nuclear pattern was generally like that of the segmented neutrophil but had less lobes, mostly two but not more than four. Nucleoli not visible. Parachromatin scant but distinct.

(c) Basophilic. Size, nine to fifteen microns. The nucleus occupied the major portion of the basophilic cytoplasm. The metachromatic specific granules varied in size, shape, and distribution throughout the cytoplasm. The nucleus was roughly spherical, somewhat indented, lobes were not discernible. The chromatin stained light blue but looked faded. Parachromatin was indistinct. Nucleoli not visible. The specific granules were water soluble which may be responsible for vacuoles sometimes seen.

8. Plasmacyte (Türk Cell, myeloma cell). Size, fifteen to thirty microns. The very abundant cytoplasm was basophilic, the nucleus round or oval, relatively small, eccentric, may contain one or two nucleoli. The chromatin formed dense blocks arranged concentrically with distinct but somewhat irregular interstices. The name "Radkern" has been applied to this type of nucleus. A small amount of parachromatin, no specific granules, azurophilic granules were seldom seen. When present the cytoplasm stained correspondingly less basophilc (Rohr, 1949). The author found one of these in a direct smear and is shown in Figure 15.

9. Megakaryocyte (megalokaryocyte). Size, thirty to seventy microns. This was a unique nucleated cell. Each individual develops from a stem cell. The cytoplasm was acidophilic and was the source of blood platelets. Azurophilic granules aggregate in patches separated by nongranular cytoplasm. The nucleus of the mature megakaryocyte was multilobular and complex. The chromatin strands were so thickened that they formed heavy, dark-staining clumped masses with little or no parachromatin visible. No nucleoli visible.

10. Reticulum cell (reticulo-endothelial cell, stem cell of modern pathologists, mesenchymal cell, hemohistioblast). Aschoff (1920), Ferrata (1910), Ferrata and Negreiros (1914), Maximow (1909), Alexandrov (1930), Rohr (1940).

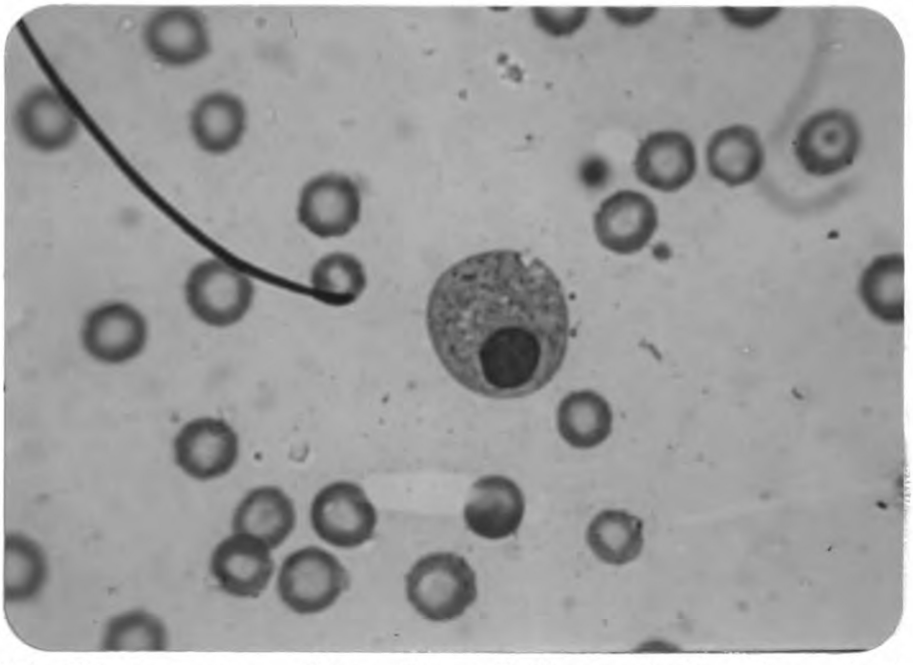

Figure 15. Plasmacyte. Note the relatively light color of the cytoplasm and the azurophil granules. X 1125.

This was probably the most primitive and least differentiated of all cells of the mammalian body. Size, fifteen to twenty-five microns. The delicate abundant cytoplasm was usually of irregular shape, pale to dark blue, may be mottled with considerable colorless or slightly acidophilic hyaloplasm. It may contain some azurophil granules. The nucleus was round or oval, the chromatin network consisted of fine, loosely knitted strands, giving almost a vesicular appearance. The color was reddish violet. Three to six nucleoli were usually present.

Parachromatin was dispersed in the irregular chromatin interstices and well contrasted. In marrow films these cells may have to be recognized by the nuclear characteristics; the cytoplasm was so delicate that it frequently ruptured. Naked nuclei were not uncommon in marrow smears.

To aid in identification of cells the author used: (1) the color atlas, Hamatologische Tafeln, by Sandoz (no date given); (2) Standard Values in Blood, by Albritton (1952); and (3) oral communications from Rebuck, in 1952, 1953, and 1954.

The method used in executing the marrow differential cell count was as follows: An area near the feather-edge of stained smears was located under low-power magnification. The oil-immersion

objective was used to count the cells, traversing the slides cross-wise until four hundred of the white cell series were counted. All cells of the red cell series in the respective fields were counted.

The Myelograms

All cells counted were tabulated as indicated in the individual myelograms for ten dogs used in this investigation (Table IV).

Table V contains a comparison of ranges of this study with similar investigations carried on elsewhere.

TABLE IV

PERCENTAGE OF DISTRIBUTION OF THE MARROW CELLS FROM THE FEMURS OF TEN DOGS

Cell Types	Case Number			
	I	II	III	IV
Rubriblasts (pronormoblast)	0.00	0.50	0.18	0.33
Prorubricytes (basophilic normoblast)	4.55	4.97	3.86	4.84
Rubricytes (polychromatophilic normoblast)	14.00	16.92	16.15	16.23
Metarubricytes (orthochromatic normoblast)	2.42	4.32	3.32	5.14
Red series total	21.00	26.67	23.50	26.55
Myeloblasts	0.65	0.00	0.37	0.33
Progranulocytes (promyelocyte) . .	1.14	1.98	1.84	1.66
Myelocytes, neutrophilic	6.69	4.15	4.04	4.97
Metamyelocytes, neutrophilic (juvenile)	7.67	9.64	8.45	12.78
Band cells, neutrophilic (stab cell)	26.60	29.83	27.02	27.10
Segmented cells (polymorphonuclear)	16.80	14.60	16.55	12.25
Eosinophilic cells, all types	7.02	6.80	5.52	6.13
Basophilic cells, all types	0.00	0.00	0.00	0.00
Lymphocytes	10.02	5.48	6.98	4.97
Monocytes	0.81	1.66	0.00	0.66
Megakaryocytes	0.00	0.00	0.00	0.00
Plasmacytes (plasma cell)	1.30	0.33	0.73	0.50
Reticulum cells	0.00	0.16	0.55	0.33
White series total	78.70	73.00	74.20	73.40
Myeloid-Erythroid ratio	3.76	2.74	3.16	2.76

TABLE IV (Continued)

Case Number						Range
V	VI	VII	VIII	IX	X	
0.36	0.32	0.18	0.60	0.36	0.37	0.00 to 0.60
4.37	5.64	2.84	2.61	2.74	5.55	2.74 to 5.64
17.45	19.65	12.45	10.65	14.42	15.35	10.65 to 19.65
2.18	4.83	5.17	3.61	2.56	4.82	2.18 to 5.17
24.40	30.49	26.50	17.48	20.15	26.10	17.48 to 30.49
0.73	0.16	0.53	0.20	0.36	0.00	0.00 to 0.73
1.45	1.12	1.78	1.00	1.28	1.66	1.00 to 1.98
9.47	10.08	14.42	13.88	12.98	9.62	4.04 to 14.42
6.55	14.80	18.15	12.28	12.61	11.29	6.55 to 18.15
25.48	21.90	17.25	23.75	19.20	22.75	19.20 to 29.83
15.29	11.25	16.20	16.30	17.92	16.84	11.25 to 17.92
5.10	3.55	5.52	3.01	5.31	3.29	3.01 to 7.02
0.00	0.00	0.18	0.00	0.18	0.18	0.00 to 0.18
8.74	5.32	3.56	10.45	7.50	5.74	3.56 to 10.45
1.45	0.80	1.06	1.00	1.10	1.48	0.00 to 1.66
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.73	0.32	0.35	1.00	0.73	0.92	0.32 to 1.30
0.73	0.00	0.18	0.00	0.36	0.18	0.00 to 0.73
75.70	70.20	79.30	83.00	79.60	74.10	70.20 to 83.00
3.10	2.30	2.98	4.75	3.95	2.84	2.30 to 4.75

TABLE V

COMPARISON OF DOG DATA WITH ANOTHER FEMORAL MYELOGRAM

Cell Types	Van Loon et al., 1943 (8 dogs)	Author, from Table IV (10 dogs)
Rubriblasts (pronormoblast)	0.0 to 0.7	0.00 to 0.60
Prorubricytes (basophilic normoblast)	4.2 to 8.4	2.74 to 5.64
Rubricytes (polychromatophilic normoblast)	13.3 to 19.8	10.65 to 19.65
Metarubricytes (orthochromatic normoblast)	7.6 to 21.1	2.18 to 5.17
Red series total		17.48 to 30.49
Myeloblasts	0.2 to 1.0	0.00 to 0.73
Progranulocytes (promyelocyte)	0.2 to 1.1	1.00 to 1.93
Myelocytes, neutrophilic	1.7 to 8.0	4.04 to 14.42
Metamyelocytes, neutrophilic (juvenile)	2.0 to 4.3	6.55 to 18.15
Band cells, neutrophilic (stab cell)	10.3 to 18.7	19.20 to 29.83
Segmented cells (polymorphonuclear)	30.1 to 39.7	11.25 to 17.92
Eosinophilic cells, all types	0.2 to 3.3	3.01 to 7.02
Basophilic cells, all types		0.00 to 0.18
Lymphocytes	0.0 to 3.4	3.56 to 10.45
Monocytes	0.0 to 0.1	0.00 to 1.66
Megakaryocytes	0.0 to 0.3	
Plasmacytes (plasma cell)		0.32 to 1.30
Reticulum cells		0.00 to 0.73
White series total		70.20 to 83.00
Unclassified cells (disintegrated cells)	0.4 to 3.1	
Myeloid-Erythroid ratio		2.30 to 4.75

DISCUSSION

Dogs are subject to a wide variety of diseases, which necessitates that the veterinarian have at his disposal an adequate armamentarium for making differential diagnoses as well as early prognoses. Blood examination, which has become an important adjuvant to both diagnosis and prognosis, has always suffered from the weakness of depending for its value on the inferences drawn from it. Knowledge of the changes in the formative tissues and the interpretations thereof have not been available to the veterinarian for practical application.

Marrow puncture is now used in human hospitals all over the world. This procedure should be available to the veterinary practitioner also so that he may correlate blood changes with those in the marrow.

A safe and practical method for doing marrow puncture, and trephination if necessary, must be provided.

Sternum, iliac crest, and dorsal processes of vertebrae are used in human medicine.

In the canine Bloom and Meyer (1944) proposed the sternum and iliac crest; Horn et al. (1953) suggested the sternum as sites for marrow puncture.

The author investigated the foregoing sites. While the quality of marrow in these bones was good, the size and the difficulty of executing the puncture on all sizes of dogs make them unacceptable in his opinion. Added to this was an admitted uncertainty of obtaining sufficient marrow plus a certain degree of danger of injury or even death to the patient.

The proximal third of the femur has been found to be a suitable site for marrow puncture as described in the section on Materials and Methods. Custer (1932) has stated repeatedly that the femur was a source of a good quality of marrow and has the advantage of being one of the first bones to respond to stimuli affecting bone marrow. Menkin (1943) also observed the early response of femoral marrow to stimuli. Femoral puncture was preferred because this site was easily accessible; it was a minor surgical procedure which did not cause the patient discomfort. There was absolutely no danger that a body cavity might be punctured while executing a femoral marrow biopsy. One can always be confident of obtaining a marrow sample in sufficient amount to prepare six or eight smears and have some

material for making either paraffin or frozen sections. Preparation of a few extra smears enhanced the chance of getting superior quality marrow films. Many of the marrow cells were very delicate, which may result in films containing many cells with ruptured cell membranes.

Sections were desirable and even indispensable in some cases. In the section on Presentation and Evaluation of Findings a case was cited where an opinion based on cytology alone resulted in a wrong diagnosis (Block, 1950). Hutchinson (1953) reported the advantage of marrow sections in determining stainable iron by a histochemical test using Prussian blue. He stated that not only does it indicate when iron was required but also when enough has been given and so can be used to obviate the danger of overdosage when iron was being intravenously administered. This method could be used to differentiate between iron deficiency and chronic intoxication anemias.

Another clinical procedure to which marrow substance lends itself was a determination of phosphatase content if malignancy was suspected (Kerppola, 1951).

Wolff and Limarzi (1945) stated that the most accurate criterion for the diagnosis and treatment of the true anemia of pregnancy was the study of bone marrow. The changes in the peripheral blood were of a secondary nature, and in many instances were misleading.

Several cases of bacterial and protozoon diseases have been diagnosed as a result of bone marrow findings (Anon., 1940).

It is only reasonable to hope that the practicing veterinarian will take advantage of the many opportunities bone marrow offers in arriving at more correct diagnoses.

The importance of the reticulo-endothelial system of the mammalian body as a mechanism of defense against disease is well known. Bone marrow offers an easy, safe, and sure way to examine this highly specialized mechanism. The reticulo-endothelial system of bone marrow is more widely distributed than that of any other single organ. Bone marrow biopsy as a means of getting a look into this mechanism is unquestionably much safer than the highly dangerous liver and spleen biopsies. Thus marrow examination may be of inestimable value diagnostically and prognostically. It may prevent more costly diagnostic examinations and sometimes surgical expense.

The author realizes that the practical value of bone marrow examination in veterinary medicine will have to be proven. At the present time it appears that marrow biopsy evaluation will have to be based on qualitative findings, myelogram and sections of small portions of the total marrow substance.

Quantitative studies of bone marrow have been carried out during the last twenty years. Isaacs (1937) reported a method of counting the cells in one cubic millimeter of marrow, claiming good results. Meyer and Bloom (1941) stated that their counts per cubic millimeter of marrow varied from 56,000 to 317,000 cells, almost 600 per cent. They believed their method had diagnostic value. In the opinion of the author the approach of Reich and Kolb (1942) to the problem of quantitative study of bone marrow was truly scientific in every way. Multiple sternal marrow samples were taken from twenty-six patients. Statistical analysis indicated that quantitative determinations on aspirated marrow samples were inaccurate. They stated, however, that qualitative marrow studies were invaluable in establishing hematologic diagnoses.

The author has called attention to the variation of the quality of marrow in dogs within areas of only a few millimeters. Custer (1932) pointed out a similar variation in human bone marrow. Calhoun (1954) observed similar variation in equine and bovine bone marrow. Quantitative counts do not appear to have any value diagnostically because of the nature of myeloid tissue. However, much difference of opinion exists as has been pointed out by Calhoun (1954).

Determination of the ratio of the total number of myeloid cells to the number of erythroid cells appeared to be a good criterion of bone marrow activity. It should be a valuable clue to some of the anemias, leukopenias, leukocytoses, and other blood dyscrasias. A comparison could be made between the ratio of the count of the smear and the picture obtained from sections.

Rohr (1949) has found the myeloid-erythroid ratio of the human adult to be three to one.

Some discrepancy existed between the two cases on dogs reported in the literature and the findings of the author.

It was appreciated that the number of cases examined during this investigation was not sufficient to be conclusive. A comparison might be permissible because the dogs used in this investigation were known to be healthy animals as far as could be determined.

The object of the cytologic study of bone marrow of the dog by Stasney and Higgens (1937) was to determine if any difference existed in the quality of marrow from different sites. The dogs used had been subjected to other experimental work, which could have been responsible for the results: a myeloid ratio of 0.53:1.00 average.

The dogs of Meyer and Bloom (1943), as far as could be determined, were out-patient cases apparently in good health. The

myeloid-erythroid ratio varied from 0.76:1.00 to 2.53:1.00 in ten cases.

The author's cases showed a myeloid-erythroid ratio of approximately three to one. This agreed with the conditions reported in human medicine. The life span of the red blood cell is one hundred and twenty-seven days in the human subject (Shemin and Rittenberg, 1946); for the dog this has been found to be one hundred and twenty-four days (Hawkins and Whipple, 1938). It can be seen that the blood picture of the dog is not too dissimilar to that of man.

Recently the author determined the myeloid-erythroid ratio of a known frank case of anemia in a dog: it was two to one. The animal had had melena for several months. An abdominal tumor was shown to be a leiomyosarcoma originating in the muscular wall of the jejunum. The melena and anemia were due to the hemorrhaging tumor.

The values given by Stasney and Higgins and the lowest values of Meyer and Bloom would indicate that the number of cells of the erythropoietic system of bone marrow was greater than those of the leukopoietic series. This appeared incredible inasmuch as no observation had been made which could even approach such low ratios in normal animals.

SUMMARY

A necessity for improving and expanding diagnostic facilities in the field of veterinary practice has been realized, and a step in that direction has been attempted.

Evidence has been presented to prove that diagnoses based on cytology alone are apt to be unreliable. It is suggested that information derived from sections and smears should be correlated with findings in the peripheral blood.

The clinician is cautioned to correlate anamnesis, signalment, present illness, peripheral blood, and bone marrow findings. He should also take advantage of necropsy material whenever it is available.

Anatomy, histology, and cytology are described, pointing out some features peculiar to the dog.

The latest nomenclature for cells of the blood and the blood-forming organs is presented in Table III.

It is emphasized that a wide variation existed in the quality and distribution of marrow in the same bone. Qualitative cell counts of marrow were an invaluable aid to hematologic findings, whereas quantitative counts were inaccurate.

A method was devised for making whole marrow sections for microscopic study.

A new site for doing marrow punctures has been suggested, and a method for evaluating gross and microscopic specimens has been outlined.

An improved instrument has been made to facilitate biopsy procedures in small animals.

CONCLUSIONS

1. A technique of femoral puncture was described which can be done with absolute safety and a minimum of discomfort to the patient.

2. A series of conditions were reported which show that study of the bone marrow obtained in this way is of great assistance in clinical diagnosis.

3. Five types of marrow were described to aid in differentiation of normal and abnormal tissues.

4. Bone marrow biopsy gives the clinician an insight into the fundamental processes underlying his findings in the peripheral blood, and permits him to observe the direct effect of therapy on the formative tissue.

5. Finally the author sincerely hopes and expresses herewith the genuine desire that the study of bone marrow may take the place in veterinary diagnostic procedure it rightfully deserves.

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