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A cytological study of the costal marrow of the adult horse and cow

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A CYTOLOGICAL STUDY OF THE COSTAL MARROW OF THE ADULT HORSE AND COW

by

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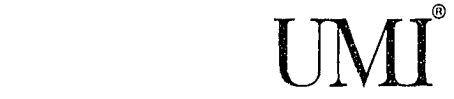

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INTRODUCTION

Important contributions have been made to the microscopic anatomy of bone marrow in man, monkey, and small laboratory animals. A search of the literature failed to reveal any similar readily available studies on farm animals. In view of the increasing importance of bone marrow as a diagnostic agent, and as a contribution to a field of histology relatively unexplored, this study of the bone marrow of the horse and cow was undertaken.

HISTORY

According to Michels (1931a), Jan Swammerdam first observed the red blood cells in 1658. Wiseman (1934) stated that hematology was first established as a branch of scientific medicine in 1770, but it was not until 1838 that Weber first saw nucleated red blood cells in man. Scott (1939) found that Robin described nucleated cells of the red marrow in 1849. Historians such as Sabin (1928), Michels (1931a) and Scott (1939) agreed with Stricker (1870) that Neuman (1868) was the first to associate blood formation with bone marrow, that Bizzozero (1868) confirmed his work that same year, and that Claude Bernard reached the same conclusions the following year. They all worked on human and rabbit material. According to Wilson (1942) the bone marrow picture in pernicious anemia was first described by Pepper in 1875 and Cohnheim in 1876. Doan (1939) felt that real progress in hematology began in 1891 when Ehrlich reported the effects of specific dyes on the blood cells. Sabin (1922) imputed the formation of our knowl-

edge of blood to Ehrlich. Wolff (1903) performed bone marrow punctures on experimental animals. According to Scott (1939) and Wintrobe (1942), Pianese executed a human marrow biopsy in 1903. Ghedini (1910) conducted similar clinical biopsies on human subjects in 1908. These workers and their followers all used tibial or femoral marrow and it was not until 1923 that Seyfarth (1923) first introduced a sternal trephine method. Arinken (1929) followed with the sternal puncture technic which has become so popular at present.

Classic among the early works on bone marrow are: Pappenheim's (1899) article which dealt with a general comparison of one type of bone marrow cell with another in various ages, using rabbits and one dog; Dominici's (1901) papers on the structure of the hematopoietic system of mammals; Dantschakoff's (1908) thesis on the development of blood in the bird; Maximow's (1910) investigations including the embryonal histogenesis of the bone marrow of mammals; Ferrata's (1918) treatise on hematopoiesis and Sabin's (1922) dissertation on the origin of the cells of the blood. Further monumental publications on the subject of bone marrow include those of Schilling (1925), Askanazy (1927) and Sabin (1928). Gilmour (1941) made a remarkable contribution to the general subject of human intra-uterine and neonatal hematopoiesis.

Plum (1936) said that Rudolph Wagner attempted to count the formed elements of the blood in 1849 but that the first real count was made by Vierordt in 1852. Garrey and Bryan (1935) claimed that Nasse had been interested in blood counting prior to 1842 and had written a section on the subject in Wagner's "Handwörterbuch der Physiologie." Plum (1936) attributed the counting chamber to a Dutch investigator named Cramer working in

1855, with further modifications by Gower in 1877 and Alferow in 1884. In 1864 Welcher was using distilled water as a diluent for leucocyte counting but Thoma later discovered that the addition of acetic acid reduced the amount of water necessary (Plum, 1936). Potain introduced the pipette in 1867 (Garrey and Bryan, 1935). These same historians credited the development and precision of the pipette and cross-ruled chambers to Welcher in 1854, Abbe in 1878 and Lyon and Thoma in 1881.

LITERATURE REVIEW

The study of hematology has only recently come to the front in veterinary medicine. Wittmann (1928) stressed its importance in the field of animal disease, veterinary surgery, and animal breeding. However, as early as 1906, Zuntz, et al, made histological studies on the bone marrow of dogs at sea level, and on animals acclimated to higher levels. Notable contributions on the subject of normal monkey bone marrow have been made by Suárez, Diaz-Rivera and Hernandez-Morales (1943) and Stasney and Higgins (1936). Science is indebted to Alexandrov (1930), Fairman and Whipple (1933), Stasney and Higgins (1937), Mulligan (1941, 1945), Van Loon and Clark (1943), Eberl (1943) and Bloom and Meyer (1944) for studies on the bone marrow in the dog. In the first decade of this century Külbs (1909) worked on the development of bone marrow using the dog as an experimental animal. Bloom (1944) compared blood and bone marrow in relation to pyometra in the dog.

A variety of studies has been carried out on rabbit bone marrow including the work of Lim, Sarkar and Brown (1922) which entailed the establishing of the normal histological picture in a study of the effect of feed-

ing thyroid, Sabin and Doan's (1927) work on the bone marrow and blood in developing rabbits by Sabin, et al, (1936), the lymphocyte content by Yoffey and Parnell (1944), the distribution of bone marrow, bone and bone ash (Dietz 1944), and Jordan's (1920) study of the giant cells of the rabbit and guinea pig bone marrow. Sundberg and Downey (1942) compared the lymphoid cells of lymph nodes and bone marrow of rabbits and guinea pigs. Epstein and Tompkins (1943) used the guinea pig as an experimental animal in a comparison of techniques for the differential counting of bone marrow cells. Mouriquand, Revol and Edel (1944) chose the femur as the site for a study of the myelogram in normal guinea pigs. Similar studies were made by Fairman and Corner (1934), Milman, Listengarten and Kurbanaliew (1934), Stasney and Higgins (1935), Kindred (1940) and (1942), Plum (1943) and Endicott and Ott (1945) on the albino rat. A normal bone marrow study on white mice was made by Petri (1934). Hammon and Enders (1939a and b), Lawrence et al (1940) and Riser (1943) studied the bone marrow in connection with infectious panleucopenia of cats. Corradetti (1934) studied the bone marrow of new born cats in connection with some work on the blood and bone marrow of healthy human beings. Gütig (1908) made a survey of the bone marrow in swine while studying the morphology of the blood of the species. Downey (1915) used the adult guinea pig to determine the origin and development of both the eosinophil and the "hematogenous mast cell." Barbieri (1935) studied the alteration of the bone marrow in association with hepatic distomoniasis in sheep. Ringoen (1921) investigated the bone marrow of the sheep and pig to trace the origin of the eosinophil.

Other investigations which serve to stress the growing importance of the bone marrow as an experimental organ and the need for knowledge of the normal, include such works as the following: Beilicke (1938) observed

the effect of iron on the blood and bone marrow of rabbits; Castrodale, et al, (1941) used dogs to study the comparative effects of estradiol and stilbestrol upon the blood, liver and bone marrow; Menkin (1943) studied the effect of the leucocytosis-promoting factor on the femoral bone marrow of dogs; Iantria (1934) investigated the cytology of the erythroblast in the growing guinea pig embryo; Smith and Hastings (1935) made a study of the megakaryocyte and blood platelet of the rat; Dougherty, Williams, and Gardner (1943) reported the changes in the myeloid and lymphoid tissues of estrogen treated dogs; temperature variations between central and outlying bone marrow of the rabbit, pigeon and albino rat were discussed by Huggins, Blocksam and Noonan (1936); Huddleson and Munger (1937) gave their attention to the phagocytic activity of bone marrow cells with the guinea pig as an experimental animal; Potter and Ward (1940) investigated the development of the megakaryocyte in adult mice, and Nettleship (1942) discussed the rabbit-bone-marrow changes produced by specific antibodies. Likewise Holderlin (1938) dealt with the bone marrow and blood picture in the sensitized rabbit. The effects of sulfanilamide on the bone marrow of rats was presented by Higgins and Machella (1939). Damade and Leger (1939) gave the cellular percentages in bone marrow following experimental anemia in the rabbit.

Little work has been done on the horse and cow. Due to war conditions and failure to receive foreign journals part of the work that has been done is not available. Ackernecht (1912) was probably the first to work on the horse. He spoke of the gross changes occurring with age and gave a brief description of the marrow cells. Varicak (1935) studied the marrow of bones of trunk of horses, cattle, swine, dogs and cats. Hjarre and Berthelsen (1938) presented detailed cellular counts of the bone marrow of ten nor-

mal horses and compared that with a similar count in horses with infectious anemia. Tkachenko* (1940) published a paper on the morphology of erythroblasts and myeloblasts in normal horse bone marrow. Hjärre (1943) described the normal sternal punctate in the domestic animals including a comparison of the dog, swine, cow and horse with man. Other studies on bovine marrow include a thesis on bone marrow puncture by Hölzel* (1939), the development of marrow in the metatarsus by Hrestak* (1941) and the work of Marcato (1941a) on the normal bone marrow. The same year Marcato (1941b) made a study of bone marrow in bovine fascioliasis. Mitchell (1940) discussed hyperplasia of the bone marrow and osteohematochromatosis in a yearling steer and stated that the bone marrow "exhibited histological changes." No normal picture was given. Stasney and Feldman (1938) made hematologic and histologic studies of the bone marrow from the femur of a calf with leukemic lymphoblastoma but again no normal was used for comparison. Richter (1938) in discussing leucemia in animals said of cattle that lesions were not found in the bone marrow. According to Jarmai (1934) histological investigations of the bone marrow of the cow in leucosis were made by Endres (1921), Lengwenant (1931) and Töllner (1931). However, they did not mention any histological studies being made. Jarmai further stated that in the literature review of leucosis in the horse, "bone marrow was seldom mentioned perhaps because no variation was apparent." Ellenberger (1931) gave a brief resume of the bone marrow in domestic animals with scant reference to any specific animal.

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*Not available

GROSS AND MICROSCOPIC ANATOMY OF BONE MARROW

The medullary cavity of the bones of the young of all species is filled with red marrow. As age increases red bone marrow is gradually replaced by a labile,yellow,fatty marrow until in the adult, red marrow is confined largely to the axial skeleton. According to Ackerneckt (1912) red marrow of the adult horse is confined to the proximal ends of the femur and humerus, the pelvic girdle, vertebrae, ribs and sternum. Varićak (1935) studied the bones of 100 horses and 300 head of cattle macroscopically. He found that the deposition of fatty marrow occurred much earlier in cattle than in horses. This process started in the axial skeleton as early as 9 months in cattle. While he determined no definite age for the transition from red to yellow marrow in horses he rarely observed fatty areas in the axial skeleton of any horse under 8 years of age. In a study of the ribs of a 10 year old horse,Varićak observed that active marrow was present for a distance of 20 cm. in the vertebral end, that the central part had both red and yellow marrow, while the sternal end was filled with fat. The 14th rib of the same animal had only a little fatty marrow in the ventral end. In one 30 year old horse no fatty marrow was apparent macroscopically in the ribs. Varićak would explain the few granulocytes and the large numbers of lymphocytes in the blood of cattle by the large amount of fatty marrow. Some of the thoracic vertebrae were completely filled with fatty marrow. As in the horse, the dorsal ends of the ribs retained active marrow. The central part had both red and yellow marrow and the sternal end became a reservoir for fat. The sternum too had areas of fatty marrow in it. By microscopic examination he observed that some of the fatty marrow centers contained foci of red marrow. Trautman and

Fiebiger*(1931) found gelatinous marrow in older horses. Varicek (1935) did not find gelatinous marrow in healthy, active,aged horses. According to Cowdry (1942) gelatinous marrow should be expected in very old people. By studying the tibia, femur, rib, sternum and vertebrae of man, Custer and Ahlfeldt (1932) ascertained that the cellularity of red marrow decreased with advancing years. Specific gravity of bone marrow was found to be only slightly more than 1.0 (Yoffey and Parnell, 1944).

Sisson and Grossman (1938) attributed the blood supply of the long bones to the large medullary or nutrient artery which enters the nutrient foramen, extends through the canal in the compact bone and ramifies in the marrow. A satellite vein follows the opposite course. Doan (1922) found that periosteal vessels along the shaft and some of the vessels near the extremities furnished additional blood supply to the bone marrow. The venous drainage corresponds to the arterial supply. According to Doan (1922) the thin walled venous sinusoids making up the vascular bed of the marrow are the most characteristic feature of the gross circulation. He and Doan, Cunningham and Sabin (1925) contended that the vascular system is a closed system.

Osgood and Seaman (1944) described the human marrow as the "largest, most widely dispersed, and least homogeneous organ in the body" with a volume one or two times that of the liver. Cowdry (1944) stated that marrow makes up about 5 per cent of the total body weight. Mechanik (1926) gave the weight of the bone marrow as 3.4 - 5.9% of the body weight and estimated 5/9 gm. of marrow to 1 gm. of blood. Dietz (1944) gave some interesting statistics on the relative weight of rabbit bone marrow; 1/3 the weight of the skeleton, 2.2% of the total body weight, 2/3 the weight of the liver and 50 times that of spleen.

*See Ellenberger (1931)

According to Piney (1922), Trautman and Fiebiger*(1931) and Wolff (1933) there are no lymphatics in the marrow. Fischer (1917) found lymph nodules in 38 out of 61 human cases investigated. Lymph nodules were considered by Mayer and Furuta (1924) and Williams (1939) to be a normal but variable constituent of human marrow. Trautman and Fiebiger*(1931) credited man and cat marrow with lymph nodules. Wolff (1933) did not find nodules with any regularity. Drinker and Yoffey (1941) concluded that "follicular accumulations in the marrow do not seem to be true lymphoid nodules with cells showing active division."

Apparently the nerve supply in man is confined to the walls of the blood vessels and no ganglion cells have been found (Wolff 1933). Ackerneckt (1912) found nerve bundles in the humerus of the horse.

MATERIAL AND METHODS

Obtaining the Samples

The cattle used in this investigation consisted of 13 cows and 1 bull in a herd used by the Department of Veterinary Obstetrics at Iowa State College. The horses were a miscellaneous group of animals, five of which were brought in to the Department of Veterinary Anatomy at Iowa State College and the other two to the clinic at Michigan State College. All the horses were old but apparently free from disease as far as could be determined by general appearence and blood determinations.

The first problem was to determine a satisfactory place to secure a marrow sample. Since both the horse and cow have a heavy musculature covering the sternum, it seemed wise to try to find an area more accessible. Hjärre and Berthelsen (1938) contended that a sternal puncture was easily executed in the horse and they did not observe any complications from the proce-

*See Ellenberger (1931)

dure. The animal was confined in a prone position and an especially constructed trocar was driven into the middle or back sternebra by a "light" tapping with a hard rubber hammer. In any sternum that was examined by the author no light tapping would succeed in penetrating the wall of the sternebrae. Also to have to confine such large animals in a lying position seemed a distinct disadvantage. According to Varicak (1935) the sternum of the horse was not suitable because of large muscles and such a procedure in the cow was awkward and time consuming. According to Ackerneckt (1912) the red marrow of old horses is confined to the sternum, ribs, vertebrae, proximal ends of the femur and tibia and to the ilium. After several unsuccessful attempts to obtain marrow from the femur, ilium and mandible of the horse, the ribs were chosen as the best site. Plate I, Fig. I shows the general area and Plate I, Fig. 2, illustrates the fact that parts of the 8th to 18th ribs are relatively exposed in that region being covered only by skin and fascia. It is well to go as high as possible and still avoid the latissimus dorsi (k) and serratus posticus (m) muscles because it seemed to be difficult to obtain sufficient marrow more ventrally. The same technic was applied to the cow using the 11th, 12th or 13th rib. (Plate II, Figs. 1 and 2) The animal may be confined in a stock or restrained against one side of a stall. Little or no resistance to the operation was ordinarily encountered. It is advisable to brush the back and side of the animal with a grooming brush. Wiping the surrounding area, particularly above the prospective operative site with a damp cloth may also aid in removing some dust and particles which might later fall into the open wound. The general area was palpated until the rib which had the least amount of fascia covering it was located. The chosen site was shaved or the hair clipped closely, the area was washed with a soap solution and iodine applied. A local anesthetic such as 2%

procaine hydrochloride was next administered. The skin was anesthetized first, the underlying fascia next and finally the periosteum, using about 10 cc. of the procaine solution. After a few minutes a short incision was made in the skin and then the fascia and periosteum were incised. A No. 487 Goodell-Pratt* hand drill as shown in Plate III, equipped with a straight shank 3/32" jobbers' drill, was used to bore into the marrow cavity. A point midway between the anterior and posterior borders of the rib should be chosen for insertion of the drill because there is danger of missing the marrow cavity completely if the drill goes through either border. Such an accident would entail the danger of penetrating the thoracic cavity. The drill "gives" when it hits the marrow, so it was not difficult to sense when the marrow was reached. The drill was removed from the rib and a cannula with stilet with the same outside diameter as the drill was inserted into the drill hole. A Jen-Sal needle trocar, J. S. 39121 in the 1940 catalogue** was used. The stilet was removed and an air tight 10 cc. syringe attached to the cannula. One cc. or less of marrow was then drawn into the syringe.

The syringe was separated from the cannula and the marrow ejected into an oxalate tube to prevent coagulation (See page 13). The tube was held in a horizontal position and tapped to mix the marrow and oxalate. After obtaining the sample the cannula was withdrawn, iodine applied and the incision left open. Healing took place rapidly and after the hair had grown out the site of the incision could not be determined. Varicák (1935) found that trephining the rib need not leave a scar. With the exception of the necessity for drilling through bone and the use of a cannula and stilet the above procedure was

*Goodell-Pratt Company, Greenfield, Mass.
**Jen-Sal Laboratories, Kansas City, Missouri.

patterned after that of Osgood and Brownlee (1937) for sternal puncture in man. All the necessary instruments and supplies are illustrated in Plate III.

There has been some criticism of withdrawing more than 1 cc. (Isaacs, et al, 1940) on the basis that the actual marrow withdrawn is too diluted with circulating blood. Jaffé (1936) suggested 0.1 or 0.2 cc. According to Hjärre (1943) the sample should be more viscous than blood and greyish red in color.

It is possible to enter a large sinusoid and withdraw material that differs little from peripheral blood, (Jones 1940 and Hjärre 1943). Should this occur, the procedure should be repeated at a different level or on a different rib. Slides were taken to the operative site in event fresh smears were ever desired.

The state of the marrow can only be determined after having analyzed the blood picture (Sabin 1928). Managh (1940) suggested that correlation of the marrow picture with that of the peripheral blood might eventually result in the ability to read the marrow hemogram by the blood findings alone.

Blood samples were obtained prior to procuring the marrow as the latter procedure might excite the animal enough to alter the blood picture. The jugular vein was chosen as the logical place to obtain such a sample. The blood was directed into an oxalate tube and shaken well. The usual aseptic venipuncture technic for large animals was followed. Conner (1945) described this technic.

The samples were taken to the laboratory and blood and bone marrow smears made immediately. Then total red and white counts were done on the blood and the amount of hemoglobin determined. Osgood's (1940) technic was

followed. Total red and nucleated cell counts on the marrow samples were purposely omitted because there was such a large variation due to dilution with circulating blood that the data seemed to have little or no value. Nordensen (1935) and Kandel and Leroy (1939), working with human bone marrow, found this to be true also.

The above technic was designed for the living animal and gives the optimum normal cytological picture. However, the occasion may present itself that postmortem-marrow examination would be necessary. Wintrobe (1942) felt that the sample should be secured within 2 hours after death to get good preparations as autolysis occurs soon. Rohr and Hafter (1937) made a special study of postmortem changes in human bone marrow. They concluded that death had little effect on the myeloid elements, but the erythroblast matured with a subsequent change from polychromatic to oxyphilic forms and extrusion of the nuclei. They also observed a decrease in the number of neutrophils beginning a few minutes postmortem. The nuclei of the remaining neutrophils swelled and became vacuolated after 2 hours.

Oxalate Tubes

The blood was collected in ordinary test tubes. Dunham fermentation tubes were used for the marrow because they were short and had a relatively small bore. Consequently the marrow and oxalate could be mixed more thoroughly.

The oxalate tubes were prepared by evaporating to dryness 0.1 cc. of a 2% potassium oxalate solution for each cubic centimeter of blood or marrow (2 mg. of dry potassium oxalate per cc.). The requisite amount of oxalate solution was measured into the tubes and they were placed in a drying oven.

Maurer and Jones (1943) evaporated the oxalate solution at a temperature below 80° C. since higher temperatures converted some of the potassium

oxalate to carbonate and consequently coagulation was not inhibited. Too much oxalate tended to distort the normal cell structure. The cells appeared shrunken and the staining was too intense.

Osgood, Haskins and Trotman (1931) recommended oxalated blood because of greater convenience and believed its use resulted in a higher degree of accuracy since the sample was larger, more time could be taken and duplicate estimations could be run. They suggested a 24 hour time limit for hemoglobin readings and total red and white blood cell counts, and a one hour limit for the smear differential. In some investigations at the University of Minnesota, Kernkamp (1942) concluded that if oxalated blood samples were examined within 60 to 90 minutes after obtaining the blood, few changes resulted. Maurer and Jones (1943) agreed with this view.

Staining and Counting Technic

The slides were soaked in sulphuric acid-potassium dichromate cleaning solution 24 hours, rinsed in running tap water for another 24 hours, rinsed in distilled water and stored in 70% alcohol. They were dried as needed.

A 24-gauge bacteriology platinum wire loop was used to remove the blood and marrow from the tubes in making the smears. To prepare the smears, the drop of blood or marrow was placed on the right end of a clean slide. A second glass slide, resting at an angle of about 45° with the first one, was moved along the first slide from the left until contact of the second slide was made with the drop. This junction spread the blood out along the width of the slide. With the second slide still at the same angle, it was pushed to the left and the blood spread out in a smear behind it. The smear was waved in the air to insure rapid drying. In a properly prepared smear the cells should not touch and the end of the smear should reflect rainbow colors.

To stain, the smear was marked off with a wax pencil thus requiring a minimum of staining solution. Wright's staining technic as outlined by Osgood and Ashworth (1937) was followed. One-tenth gm. of Wright's dry stain was mixed with 20 cc. of acetone-free methyl alcohol and left over night before use. A buffer solution with a pH of 6.4 was prepared by dissolving 6.63 gms. of monopotassium phosphate and 3.56 gms. of anhydrous disodium phosphate in a liter of distilled water. One cc. of chloroform was added. Six to 12 drops of the dye, depending on the size of the smear, were left on the slide for two minutes, then diluted with an equal number of drops of buffer and left seven minutes longer. With the slide in a horizontal position the staining solution was rinsed off and the slide washed for thirty seconds with a brisk stream of running tap water. The slides were air dried. No cover slip was applied. Instead the smear was covered with a thin film of immersion oil at the time of examination.

In this study the filled counting chambers were examined with the low power objective for general uniformity of distribution, bubbles, or any foreign material. If the initial survey revealed no inequality of cell dispersion, bubbles or other artefacts the counts were made. Differences exceeding the "standard limits" were not discredited. Both sides of the chamber were counted and totals were determined from those figures.

Hemoglobin readings were made on a Dare hemoglobinometer. Although this instrument would not be precise enough for research specifically on hemoglobin it was deemed sufficiently accurate for use here.

Plum (1936) was of the opinion that the unequal distribution of the cells in the blood smear played the predominant role in the error of individual counts. McGregor, Richards and Loh (1940) claimed that the "battle-

ment edge" count gave the greatest accuracy. This consisted of starting on the edge of the smear, going a millimeter in from the edge, the same distance across, another millimeter back to the edge and an equal distance horizontally along the edge, continuing this pattern in the same direction until 300 cells had been counted.

Hay (1942) recommended counting 300 or 400 white blood cells to reduce the error of random sampling. He found further that the choice of area in which to count the cells did not play as important part as thought by earlier investigators. With the low power magnification a field was chosen in which there was an even distribution of nucleated cells, the red cells did not overlap and staining was sharp. The edge of the smear was avoided. In the blood smears 300 cells were counted at random by going back and forth across the smear.

A Spencer research microscope with widefield 9x oculars, and 16, 8, 4 and 1.8 mm. objectives was used. All differential counts were made with the 1.8 mm. oil immersion objective, magnification 95x, making a magnification of 855x. A Spencer lamp model 370 was the source of light. A 100 watt bulb was customarily used but for cytological details a 200 or even a 300 watt bulb will bring out special structures.

Thoma pipettes were used throughout for diluting the blood for total counts. The Neubauer "Bright Line" counting chamber was employed. Separate counting chambers were used for the red and white counts. This avoided any possibility of carrying over any of the acetic acid used in the white count to that of the red count.

Standard textbooks on hematological technics call for a variation of less than 18 to 20 cells between the 5 groups of squares for the red count and

variation not to exceed 8 or 10 cells between squares in the white count. This would be within limits of 180,000 to 200,000 and 160 to 200 cells respectively for total counts. Windle, Sweet and Whitehead (1940) accepted an agreement of two counts within limits of 100,000 for the red blood cells and 250 for white blood cells. Berkson, Magath, and Hurn (1935) and Magath, Berkson and Hurn (1936) showed that such stringent limits are not statistically probable. To the contrary they found that greater differences than those would be normally expected in from 50 to more than 90 percent of the counts. Ohlson (1945) made a study of sampling methods in connection with erythrocyte determinations. From data obtained with haemocytometers that presented a uniform distribution of cells and no bubbles, the following conclusions were drawn: (1) significant differences occurred between subjects; (2) differences bordering on significance might occur between pipettes; (3) with the trained technician there was no statistically significant difference between successive counts on the same sample even though differences exceeding 20 cells existed between the 5 groups of squares.

In the differential cell count of the marrow 500 cells were enumerated. Stasney and Higgins (1935) (1939), Osgood (1937), Kingery, Osgood and Illge (1937), Pitts and Packham (1939) and Bloom and Meyer (1944) enumerated 500 cells. Wintrobe (1942) stated that 500-1000 should be counted. Davidson, Davis and Innes (1942) suggested observing 400 or 500 cells, Hjärre (1943) counted 800 cells and Wilson (1942) and Thomson (1944) counted 1000 cells. Reich, Swirsky and Smith (1944) did not do differential counts "owing to the inaccuracy of the method." In a subsequent personal communication Dr. Reich said that the inaccuracies that they found might not exist in this work.

According to Cowdry (1944) a bone marrow smear will contain endothelial cells from the capillaries, some reticular cells, and connective tissue fibers in addition to the developing blood cells. Cells are frequently injured and the cytoplasm may have been torn and the nucleus drawn out in strands. Other cells appear to be dying. These injured and dying cells have been given various names such as degenerate, smudge, smear, or basket cells. Since Hynes (1939) considered these cells to be "largely fortuitous and merely distort the picture if they are included in the differential count" and Bloom (1943) thought them artefacts and not necessary to include, it was decided to omit them from the differential count.

RESULTS

Blood Studies

Miller (1932) made 81 determinations of the blood volume of cattle. The average quantity of blood per pound of body weight was 27.07 cc. An increase in blood volume occurred in pregnancy.

Wirth and Mader (1938) and Zemljič (1935) stated that the ox has a lymphocytic blood picture.* They characterized ox blood as having azure granules in the lymphocytes, occasional stab cells, small eosinophilic granules, and commented on the especial size of the neutrophil. According to Goodall (1910) the general morphology of ox blood does not differ greatly from human blood. Du Toit (1916) also found ox blood similar to human blood except that it had a high lymphocyte count and a low neutrophil count when compared with man. Gudim-Lewkowitsch (1929) in a study of the differential number of the neutrophils in 15 normal cows gave the following data: juveniles, 0.5%; stab, 45.7%; 2-lobed, 35.7%; 3-lobed, 14.9%; 4-lobed, 2.9%; and 5-lobed, 0.6%. Zemljič's (1935) results on 50 animals differed somewhat with 12.3% juveniles,

*Majority of white blood cells were lymphocytes.

21.6% stab cells and 66.1% segmented. Fraser (1930) found 0.1% juveniles, 3.5% stab and 96.3% segmented. Basel and Levek (1928) stated that there were no myelocytes, 0.1% juveniles, 2.8% stab cells and 41.7% segmented neutrophils in normal ox blood. Kennedy and Climenko (1931) reported the normal Arneth count for the cow as 2.34 (weighted mean).

Goodall (1910) characterized the neutrophil of horse blood as having an unusual degree of lobation and especially fine granules. He found the eosinophil to contain large spherical or ovoid granules. Wirth (1931) mentioned the large eosinophil, large basophil, large platelets and preponderance of neutrophils in the horse.

It seems feasible, since any so-called "normal" limits of either blood or bone marrow must be arbitrarily chosen, that some animals without evidence of abnormality may have counts that do not fall within the range ordinarily considered normal. Shukers, Langston and Day (1938) were of this opinion. Tables 1 and 2 summarize the literature on blood studies of cattle and horses. Tables 3 and 4 present the hemograms of the experimental animals used in this study.

Table 1

Hemograms reported on cattle

Author	No. of Animals	Hemoglobin content*** %	Hemoglobin content*** Gm. per 100 cc.	R.B.C. millions per cu.mm.	W.B.C. hundreds per cu.mm.	Differential count, per cent: Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Dimock & Thompson (1906)	**3M 18F	59.75	8.25	6.1526	54.86	30.49	54.22	1.47	13.15	0.59
DuToit (1916)	7F	62	8.5	6.54	78.6	38.8	49	3.7	8	0.5
Kuhl (1919)						24	65	10	5	0-1
Meyer (1924)						44.3	46.7	5.3	3.7	
Basel & Lewek (1928)						44.6	44	1.6	9.6	0.2
Kohanawa (1928)	12			6.779	82.1	33.0	51.7	4.3	10.9	0.1
Sergent et al (1929)	39				10.0	22	59.5	7.5	10	1.0
Canham (1930)	12F dry			6.66	97	34.0	53	4.0	8.0	1.0
Fraser (1930)	41					28.4	54.7	6.7	9.9	0.1
Scarborough*(1931-32)		60	8.26	6.6	93	31.9	55.4	5.2	7.7	0.62
Wirth (1931)		60-80	8.26-11	5-7	50-100 Av.	25-50 30	37-63 50	3-10 5	3-8 6	0-0.5 0.1
Miller (1933)	56F	87.1	12	6.325	86.16					
Thormahlen (1935)				6.0	82.2	30-40	50	7	10	1.0
Zemljic (1935)	50			6.1169	70.08	32.42	52.52	5.79	8.96	0.8
Thijn (1936)	12				46-109.6	32-54	30-48	1-5	6-23	0-1
Bell & Irwin (1938)				6.1228	95.74	34.89	42.27	9.46	12.4	1.003
Wirth & Mader (1938)				5-7	50-100	39	50	5	6	0.1
Delaune (1939)	5			6.39	102.25	25.9	58.1	8.0	7.0	
Delaune & Mayhew (1941)	4			6.05-6.80	95.30-107.63	19.1-29.8	56-61.6	7.2-9.0	5-15	
Ferguson et al (1945)	25F			6.3291	89.1152	34.73	41.24	7.94	14.87	0.62

*Literature review

**F-female, M-male

***The percent of hemoglobin was converted to grams from conversion tables of Bausch and Lomb Optical Company. 1930

Table 2

Hemograms reported on horses

Author	No. of Animals	Hemoglobin content*** %	Hemoglobin content*** Gm. per 100 cc.	R.B.C. millions per cu.mm.	W.B.C. hundreds per cu.mm.	Differential count, per cent: Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Burnett* (1917)		62-11	9-11	5.5-10	65.-110	50-70	35-45	1.5-3.5	1.5-4	.2-.7
Habersang* (1921)				6.5-9.5	69.-110	50-75	15-45	1.5-14	.5-5.8	0-.7
Hauber (1924)	25F 21p**			7.8-11.0	67.-110	50-62	32-46	1.0-2.2	1.2-2.5	0.5-1.6
Meyer (1924)						60-70	28	3.3	4	0.3
Kohanawa (1928)	12			7.201	80.68	54.2	38.1	2.6	4.7	0.4
Dremjatsky et al (1929)	139	65-77	9-11	7.8-8.9	70-93	50-60	26-40	2.4-4-5	3.2-8	1.5
Scarborough* (1931-32)		70-95	10-13	7.8	92.6	56.8	30.4	8.5	3.7	0.46
Wirth (1931)				7-10	70-100	55-65	16-43	0.3-6	2-4	0.1-0.6
					Av.	60	35	3	3	0.5
Neser (1923)						45-60	30-45	2-8	3-9	0-1
Stewart (1940)	36			6-7	66.-118	36-72	13-56	1-8	1-28	0-3
Lamarre (1944)						45-60	30-45	2-8	3-9	0-1

*Literature review
**F-female, p-pregnant
***The percent of hemoglobin was converted to grams from conversion tables of Bausch and Lomb Optical Company. 1930.

Table 3

Hemograms of the cows used in this study

Animal Number	Hemoglobin content* %	Hemoglobin content* Gm. per 100 cc.	R.B.C. millions per cu.mm.	W.B.C. hundreds per cu.mm.	Differential count, per cent: Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
42243			7.545	14.14	46.7	37.3	10.0	6.0	0.0
42743			6.850	6.72	23.7	55.3	8.7	11.7	0.6
5643	120	16.51	7.940	7.06	19.3	67.3	5.3	7.7	0.3
52043	93	12.81	6.125	6.36	28.7	54.3	8.3	8.0	0.7
52743	78	10.74	6.790	6.86	19.7	64.7	6.0	9.0	0.6
61643	88	12.12	5.280	8.08	27.0	64.0	4.3	4.0	0.7
61843	85	11.7	6.865	4.26	41.67	44.0	4.3	9.7	0.3
62343	67	9.22	4.775	6.76	40.0	40.4	13.3	5.0	1.3
62843	63	8.67	4.530	6.82	31.0	52.4	7.3	9.0	0.3
7543	108	14.87	6.900	8.90	23.33	49.7	11.7	14.3	1.0
7743	85	11.7	6.760	10.32	17.33	63.0	4.7	14.0	1.0
71243	83	11.4	6.615	7.14	27.0	56.7	0.7	14.3	1.3
72143	75	10.33	5.110	7.66	51.0	33.0	2.0	13.3	0.7
72843M	90	12.39	5.880	7.54	37.7	53.3	5.0	3.7	0.3
Range	63-120	8.67-16.51	4.53-7.94	4.26-14.14	17.3-51.0	33.0-67.3	0.7-13.3	3.7-14.3	0.0-1.3
Average	86.3	11.87	6.2832	7.7585	31.01	53.17	6.61	9.33	0.68

*The percent of hemoglobin was converted to grams from conversion tables of Bausch and Lomb Optical Company. 1930.

Table 4

Hemograms of the horses used in the study

Animal Number	Hemoglobin content* %	Hemoglobin content* Gm. per 100 cc.	R. B. C. millions per cu. mm.	W. B. C. hundreds per cu. mm.	Differential count, per cent: Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
6943w	58	7.98	8.020	8.4	66.7	26.7	3.3	9.0	0.3
5843b	68	9.36	4.905	clotted	61.0	30.0	4.0	4.0	1.0
6843w	88	12.13	11.350	clotted	85.0	10.0	3.0	0.0	2.0
7943b	58	7.98	8.440	8.7	66.7	31	0.7	1.3	0.3
6943s M**	54	7.55	6.525	8.04	68.0	25.4	1.3	4.3	1.0
8344 M	80	11.02	12.040	9.92	43.4	44.0	2.3	9.0	1.3
92944	data lost before recorded				78.3	14.0	3.7	3.3	0.7
Range	54-88		4.905-12.040	8.34-9.92	43.4-85.0	10.0-44.0	0.7-4.0	0.0-9.0	0.3-2.0
Average	67.6	9.33	8.546	8.84	67.01	25.87	2.61	4.41	0.94

**M-male castrate

* The percent of hemoglobin was converted to grams from conversion tables of Bausch and Lomb Optical Company. 1930.

Bone Marrow Studies

Terminology

In 1912 Ackernecht remarked that no unity existed in the morphologic and histogenetic classification of marrow cells. In 1944 Osgood and Seaman were still pleading for "an approved 'Standard Nomenclature for Hematology'."

With the exception of Osgood and Ashworth (1937) and Pitts and Packham (1939) most authors have adhered to a generally uniform set of terms in describing the bone marrow cells. Unfortunately the same term did not always apply to the same type of cell. Sanchez (1941), Maximow and Bloom (1942), Israels (1943), and Piney and Hamilton-Paterson (1944) preferred the term "hemocytoblast" to "myeloblast" used by most all others. Mulligan (1941) chose the term "stem cell" which included the very early cells in all series. According to Osgood (1937) the monoblast, myeloblast, lymphoblast, plasmablast and megaloblast are distinct types of cells but Jordan (1939) stated: "to differentiate the various "blast" cells is to ignore individual differences between cells in a given series." Stasney and Higgins (1937) used in addition to myeloblast the word "leukoblast" to designate a cell between the myeloblast and the promyelocyte. Mallarme (1937) adhered to the term "leukoblast" instead of either myeloblast or hemocytoblast. Most investigators agreed on a "promyelocyte." Wintrobe (1942) divided the promyelocytes into "A" and "B" types. The nomenclature followed by the majority of authors to describe the developing granulocyte series included a myelocyte, a metamyelocyte and the adult granulocyte for the neutrophil, eosinophil and basophil. Reich (1935), Suarez (1936), Kandel and LeRoy (1939), Scott (1939) and Van Loon and Clark (1943) had a "band" neutrophil, Segerdahl (1935), Thaddea and Bakalos (1940) and Mulligan (1941) a "stab" neutrophil and Lichtenstein and

Nordenson (1939) preferred to use "rod" form instead of "stab" or "band". Doan (1939) and Rhoads and Miller (1938) used the letters "A", "B" and "C" to represent stages in the development of granulocytes or myelocytes. Yaguda (1936) used the terms "non-granular" and "granular" myelocyte to indicate stages in development. Holmes and Broun (1933) carried the Roman numerals II - V or VI with the neutrophils to indicate the number of lobes.

The technical expressions designating the red blood cell series may be divided into two main groups; the one, "proerythroblasts," erythroblasts," and "normoblasts" accepted by Arinkin (1929), Reich (1935), Markoff (1936), Suárez (1936), Yaguda (1936), Vogel, Erf, and Rosenthal (1937), Doan (1939), Davidson (1941a and b) and Sanchez (1941); the other "basophilic," "polychromatic" and "orthochromatic" erythroblasts or normoblasts as used by Mallarme (1937), Kandel and LeRoy (1939), Scott (1939), Lichtenstein and Nordenson (1939), Thaddea and Bakalos (1940), Maximow and Bloom (1942), Wintrobe (1942) and Van Loon and Clark (1943). Japa (1945) divided them into two groups "early" and "late" erythroblasts depending on the cytological appearance of both nucleus and cytoplasm. Almost all authors included the lymphocyte, monocyte, and plasma cell in routine counts. Many involved the megakaryocyte and some enumerated the reticulo-endothelial cell or a reticulum cell. Only Thaddeas and Bakalos (1940) counted monoblasts and promonocytes. Sabin and Doan (1927) and Doan (1939) included the clasmatocyte in the cells counted.

The exceptions to the above, Osgood (1937) and Pitts and Packham (1939), used a set of terms not in general use such as "progranulocytes A and B", and "rhabdocytes" and "lobocytes" for the unsegmented and segmented polymorphonucleers. In the red blood cells series they used "karyoblasts", "prokaryocytes," "karyocytes" and "metakaryocytes".

With the idea in mind of having as simple a terminology as possible and yet be useful, the following list of terms to specify the cells observed in bone marrow was decided upon.

"Stem cell" - All the immature cells which could not be classified into any given series.

"Erythroblast" - All cells in the red blood cell series from the youngest that could be identified with that series to the normoblast.

"Normoblast" - Those in the red blood cell series that contained hemoglobin in comparable amounts to the adult red blood cells as indicated by similar staining properties and containing nuclei.

"Promyelocyte" - Young cells with non-specific azurophilic granules.

"Eosinophilic" and "neutrophilic myelocytes" - Cells in these respective series included the metamyelocytes, juvenile cells and stab, rod or band forms of other authors.

"Eosinophil" and "neutrophil" - The adult cells of these granulocyte series.

"Basophil" - There were so few basophils that both developing cells and adults of that series were grouped together.

"Lymphocyte" - Any developing cells in this series were included.

"Monocyte" - All promonocytes and distinguishable monoblasts were added here.

"Plasma cell" - If any developing plasma cells were observed, this heading included them.

The list makes a total of twelve headings and it is not so formidable that it should discourage anyone from attempting to make bone marrow cell counts. According to Bloom (1945) anyone familiar with the morphology of blood cells should have little difficulty in the recognition of bone marrow cells.

Myelograms

Tables 5 and 6 indicate the individual myelograms for all the experimental animals in this investigation.

Tables 7 and 8 contain a summary of this study and a comparison with similar investigations carried on elsewhere.

Description of cells

It is inevitable that all the slides will not be stained exactly alike principally as a result of variation in the dilution of stain, and in timing. Frequently there are darker and lighter stained areas on the same slide due to the variation in the mixing of the buffer with the dye. Fainter stained preparations tend to show the nucleoli more clearly and the nuclear structures may not be masked by the staining of superimposed granules. Heavily stained smears, though the nuclear structures may be masked, show the granules in the cytoplasm well.

The younger the cell the more homogeneous the protoplasm of the nucleus. Nucleoli also indicate a young cell since they disappear as the cell develops. Nucleoli are greyish-blue spherical or ovoid bodies up to 2 micra in diameter.

Frequently cell nuclei appear like doughnuts but it is only the ends of u- or rod-shaped nuclei touching or overlapping which give this appearance.

In this study the slides were examined in the following manner. A low power preview was made to get an idea of distribution, staining and anything unusual. A representative field was chosen for the differential counts, measurements, color comparisons and cytological details and was explored under oil immersion.

Table 5

Percentage distribution of the marrow cells from the ribs of 14 cows

Cell Types	*42245	42743	5643	52043	52743	61643	61843	62343	62843	7543	7
Stem cell	1.4	2.0	3.4	0.8	0.2	0.0	2.2	3.0	2.2	3.2	
Erythroblast	15.6	36.2	29.2	20.6	30.2	11.8	42.4	20.0	36.6	37.0	3
Normoblast	21.4	19.8	18.0	13.6	27.8	9.2	28.6	7.2	20.6	15.4	3
Total erythroid cells (E)	37.0	56.0	47.2	44.2	58.0	21.0	71.0	27.2	57.2	52.4	7
Promyelocyte	3.0	1.2	0.6	1.4	0.0	1.8	2.0	6.8	1.6	0.8	
Neutrophilic myelocyte	19.8	24.8	16.2	32.0	21.0	28.8	16.4	29.2	17.2	13.0	1
Neutrophil	9.4	5.0	4.8	4.6	7.2	12.2	1.4	10.0	4.0	8.4	
Eosinophilic myelocyte	4.6	5.6	9.2	7.8	3.0	9.0	2.8	9.8	9.0	10.4	
Eosinophil	7.6	0.0	1.0	0.6	1.0	2.4	0.0	4.2	0.2	3.0	
Basophils (all)	0.2	0.0	0.4	1.0	0.8	0.2	0.4	0.4	0.8	0.0	
Total myeloid cells (M)	44.6	36.6	32.2	47.4	33.0	54.4	23.0	60.4	32.8	35.6	1
Monocyte	0.0	1.2	5.6	5.2	1.2	7.6	1.4	1.6	3.4	2.2	
Plasma cell	0.8	0.8	0.4	2.0	1.0	0.2	0.4	1.4	0.8	1.0	
Lymphocyte	17.2	3.4	11.2	10.4	6.6	16.8	2.0	6.4	3.6	5.6	
Megakaryocytes in 300 sq. mm.	21	41	23	20	0	8	0	121	7	8	
Mitoses	3	7	2	4	5	2	5	0	4	9	
Myeloid-erythroid ratio	1.2	.65	.68	1.07	.57	2.59	.32	2.22	.57	.68	

*All female except 72843

s from the ribs of 14 cows

61643	61843	62343	62843	7543	7743	71243	72143	72843	Range	Mean
0.0	2.2	3.0	2.2	3.2	5.0	2.4	2.8	1.4	0.0- 5.0	2.14
11.8	42.4	20.0	36.6	37.0	38.6	30.8	42.8	31.8	11.8-42.8	30.26
9.2	28.6	7.2	20.6	15.4	33.6	22.2	27.0	39.2	7.2-39.2	21.69
21.0	71.0	27.2	57.2	52.4	72.2	53.0	69.8	71.0	21.0-72.2	52.66
1.8	2.0	6.8	1.6	0.8	0.6	0.6	0.8	0.0	0.0- 6.8	1.51
28.8	16.4	29.2	17.2	13.0	10.4	15.8	16.2	10.6	10.4-32.0	19.39
12.2	1.4	10.0	4.0	8.4	2.4	6.2	1.2	3.4	1.2-12.2	5.73
9.0	2.8	9.8	9.0	10.4	6.0	8.4	6.2	1.8	1.8-10.4	6.69
2.4	0.0	4.2	0.2	3.0	0.2	3.0	0.2	3.6	0.0- 7.6	1.92
0.2	0.4	0.4	0.8	0.0	0.0	0.4	0.0	0.2	0.0- 1.0	.34
54.4	23.0	60.4	32.8	35.6	19.6	34.4	24.6	19.6	19.6-60.4	35.59
7.6	1.4	1.6	3.4	2.2	1.2	4.4	0.2	1.8	0.0- 7.6	2.64
0.2	0.4	1.4	0.8	1.0	0.6	1.0	0.2	0.4	0.2- 2.0	.79
16.8	2.0	6.4	3.6	5.6	1.4	4.8	2.4	5.8	1.4-17.2	6.68
8	0	121	7	8	5	8	83	7	0-121	25.14
2	5	0	4	9	9	5	11	3	0- 11	4.9
7 2.59	.32	2.22	.57	.68	.27	.65	.35	.28	0.27-2.59	

Table 6

Percentage distribution of the marrow cells from the ribs of 7 horses

Cell Types	6943w F	6843b F	6843w F	7943b F	6943s *M-c	8344 M-c	92944 F	Range	Mean
Stem cell	0.4	0.4	1.6	3.4	2.0	2.4	1.0	0.4-3.4	1.6
Erythroblast	19.4	8.0	14.0	32.0	23.6	31.4	18.2	8.0-32.0	20.94
Normoblast	24.2	12.0	5.0	15.6	15.2	13.6	10.4	5.0-24.2	13.71
Total erythroid cells (E)	43.6	20.0	19.0	47.6	38.8	45.0	28.6	19.0-47.6	34.66
Promyelocyte	0.0	0.6	5.0	1.8	1.6	3.2	0.6	0.0-5.0	1.83
Neutrophilic myelocyte	26.2	47.8	56.0	26.6	31.6	37.8	40.4	26.2-56.0	38.06
Neutrophil	20.2	16.6	9.0	12.6	15.4	1.8	17.6	1.8-20.2	13.31
Eosinophilic myelocyte	0.8	3.4	0.4	2.6	3.6	2.6	3.0	0.4-3.6	2.34
Eosinophil	0.4	0.2	1.0	1.0	0.2	0.2	1.2	0.2-1.2	0.60
Basophils (all)	0.0	1.0	0.2	0.4	0.8	1.0	0.8	0.0-1.0	0.60
Total myeloid cells (M)	47.6	69.6	71.6	45.0	53.2	46.6	63.6	45.0-71.6	56.74
Monocyte	2.0	4.4	4.8	1.2	1.6	1.8	1.4	1.2-4.8	2.46
Plasma cell	0.8	0.6	0.8	0.8	0.6	0.8	0.0	0.0-0.8	0.63
Lymphocyte	5.6	5.0	2.2	2.0	3.8	3.4	5.4	2.0-5.6	3.91
Megakaryocytes in 300 sq. mm.	0	0	8	0	3	1	0	0-8	1.71
Mitoses	2	0	0	2	8	1	6	0-8	2.71
Myeloid-erythroid ratio	1.09	3.48	3.76	.94	1.37	1.04	2.22	.94-3.76	

*M-c-male castrate

Table 7

Comparison of cow data with other myelograms

Mercato (1941)	Middle aged cow	Old cows	Mean	Hjarre (1943)	Range	Author	Range from table 5	Mean from table 5
Hemocytoblast	4.35	4.1	4.23	Myeloblast	1.5-4	Stem cell	0.0-5.0	2.14
Erythroblast	3.7	3.68	3.69					
Proerythroblast	2.3	2.42	2.36	Pronormoblast	0-1.0			
Basophilic erythroblast	11.5	11.95	11.72	Basophilic				
Total erythroblasts	17.5	18.05	17.77	normoblast	3-7.5	Erythroblast	11.8-42.8	30.26
Polychromatophilic erythroblast	18.0	16.8	17.4	Hemoglobin				
Orthochromatic erythroblast	19.5	19.0	19.25	containing				
Total	37.5	35.8	36.65	normoblast	27-55	Normoblast	7.2-39.2	21.69
						Total erythroid		
Total erythroid series	55.0	53.85	54.42			cells	21.0-72.2	52.66
Neutrophilic promyelocyte	1.4	1.5	1.45	Promyelocyte	0.5-3	Promyelocyte	0.0-6.8	1.51
Neutrophilic myelocyte	5.6	6.1	5.85	Myelocyte	3-9.6			
Neutrophilic metamyelocyte	11.5	12.1	11.8	Metamyelocyte	3-13.5	Neutrophilic		
Total neutrophil series	18.5	19.7	19.10	Stab cells	7.6-18.1	myelocyte	10.4-32.0	19.39
				Segmented				
				neutrophil	6.3-19	Neutrophil	1.2-12.2	5.73
Eosinophilic promyelocyte	1.5	1.5	1.5			Eosinophilic		
Eosinophilic myelocyte	5.0	5.0	5.0			myelocyte	1.8-10.4	6.69
Eosinophilic metamylocyte	6.3	5.27	5.78			Eosinophil	0.0-7.6	1.92
Total eosinophil series	12.8	11.77	12.28			All basophils	0.0-1.0	0.34
						Total myeloid		
						cells	19.6-60.4	35.59
Monoblast and monocyte	8.35	9.36	8.85	Monocyte	0-1	Monocyte	0.0-7.6	2.64
Plasmacyte	0.5	0.68	0.59			Plasma cell	0.2-2.0	0.79
						Lymphocyte	1.4-17.2	6.68
Myeloid-erythroid ratio	0.58	0.66	.62	M.E. ratio	0.7	M/E	.27-2.59	.676(T.9)

Table 8

Comparison of horse data with other myelograms

Hjärre and Berthelsen (1938)	10 horses	Hjärre (1943)	Author	Range from table 6	Mean from table 6
Myeloblast	1.5-2.5	1.5-3	Stem cell	0.4-3.4	1.6
Promegaloblast like cells	1-2				
Pronormoblast	1.5-3	2.5-5.5	Erythroblasts	8.0-32.0	20.94
Basophilic normoblast	4.5-9	4.5-9	Normoblasts	5.0-24.2	13.71
Hb. containing normoblasts	40-60	40-60	Total erythroid cells	19.0-47.6	34.66
Promyelocyte	1.0-2.5	1-2.5	Promyelocyte	0.0-5.0	1.83
Myelocyte	2-5	2-6.7			
Metamyelocyte	5-15	5-15.7	Neutrophilic myelocyte	26.2-56	38.06
Stab cells	5.5-11	5-10.7	Neutrophil	1.8-20.2	13.31
Segmented neutrophils	6.5-14	6.3-16	Eosinophilic myelocyte	0.4-3.6	2.34
Eosinophil	0.5-1.5		Eosinophil	0.2-1.2	0.60
Basophil	0-0.5		All basophils	0.0-1.0	0.60
			Total myeloid cells	45.0-71.6	56.74
Monocyte	0-0.5	0-0.5	Monocytes	1.2-4.8	2.46
Plasma cell	0.1-1		Plasma cell	0.0-0.8	.63
Lymphocyte	2-6		Lymphocyte	2.0-5.6	3.91
Myeloid-erythroid ratio		0.5	M/E	.94-3.76	1.64 (T.10)

The two main sources of reference used in identifying and describing the cells were "Atlas of Hematology" by Osgood and Ashworth (1937), and the "Munsell Book of Color" (1929). "Colour Terminology in Biology" by Dade (1943) was occasionally referred to.

For the purpose of specificity color comparisons were made with the Munsell Color book and the approximate color or range of colors was determined. Comparing transmitted light with reflected light presented some incongruities. The most predominating nuclear color was chosen for comparison. The nuclear color of all cells may be approximately matched in the 5.0 red-purple color chart. The darkest nucleus with a value of 2 and a chroma of 4 (2/4) was found in the normoblast. The pale staining which was frequently found in the monocyte nucleus may be designated 7/4. Other cell nuclei matched colors lying between these two extremes depending on the stage of development and the intensity of staining.

Like that of the nucleus the cytoplasmic color varied with the degree of staining but in addition there was considerably more variation between cells. Frequently the cytoplasm was so pale that it might be designated "colorless". The pale blue of the monocyte and a color in the erythroblast series compared very favorably with a purple-blue-purple shade designated 10.0 B 8/2. At the other end of the color range was the purple-blue of the stem cells and the young erythroblast, 5.0 purple-blue 4/10 or 5/10. The cytoplasm of the erythroblast that had begun to take up hemoglobin matched 8/2 in the 5.0 green-yellow Munsell color plate. The normoblast cytoplasm compared favorably with 8/2 in the 5.0 yellow-red plate. The color of the extra-nuclear protoplasm of other cells ranged from 8/2 to 5/8 inclusive on the 5.0 purple-blue plate.

The various shades of the eosinophil granules may be designated by 5.0 red 8/4, 10.0 red-purple-red 8/6, 5.0 red-purple 7/2, 7/4, 6/2-6/8, 5/6

and 5/8. The basophilic granules which were intermingled with the eosinophilic granules were much the same color as those of the basophil itself.

The basophil granules matched colors under the classification 5.0 red-purple 2/4, 2/6, and 3/6 in the Munsell color book.

The azurophil granules of the lymphocyte, monocyte and promyelocyte were about the colors 4/10, 4/12 and 5/10 in the 5.0 red-purple plate.

The smallness of the neutrophil granules made color comparisons difficult but as nearly as could be ascertained they are the color of 8/4 in the 10R red-yellow-red set of hues.

The Kodacolor prints in Plates IV - IX only partially represent the above colors. In the parts where the background is too blue the reds are not typical. The colors illustrated in Plate X compare very favorably with those in the "Munsell Book of Color.

Generally speaking there is so little difference between horse and cow bone marrow that the cells need not be described separately.

Stem Cell: The stem cells measured varied in size from 12 x 14 to 26 x 30 micra. These included all the young cells which could not be classified in any definitive series. The reddish-purple nucleus had a homogeneous finely reticulated karyoplasm with 2 or more pale blue nucleoli. Bloom and Meyer (1944) did not find a nucleolar membrane in the stem cell of dog marrow but the nucleoli in these cells in the horse and cow appeared to have a very definite nucleolar membrane in most instances. The nucleus was large in relation to its cytoplasm. For example, one cell measuring 22 x 22 micra had a nucleus 14.5 x 16 micra. Other cells had a narrower rim of cytoplasm such as a cell measuring 13.5 x 17 micra with a nucleus 10 x 15 micra. The cytoplasm, which had a varying color range from a pale blue to a greyish-sky-blue, often

presented a mottled appearance and frequently contained a vacuole or two. Some of the stem cells contained a few azure staining granules in the cytoplasm. For illustrations of this cell refer to Plate IV, Figs. 1 and 2 and Plate X.

Erythroblast and Normoblast: Davidson, Davis and Innes (1942) have aptly defined the erythroblast as "any nucleated cell capable of differentiation towards an erythrocyte" and divided such cells into four groups including the stem cells of that series and the orthrochromatic erythroblast or normoblast. The erythroblasts here include only two groups, the basophilic erythroblasts and those beginning to take up hemoglobin (the polychromatic erythroblasts of some authors). Those measured varied in size from 7 x 7 to 15 x 20 micra. One in the process of mitosis was 15 x 23 micra. The nucleus varied from the homogeneous reddish-purple, nucleoli-containing nucleus of the most primitive erythroblast to the chromatin-clumped dark purple nucleus of the late erythroblast just prior to complete hemoglobinization of the cytoplasm. The structureless cytoplasm varied in color from the blue of the more primitive cells to the steel grey or even greenish-grey of the stage near the normoblast. The normoblast presented size variations of 5 x 7 or 6 x 6 to 9 x 9 micra. One in mitosis was 12 x 12 micra. According to Wirth (1931) the erythrocyte of the ox varies in size from 4.4-7.7 micra with an average of 5.1 and the horse red blood cell varies from 4.0 to 7.5 with an average of 5.6 micra. Consequently little if any size differences between the developing red cells in the two species would be expected. None were noted. The pyknotic nucleus was so purple as to appear almost black and was so dense no internal structure could be made out. Israels (1941 a and b) has described the maturation of the erythroblast. The cell shrinks to one half

its original size; the cytoplasmic color progresses from basophilic, to polychromatophilic to eosinophilic with the increase in hemoglobin content, and the nucleus shrinks, condenses and finally becomes a dark featureless mass. The nuclear structure changes and hemoglobinization are not necessarily synchronized into any set pattern for occasionally a small cell with a pyknotic nucleus may retain quite basophilic cytoplasm while the opposite, a large cell with an erythroblastic nucleus and complete hemoglobinization of the cytoplasm, may occur. Israels (1941a) claimed the latter was associated with some increased demand for red blood cells and should not be considered normal. For example in horse number 6843b, cells with hemoglobinized cytoplasm were as large as 13 x 14 micra. This animal had the lowest red blood cell count (4,905,000) of any of the animals studied. This variation in development need not be a matter for concern, however, as the total for the red cell series is a sufficient index to the state of the bone marrow. Endicott and Ott (1945) made a simple classification of marrow-cells in the rat and one grouping was "red cell series" which included all the nucleated precursors of the red blood cells.

Illustrations of this series are shown in Plates IV, V, VI, VIII, IX, Figs. 1 and 2, and Plate X.

Promyelocyte: The promyelocytes caried in size from 15 x 16 to 21 x 22 micra. This cell had a spherical or ovoid reddish-purple nucleus with usually 2-5 pale blue nucleoli. The light blue cytoplasm contained small fairly evenly distributed azurophilic granules. Sometimes a rim of deeper blue cytoplasm was present. For illustration see Plates VI, VIII and IX, Fig. 1, and Plate X.

Neutrophilic myelocyte: The cells grouped in this series included several developmental stages with morphological variations but all had about

the same staining properties. The size ranged from 10 x 10 to 16 x 18 micra. The reddish-purple nucleus varied in form from the spherical "finely chromatined" nucleus of the youngest cell in the series, through successive stages of oval, kidney-bean-and u-shapes to the darker staining, "chromatin-clumped" nucleus beginning to show constrictions but not segmented into lobes. These nuclear changes correspond to the myelocyte, metamyelocyte or juvenile, and staff cells of other authors. The cytoplasm varied from colorless to pale blue with fine neutrophilic granules. The early stages often retained a few larger azurophilic granules. Examples of this cell are shown in Plates IV, V and VIII Figs. 1 and 2, Plate VI Fig. 1 and Plate X.

Eosinophilic myelocyte: These cells pass through the same developmental stages as the neutrophil. They are easily identified by the eosinophilic granules which in the cow are 1 micron or smaller but in the horse vary from 1 micron to 3 micra. Because of the small size of the granules in the cow it was impossible to count them but in the horse eosinophil, from 30 to as many as 125 granules were seen. Hirschfeld (1897) counted from 20 to 40 granules. He stated that the largest ones might reach the size of the goat erythrocyte. The cells measured were as large as 26 x 26 and 22 x 30 micra in the cow and one in the horse was 23 x 33 micra. In the young cells there was considerable variation in color of the granules, some azurophilic, others taking on a blue-gray shade. Most of the granules were spherical but a few oval ones were observed in the horse eosinophil. Hirschfeld (1897) observed these elliptical granules too. According to Downey (1915) the eosinophilic granules are endogenous, being differentiated from the basophilic protoplasm. He found that they changed from small basophilic granules to even larger eosinophilic ones than were to be found in the mature eosino-

phil. The cytoplasm appeared grey-blue when not obscured by the granules. The reddish-purple nucleus changed from spherical to ovoid to u-shaped, becoming lobed in the adult cell. One or more nucleoli were observed in the most immature cells of this series. Turn to Plates IV Figs. 1 and 2, Plate V Fig. 1, and Plate X for the illustrations.

Basophils: The largest immature basophil measured was 18 x 21 micra and the smallest adult cell in this series was 11 x 11 micra. The nucleus when discernable was a reddish-purple color but it was frequently masked by the basophilic granules which were a deeper shade than the nucleus. The spherical granules varied in size, the largest being about a micron in diameter. The cytoplasm was a pale blue. According to Hirschfeld (1897) the mast cell (basophil) of the horse was large and displayed an abundance of granules such as did not occur in any other animal. He thought the granules were needle shaped ("nadelformig"). No appreciable difference could be detected between the size of the basophils of the horse and cow and needle shaped granules were not observed. Staining variations of the granules ranged from a muddy reddish color to a very dark purple with a brownish cast. Plate X contains a drawing of a basophilic myelocyte from the cow and Plate IX Figs. 1 and 2 are photomicrographs of horse bone marrow including basophils.

Plasma cell: The plasma cell of both the horse and cow was easily distinguished from other cells but its differential characteristics are difficult to describe. The blue of its cytoplasm was just a bit brighter than that of the erythroblast. This and its usually eccentrically placed nucleus with its perinuclear clear area all combined to set it apart from similarly stained cells in the lymphocytic and red blood cell series. The largest plasma cell measured was 14 x 19 micra with an 8 x 8 nucleus. A smaller cell

(11 x 11) had a nucleus 7 x 8 micra. The chromatin network was heavy and coarse textured in the older cells but of a finer structure and less dense in more immature cells. Michels (1931b) has written a review on the plasma cell including morphogenesis. Plate IV Fig. 2 shows an adult plasma cell in cow bone marrow. Plate VIII Fig. 2 shows one dividing amitotically in horse marrow and Plate X includes a drawing of a plasmablast from the horse.

Megakaryocyte: Ackerneckt (1912) described two types of megakaryocytes in the horse, one a multinucleated cell, the other a single nucleated cell having somewhat different staining properties. He found transitional stages between the two. He suggested that the multinucleated cells might be a regressive product of metamorphosis. Kingsley (1935) made a study of the development of the megakaryocyte in pig embryos. According to him the megakaryocytoblast is a small cell (8 micra) and can be distinguished from the hemocytoblast by specific cytoplasmic granules. The cell increases in size, the spherical nucleus becomes oval, then horse-shoe shaped and finally bell shaped. The disappearance of the nucleoli and "vesiculation" of the nucleus accompanies these changes. Subsequent development includes increase in size and amount of the cytoplasm and nucleus. It may have been these different stages in development that Ackerneckt observed. Limarzi and Schleicher (1940) included the steps in the maturation of the megakaryocyte in a study of thrombopenic purpura. They grouped them into young, adult and degenerated types. Levy (1945) concluded that they are abnormal cells, an outcome of malformed cell divisions and no special function should be given to them. Whatever their role, that of platelet formation or merely functionless developmental abnormalities, a description follows. The megakaryocyte is the largest cell in marrow. Its size varied from 15 x 21 to 85 x 100 micra.

The cells were spherical or ovoid. Some of them had large pseudopodia. (Plate XI Figs. 3 and 4) Very few were encountered in the horse and many of those did not seem to have any cytoplasm. In the cow many more were apparent. These presented two distinct cytological pictures. One had a dark blue to plum colored nucleus with an area of reddish-purple granular cytoplasm. At the outer edge of the cytoplasm the granules were absent or sparse and the blue cytoplasm was apparent. (Plate XI Fig. 2) One cell presented the exact opposite picture, a perinuclear area of blue and an outer rim of fine reddish-purple granules. The other type had a less dense, lobed or folded nucleus surrounded by a cytoplasm filled to the edge with tiny reddish-purple granules. In some cells the cytoplasm appeared to fray out into particles resembling platelets. Plate XI Fig. 3 best illustrates this. According to Limarzi and Schleicher (1940) platelets are formed by either a detachment of portions of the cytoplasm or by cytolysis. Schenker (1939) favored platelet formation through disintegration of mature megakaryocyte plasma and found evidence to support the theory that the platelets are of nuclear origin. Plates V Fig. 1 and XI Fig. 1 show the usual megakaryocyte.

Mitosis

Tables 5 and 6 indicate the number of mitotic figures encountered in the process of counting 500 cells. They were chiefly in the red blood cell series. Subsequent examination of the slides revealed cell division in practically all of the types of cells. According to Osgood (1939) all the "blast" and "pro" cells divide by mitotic division and the more mature erythroblasts, plasmacytes and lymphocytes divide amitotically. Kienle (1943) found that the hemocytoblasts seldom showed mitosis either normally or in leukemia, that the myeloblast exhibited more prophases than any other phase while the promyelo-

cytes occurred more frequently in metophase and telophase. Japa (1942) counted the number of dividing cells per 1000 nucleated cells and the proportions of each stage per 100 cells. He considered the normal number of dividing cells to be 15 per 1000 nucleated cells, distributed as follows: 40% prophase, 45% metaphase, 10% anaphase and 5% telophase; 97% myelocytes and 3% myeloblasts comprised 45 mitoses per 100 and 91% late and 9% early erythroblasts made up the other 55%. Plate X Fig. 7 illustrates an erythroblast in mitotic division and Plate VIII Fig. 2 shows a plasma cell dividing by amitosis.

Myeloid-erythroid ratio

The myeloid-erythroid ratio, the ratio of developing red blood cells to those in the white blood cell series, is an important index of the activity of the marrow. Should a systemic demand for an increase in one or the other of these series be reflected in the marrow the usual ratio would be upset. This ratio was determined by adding the percentages of erythroblasts and normoblasts and comparing the figure with a similar sum of the developing granulocytes. These are indicated in Tables 7 and 8 by "Total myeloid cells" and "Total erythroid cells". The myeloid-erythroid ratio, M/E, given in the same tables varies from .27 to 2.5 for the cow and .94 to 3.76 in the horse. Krecke (1941) stated that the normal myeloid-erythroid ratio for man varies from 1:2 to 1:6 in healthy adults. While there is considerable variation among individuals the mean M/E ratio for the cows used in this study was 35.59/52.86 or .676 (see Table11). The mean for the horses was 56.74/34.66 or 1.64. (see Table 12).

Bone-healing process

Plates XII to XV show the healing process that follows the use of the drill in procuring marrow from the rib of the horse. The surgical procedure of obtaining marrow was carried out at the following intervals before the animal was killed: 7 weeks 2 days, 6 weeks 2 days, 5 weeks 2 days, 4 weeks 2 days, 3 weeks 2 days, 2 weeks 1 day, 9 days, 4 days, 2 days, 6½ hours and 2 hours. At autopsy the portion of the ribs including the site of the drill hole was obtained. These were subsequently decalcified, embedded in paraffin and histological sections prepared. Plates XII and XIII show consecutive stages in the early healing process. High power magnification showed an increasing number of fibroblasts with the lengthened time interval between the operation and killing of the animal. Plate XV Fig. 1 shows the drilled area filled with a loose fibrous connection tissue. Plates XIV Fig. 2 and XV Fig. 1 show bone filling the drill site and in the latter healing is practically complete. The bony lamellae appear to extend parallel to the direction of the drilled hole. Plate XV Fig. 2 shows a longitudinal section of a rib which had not been drilled into. By reviewing these slides it appears that the healing process proceeds along the same time pattern as an ordinary fracture. Only intramembranous bone formation was observed.

Sampling experiment

A sampling experiment consisted of getting marrow from two positions about an inch apart on each of five ribs in the cow. The ribs used were the 8th to 12th and the body level was that indicated in PlateII Fig. 1. These data are shown in Table 9.

The means shown in Table 10 were treated statistically by analysis of variance. The results showed three significant differences in the 12th

Table 9

Cellular composition of marrow in various ribs and varying positions on those ribs of the same cow

Rib	8	8	9	9	10	10	11	11	12	12
	upper	lower	u	l	u	l	u	l	u	l
Stem cell	1.0	0.8	0.2	0.2	0.8	0.4	0.8	1.0	0.6	0.0
Erythroblast	20.0	16.0	14.4	8.8	20.0	12.4	6.8	13.0	4.2	3.4
Normoblast	19.0	18.0	19.8	21.2	17.2	10.0	8.2	15.4	18.2	3.6
Total erythroid cells (E)	39.0	34.0	34.2	30.0	37.2	22.0	15.0	28.4	22.4	7.0
Promyelocyte	2.0	2.0	1.6	0.6	1.2	0.8	1.6	1.0	0.4	0.2
Neutrophilic myelocyte	23.2	26.0	28.0	30.6	22.8	25.0	38.0	34.4	26.8	18.8
Neutrophil	3.6	2.4	2.6	7.6	4.4	11.6	11.0	7.6	10.4	14.5
Eosinophilic myelocyte	8.4	12.0	13.0	10.2	9.8	12.6	17.0	13.0	10.2	10.1
Eosinophil	2.6	3.4	2.8	2.4	1.8	4.2	3.0	10.0	6.0	3.0
Basophils (all)	1.0	0.4	0.0	0.0	0.4	0.0	0.6	0.2	0.4	0.4
Total myeloid cells (M)	40.8	46.2	48.0	51.4	40.4	54.2	71.2	66.2	54.2	46.8
Monocyte	2.2	2.4	7.2	3.4	3.2	3.6	1.6	5.0	0.8	7.1
Plasma cell	1.0	0.0	0.4	0.0	1.2	0.4	1.0	0.2	0.0	0.0
Lymphocyte	16.0	16.6	10.0	15.0	17.2	19.0	10.4	6.2	22.0	39.1
Myeloid-erythroid ratio M/E	1.04	1.36	1.40	1.71	1.08	2.46	4.74	2.29	2.41	6.68

Table 10

Summary of means from table 9

	8	9	10	11	12	Upper	Lower	Total
Stem cell	.9	.2	.6	.9	.3	.68	.48	.58
Erythroblast	18.0	11.6	16.2	9.9	3.8*	13.08	10.72	11.90
Normoblast	18.5	20.5	13.6	11.8	10.9	16.48	13.64	15.06
Total erythroid cells**	36.5	32.1	29.6	21.7	14.7	29.56	24.28	26.92
Promyelocyte	2.0	1.1	1.0	1.3	.4*	1.36	.94	1.15
Neutrophilic myelocyte	24.6	29.3	23.9	26.2	22.6	27.76	26.90	27.33
Neutrophil***	3.0	5.1	8.0	9.3	12.4	6.40	8.74	7.57
Eosinophilic myelocyte	10.2	11.6	11.2	15.0	10.2	11.68	11.58	11.63
Eosinophil	3.0	2.6	3.0	6.5	4.5	3.24	4.60	3.92
Basophil	.7	.0	.2	.4	.4	.48	.20	.34
Total myeloid cells	43.5	49.7	47.3	68.7	50.5	50.90	52.96	51.93
Monocyte	2.3	5.3	3.4	3.3	3.9	3.0	4.3	3.65
Plasma cell	.5	.2	.8	.6	.0	.72	.12	.42
Lymphocyte	16.3	12.5	18.1	9.3	30.6*	15.12	19.58	17.35

*Significant
**Significant negative trend among the rib averages
***Significant positive trend among the rib averages

rib and no significant difference between positions on the rib. The total erythroid cells indicated a significant negative trend from the 8th to the 12th rib and the neutrophils a significant positive trend in the same rib order. Since there was only one animal and many of the cell types occurred in such few numbers, the experiment should not be given too much weight until further work can be done to substantiate it. In the light of these meager data it would seem better to obtain samples anterior to the 12th rib. In view of Varićak's (1935) findings that the sternal end of the rib changed from red to yellow marrow earlier than the vertebral end, probably it is wise to drill the rib at as high a level as possible and still avoid the back muscles.

DISCUSSION

Hematologists are not agreed on the value of aspiration and bone marrow smear methods. Doan and Zerfas (1927) suggested conservatism in drawing deductions because of the limitations of the technic, the fallacy of drawing conclusions from counting so few of the millions of cells present, and the debated question of identification and classification. Custer (1932) found the cellular state of marrow to vary in different bones, and in different bones at the same level in the same animal. Dameshek (1935) contended that for exact cytological study, marrow smears could not be excelled. According to Young and Osgood (1935) the only limitation of aspirated samples is the loss of structural relationships. Nordenson (1935) claimed that marrow from several bones in the same patient was similar in quality and quantity. Williams (1935), studying the cellular pattern of human marrow at autopsy, ascertained that the differential count from different bones in the same case was essentially the same. Jaffé (1936) reasoned that the simplicity and lack of technical skill required outweighed the disadvanteges of the dilution with

blood and failure to give an *in situ* picture. Dameshek, Henstell and Valentine (1937) thought the chief advantage of puncture biopsy, its simplicity, was exceeded by its inaccuracy. Helpap (1937) agreed with Custer (1932) that bone marrow is not homogeneous and that samples from one part of a bone differ from those from another part. Stasney and Higgins (1937) concluded, to the contrary, that there is sufficient uniformity of hematopoiesis in dog marrow so that "the appraisal of the marrow of any one region will reveal what the trend of its cellular changes is elsewhere in the body." A later work by the same authors substantiates this view for human marrow (Stasney and Higgins, 1939). Stodtmeister and Buchmann (1939) studied the influence of sternal puncture on the circulating blood and found it had no effect on cell composition. Gordon (1941) gave as disadvantages of any aspiration method, trauma, the failure to dislodge immature cells, loss of topographic relationships and the dilution with peripheral blood. Mulligan (1942) obtained a favorable correlation by comparing marrow obtained by sternal puncture and trephine methods. Reich and Kolb (1942) found by statistical analysis that quantitative determinations on aspirated marrow samples were inaccurate. Epstein and Tompkins (1943) would invalidate differential counts made from smears on the basis of trauma and inadequate distribution of cells. Steiner (1943) felt that a marrow aspiration method might not be satisfactory in Hodgkin's disease because of the distribution of the lesions in foci. By comparing marrow smears from the ribs and femurs of dogs, Van Loon and Clark (1943) came to the conclusion that such preparations were similar in content. Osgood and Seaman (1944) pointed out that any marrow preparation whether a section, an imprint, or aspirated material, will have blood in it because blood is present in the sinusoids and vascular channels of both normal and pathologic marrow. According to Schleicher (1944) any kind of sampling from

as large and complex an organ as bone marrow is subject to errors of chance.

In spite of all this controversial evidence as to its value bone marrow examination is a useful tool to be employed when indicated just as other technics are. According to Bloom (1945) no hematological study is complete if the bone marrow is neglected.

In reviewing this study and the data presented here certain points may be noted.

The few stem cells may be readily explained by referring to some investigations of Japa (1942) on the mitotic activity of human marrow. He found that out of 100 cells in the myelocytic series only 3 were myeloblasts and in the erythroblastic system 9 were early erythroblasts. Trautmann (1940) seldom saw the stem cell in normal bone marrow.

Few young erythroblasts (the proerythroblasts of some authors) are encountered because the multiplication of older cells is sufficient to fill the physiological need (Jaffé 1933).

Most of the mitotic figures occurred in the erythroblastic series. Japa (1942) found 15 per thousand nucleated cells in human marrow. The range in the cow was from 0-11 and in the horse 0-8 per 500 cells counted.

In Fig. 1 the neutrophilic myelocytes and neutrophils in the marrow were plotted on the same graph with the blood neutrophils for each cow. The resultant curve seems to bear out the statement of Doan and Zerfas (1927) that there is a striking reciprocity between the neutrophilic myelocyte and the mature neutrophil. Fig. 2 presents a similar correlation for the horse but such deductions are not so apparent perhaps because of fewer animals.

Curves comparing the eosinophilic series with the blood eosinophil for both the cow and horse are shown in Figs. 3 and 4. There appears to be

a positive correlation between the eosinophilic myelocyte and the marrow eosinophil but no interdependence between these and the blood eosinophil. Tötterman (1936) made such a study on 66 persons with broad tapeworm and concluded that there was no parallelism between eosinophilia in the blood and in the bone marrow. Barta (1933) found that eosinophils might be increased in numbers in the bone marrow without appearing in the peripheral blood.

In similar curves for the red blood cell series (Figs. 5 and 6) there is a much more favorable correlation indicating that there is a relation between the erythroblasts, normoblasts and peripheral red blood cells.

Individual maturation curves of the neutrophils illustrated in Fig. 7 indicate that all fourteen cows showed a similar curve. A mean of these curves is plotted in Fig. 8 and compared with the data given by Hjärre (1943) and Marcato (1941). Cotti (1939) illustrated a gradually ascending maturation curve for human marrow. Figure 9 shows the individual maturation curves of the neutrophils of the seven horses used in this study. They compare favorably with each other and are like those of the cattle.

Tables 11 and 12 compare the peripheral blood with the bone marrow for both the cow and horse. Table 13 summarizes the bone marrow findings in this study.

In the last decade great strides have been made in human medicine in correlating the bone marrow findings and certain diseases. Some of those investigations are mentioned here that they may point the way to similar researches in the field of veterinary hematology. Dreyfus (1936) valued bone marrow examination in cases of myeloma, lymphoma, acute leukemia, anemias and in atypical Hodgkins disease. According to van de Merwe (1936) the absence

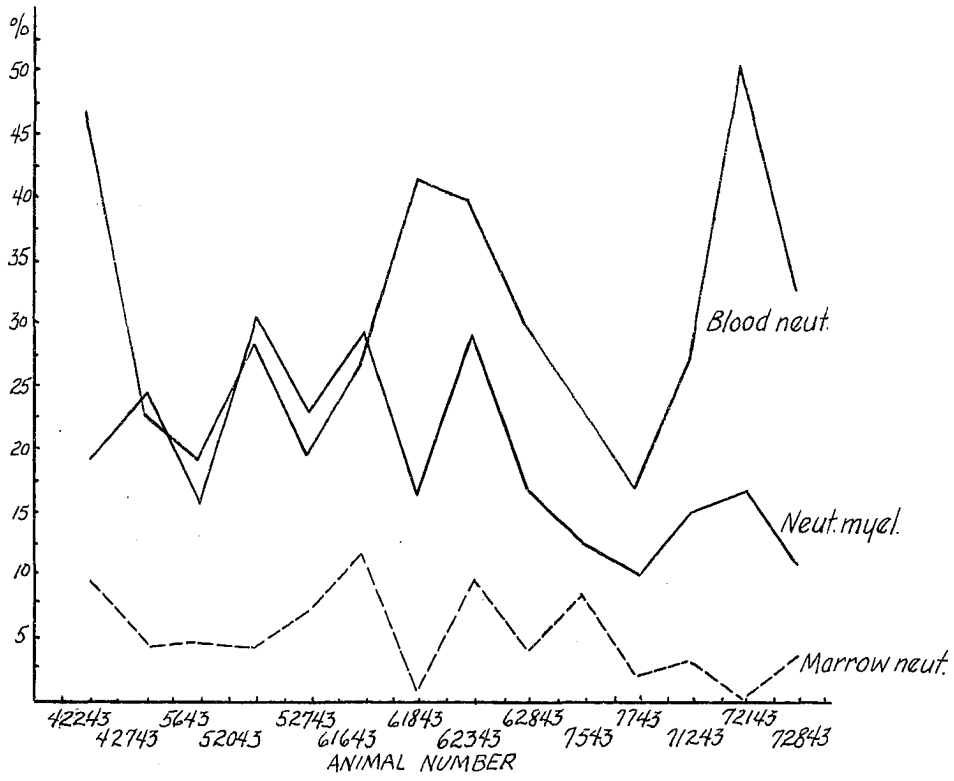

Fig. 1. Comparison of neutrophils of bone marrow and blood of the cow.

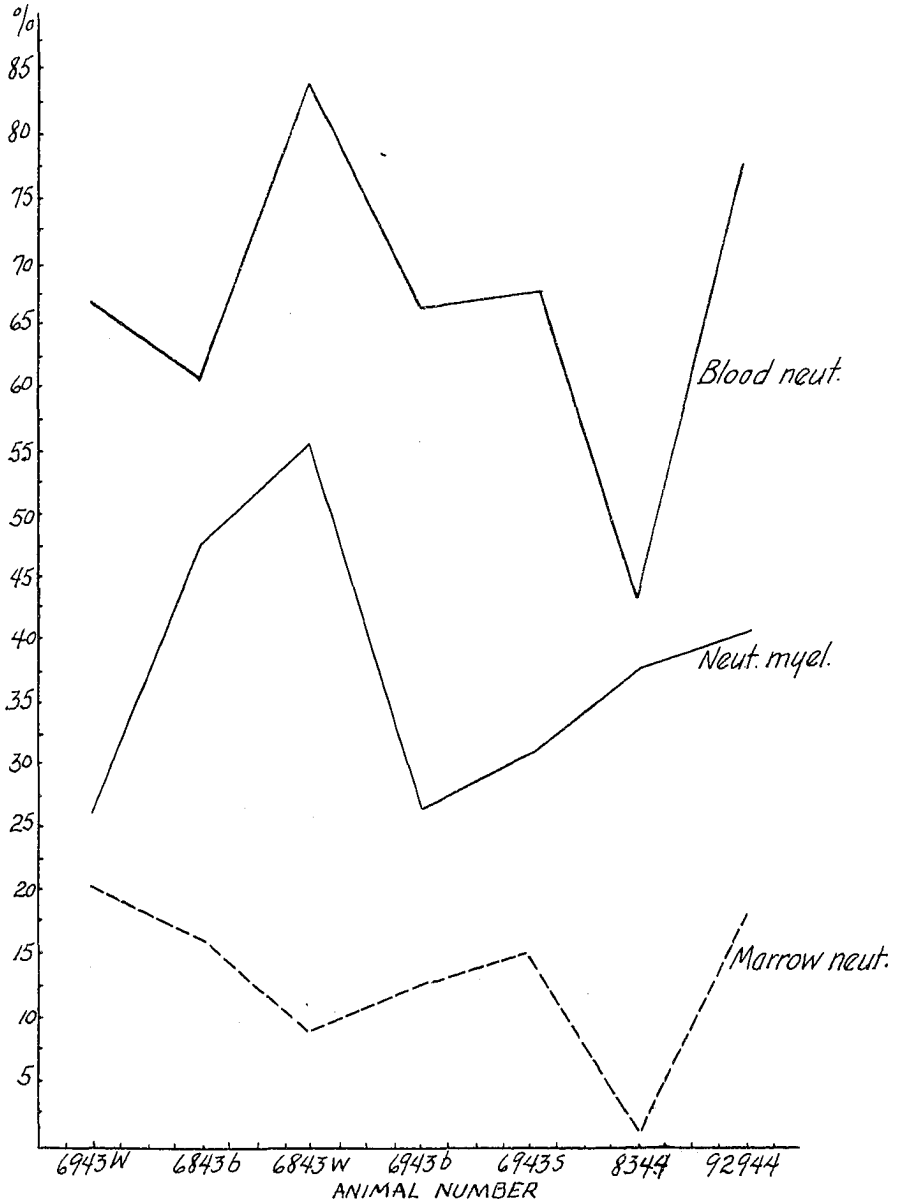

Fig. 2. Comparison of neutrophils of bone marrow and blood of the horse.

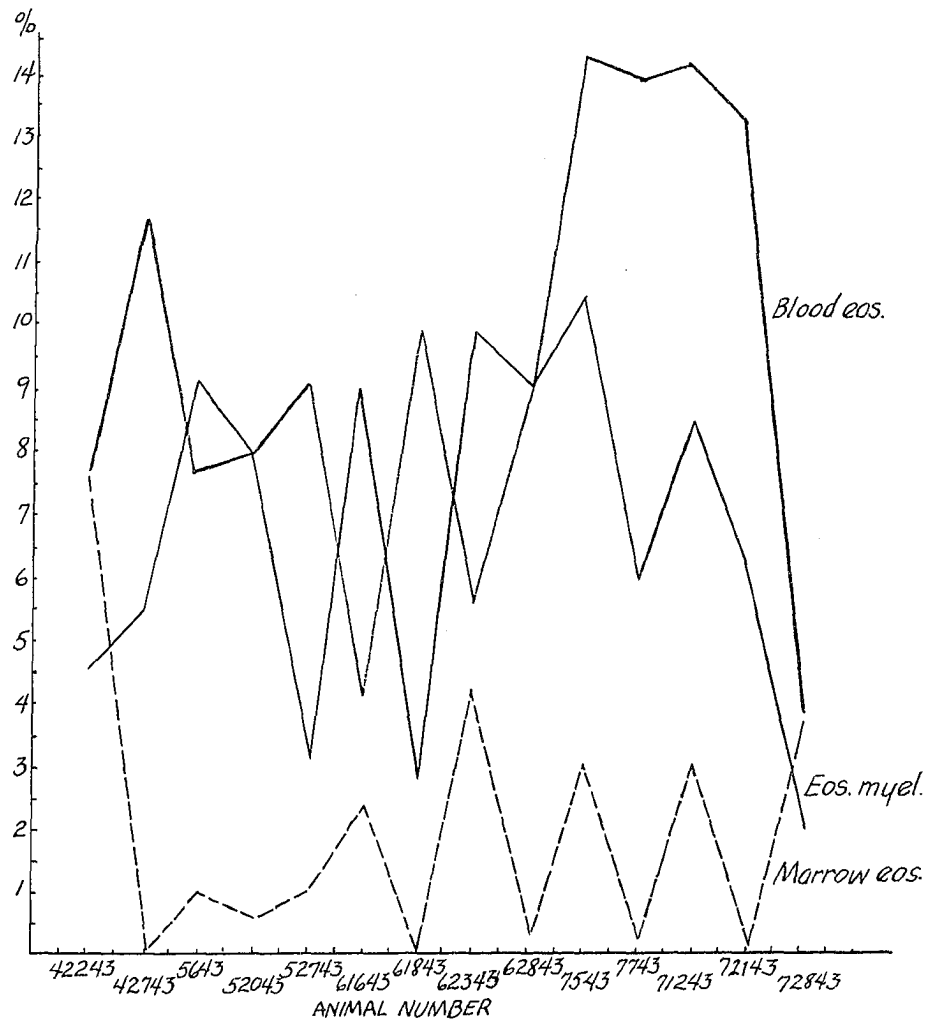

Fig. 3. Comparison of eosinophils of bone marrow and blood of the cow.

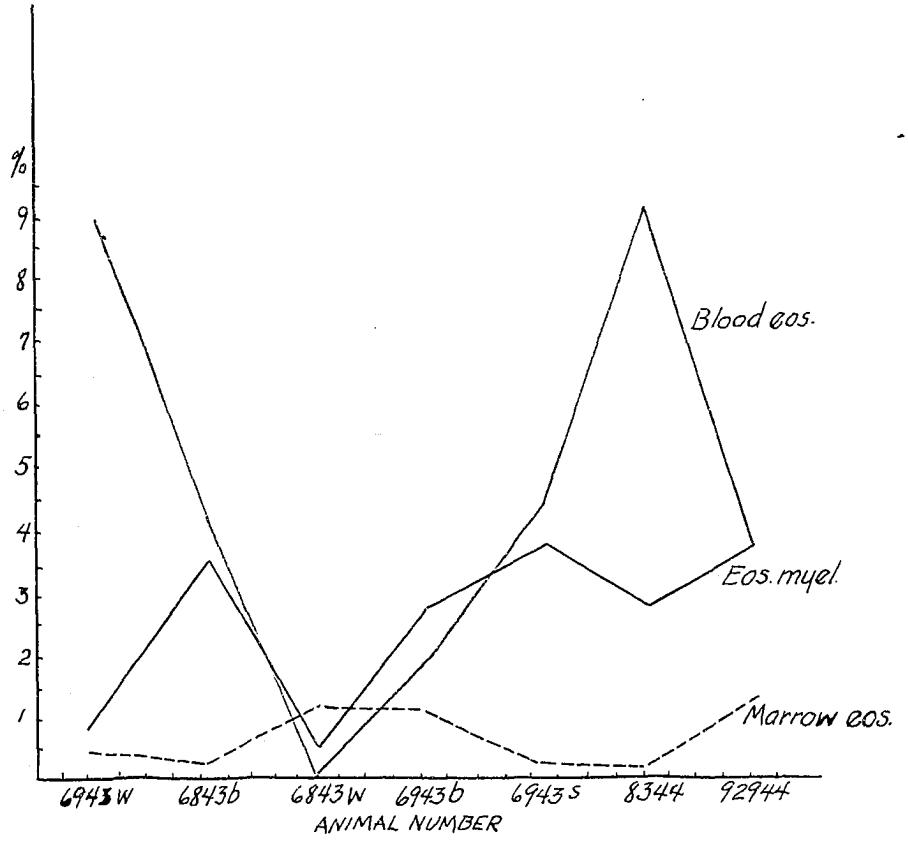

Fig. 4. Comparison of eosinophils of bone marrow and blood of horse.

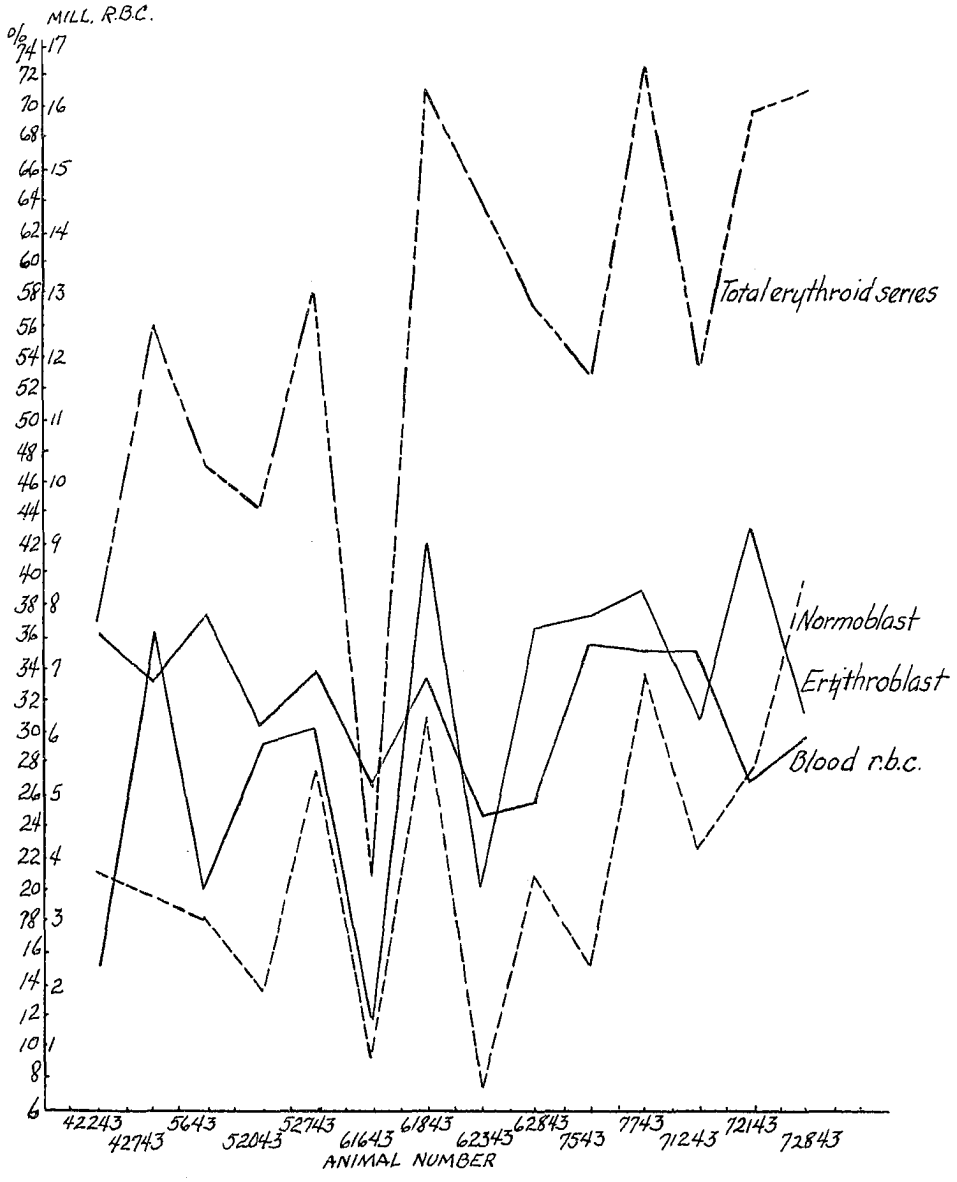

Fig. 5. Comparison of red blood cell series in the marrow with the peripheral red blood cell counts in the cows.

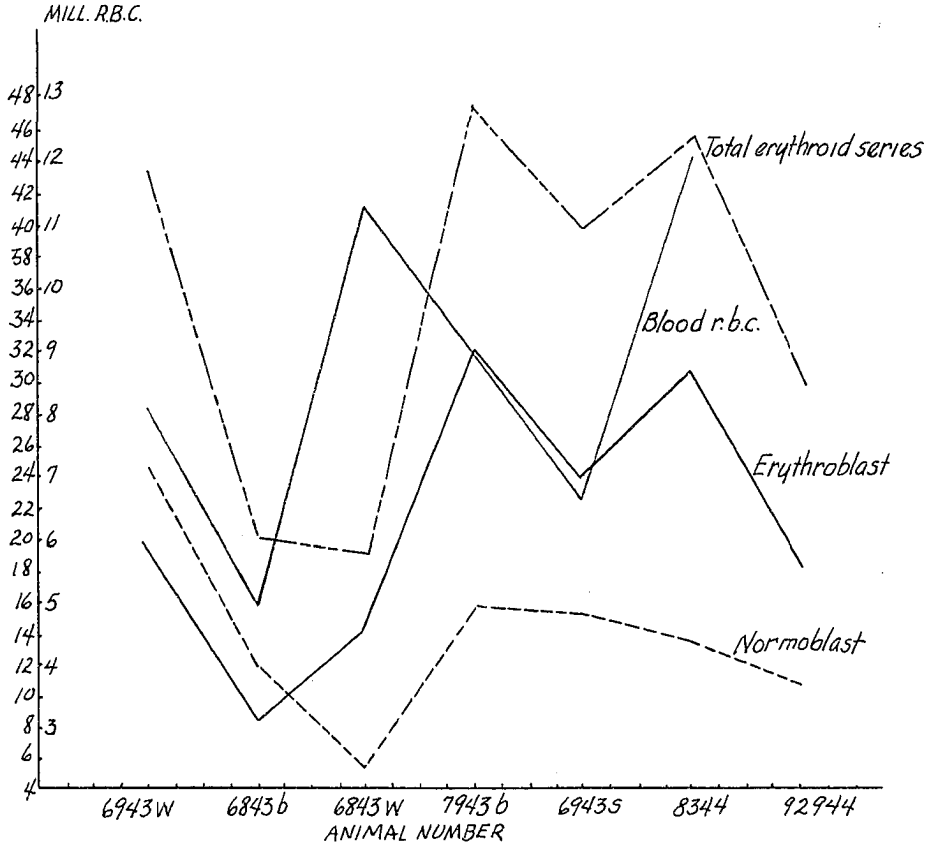

Fig. 6. Comparison of red blood cell series in the marrow with the peripheral red blood cell counts in the horses.

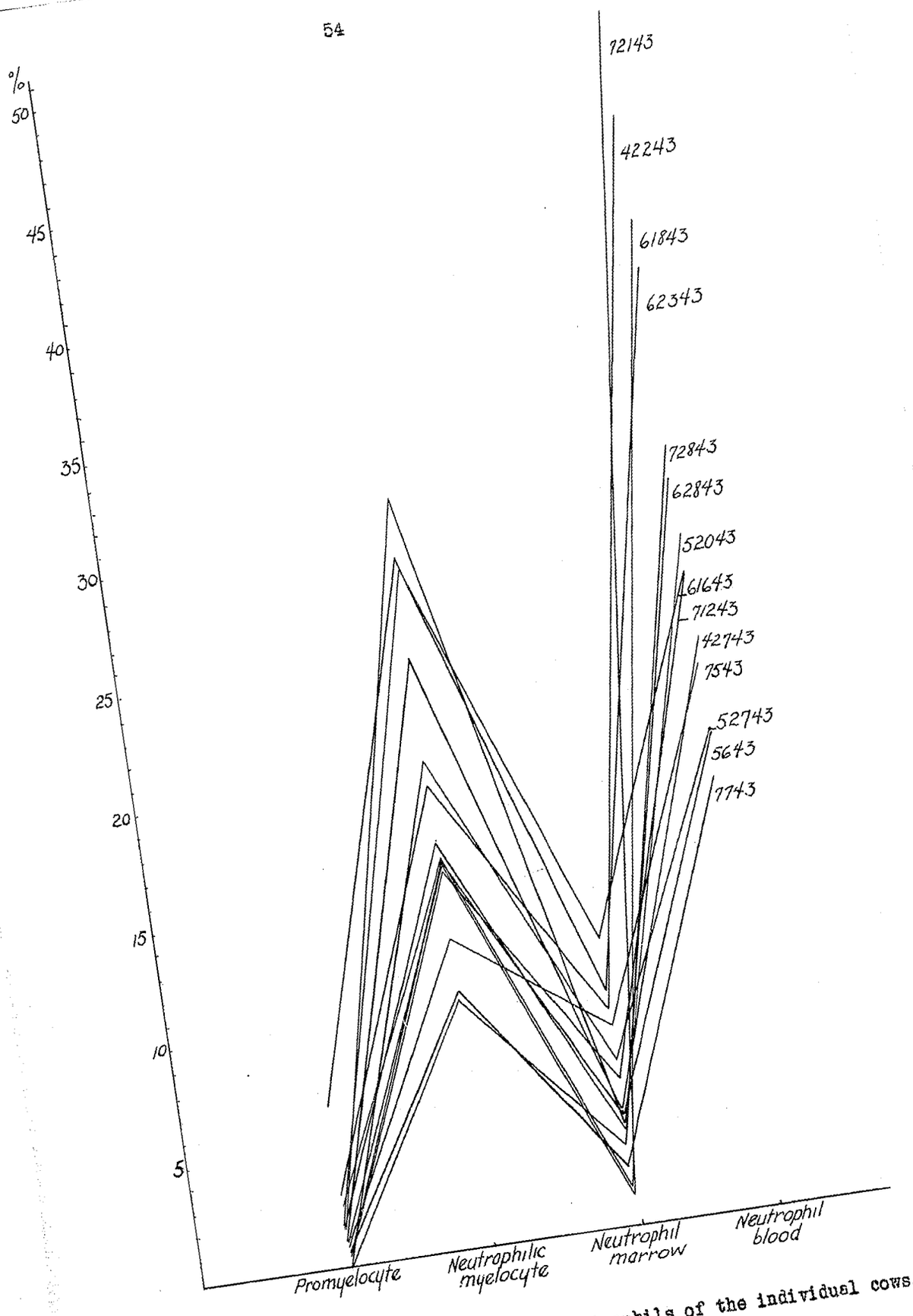

Fig. 7. Maturation curve for the neutrophils of the individual cows.

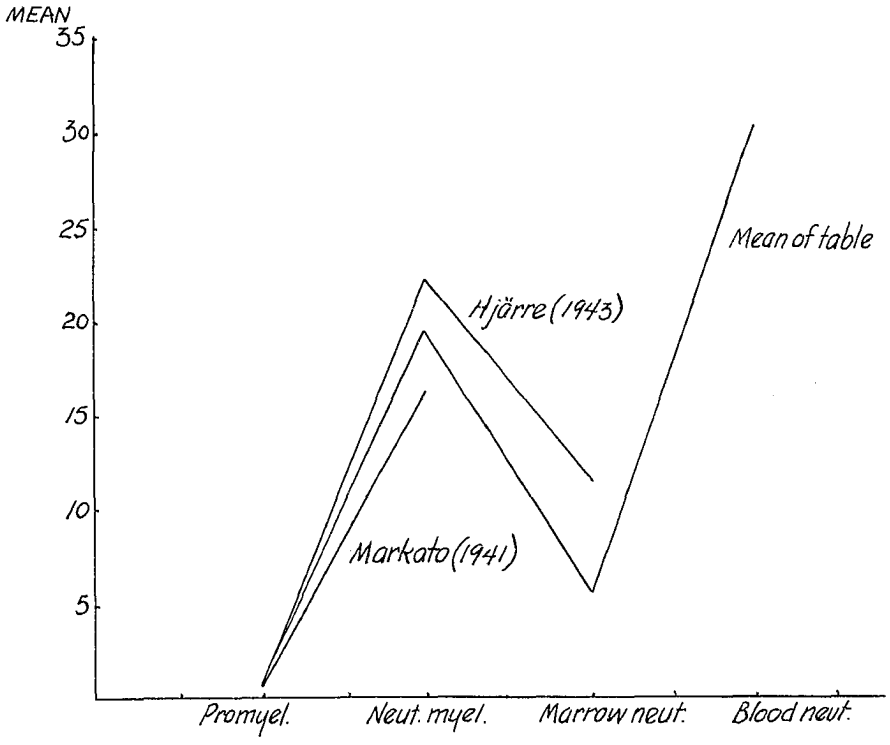

Fig. 8. Maturation curve of the neutrophils of the cow compared with that of other investigators.

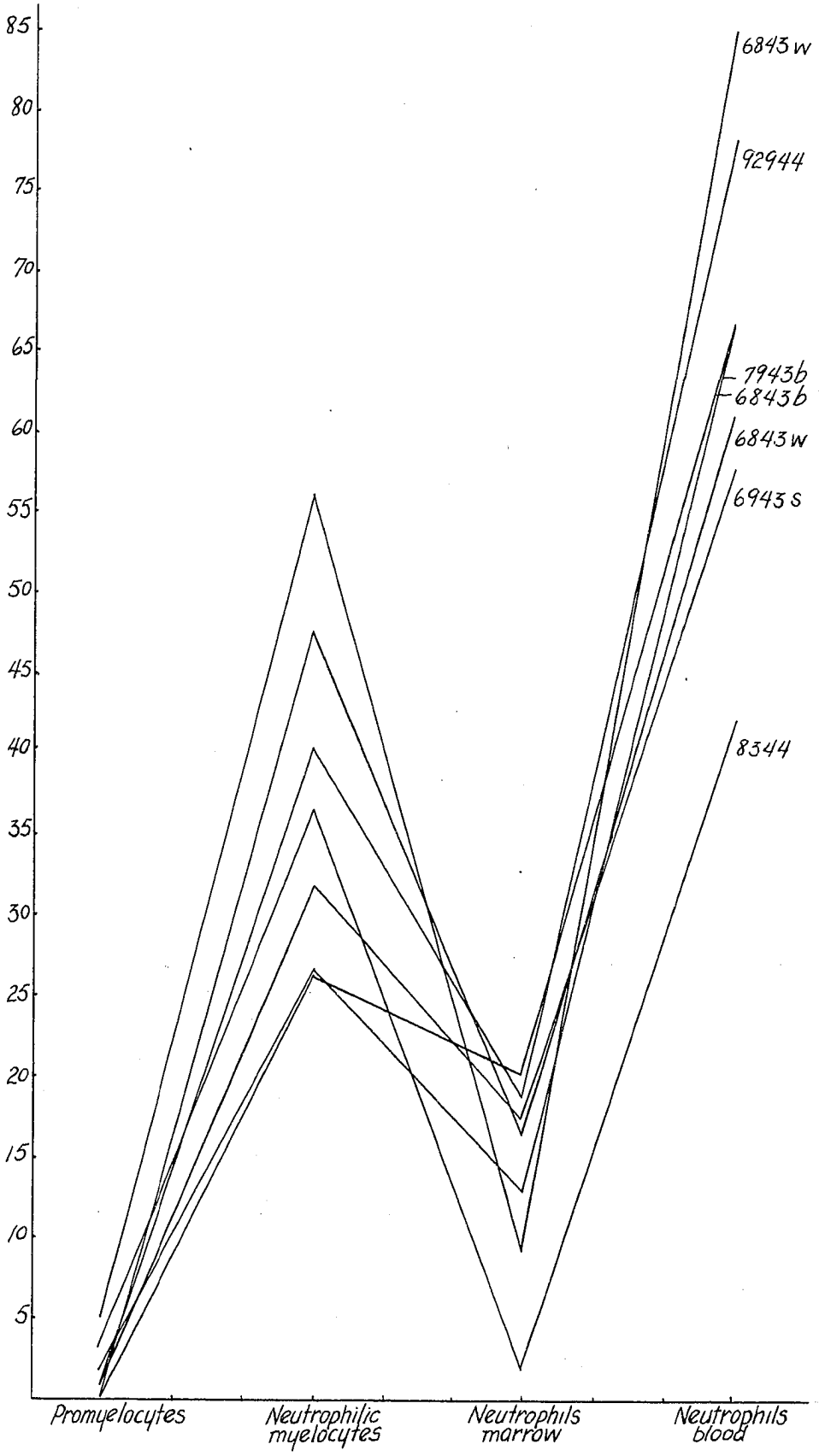

Fig. 9. Maturation curve for the neutrophils of individual horses.

Table 11

Comparison of peripheral blood with bone marrow (cow)

	Peripheral Blood from Table 3			Bone Marrow from Table 5	
Animal	Total leucocytes	% myeloid cells*	erythroid	myeloid	erythroid
42243	14,140	52.7	7,545,000	44.6	37.0
42743	6,720	36.0	6,850,000	36.6	56.0
5643	7,060	27.3	7,940,000	32.2	47.2
52043	6,360	37.2	6,125,000	47.4	44.2
52743	6,860	19.2	6,790,000	33.0	58.0
61643	8,080	31.6	5,280,000	54.4	21.0
61843	4,260	51.6	6,865,000	23.0	71.0
62343	6,760	46.3	4,775,000	60.4	27.2
62843	6,820	40.3	4,530,000	32.8	57.2
7543	8,900	38.6	6,900,000	35.6	52.4
7743	10,320	32.3	6,760,000	19.6	72.2
71243	7,140	32.6	6,615,000	34.4	53.0
72143	7,660	65.0	5,110,000	24.6	69.8
72843 Male	7,540	36.7	5,880,000	19.6	71.0
Mean	7,758.5	39.1	6,283,214.2	35.59	52.66
Myeloid-erythroid ratio				35.39/52.66 - .676	

*Neutrophils plus eosinophils plus basophils

Table 12

Comparison of peripheral blood with bone marrow (horse)

		Peripheral Blood from Table 4			Bone Marrow from Table 6	
Animal	Sex	Total White blood cells	% myeloid cells**	Total red blood cells	myeloid	erythroid
6943w	F	8,400	76.0	8,020,000	47.6	43.6
6843b	F	clotted	66.0	4,905,000	69.6	20.0
6843w	F	clotted	85.2	11,350,000	71.6	19.0
7943b	F	8,700	68.3	8,440,000	45.0	47.6
6943s	M-c*	8,340	73.3	6,525,000	53.2	38.8
8344	M-c	9,920	53.7	12,040,000	46.6	45.0
92944	F	-	82.3	-	63.6	28.6
Mean		8,840	72.1	8,546,666	56.74	34.66
Myeloid-erythroid ratio					56.74/34.66 - 1.64	

*M-c mele castrate
**Neutrophils plus eosinophils plus basophils

Table 13

Summary of bone marrow data on the cow and horse

Cells	Cow		Horse	
	Range	Mean	Range	Mean
Stem cell	0.0- 5.0	2.14	0.4- 3.4	1.6
Erythroblast	11.8-42.8	30.26	8.0-32.0	20.94
Normoblast	7.2-39.2	21.69	5.0-24.2	13.71
Total erythroid cells (E)	21.0-72.2	52.66	19.0-47.6	34.66
Promyelocyte	0.0- 6.8	1.51	0.0- 5.0	1.83
Neutrophilic myelocyte	10.4-32.0	19.39	26.2-56.0	38.06
Neutrophil	1.2-12.2	5.73	1.8-20.2	13.31
Eosinophilic myelocyte	1.8-10.4	6.69	0.4- 3.6	2.34
Eosinophil	0.0- 7.6	1.92	0.2- 1.2	0.60
Basophils (all)	0.0- 1.0	0.34	0.0- 1.0	0.60
Total myeloid cells (M)	19.6-60.4	35.59	45.0-71.6	56.74
Monocyte	0.0- 7.6	2.64	1.2- 4.8	2.46
Plasma cell	0.2- 2.0	0.79	0.0- 0.8	0.63
Lymphocyte	1.4-16.8	6.68	2.0- 5.6	3.91
Megakaryocytes in 300 sq. mm.	0-121	25.14	0-8	1.71
Mitoses per 500 cells	0-11	4.9	0-8	2.71
Myeloid-erythroid ratio M/E	0.27-2.5	0.676	0.94-3.76	1.64

of megaloblasts in the marrow ruled out carcinoma of the stomach. Dameshek, Henstell and Valentine (1937) found sternal puncture to be indicated in persistent anemia, leukopenia, thrombocytopenia, splenectomy and for a differential diagnosis of splenomegaly. Jagić and Klima (1937) thought that a marrow examination was useful in diseases of the reticulo-endothelial system. Vogel, Erf and Rosenthal (1937) determined that bone marrow studies were of diagnostic importance in Gaucher's disease, myeloma, leishmaniasis, malaria, certain leukemias and carcinomas as well as of confirmatory value in a number of other blood dyscrasias. Zanaty (1937) thought some workers exaggerated the diagnostic value of bone marrow counts but admitted their value in the study of the morbid anatomy and physiology of the blood forming organs and the effects of treatment. Hardgrove and Van Hecke (1939) resorted to bone marrow examination in questionable blood dyscrasias and certain blood disorders. Hynes (1939) observed that aplastic anemia and myelosclerosis necessitated bone marrow study. Schmid (1939) made a study of blood and bone marrow in human undulant fever. He observed "Bang nodules" in the marrow and thought the platelet forming function of the megakaryocytes was interfered with. Vogel and Bassen (1939) used sternal puncture to rule out leukemia in cases of infectious mononucleosis. Jones (1940) studied the bone marrow in hyperthyroidism and hypothyroidism and found an increase in nucleated cells in the former and a decrease in the latter. Limarzi and Schleicher (1940) worked on the reaction of peripheral blood and bone marrow in chronic hemmorhage and essential thrombopenic purpura and concluded that diagnoses of purpuric states could be more satisfactorily made from bone marrow studies. Davidson (1941b) confirmed diagnoses of cancer, Hodgkins disease, multiple myelomatosis and lipoidoses by finding specific cellular

elements in the bone marrow. Gordon (1941) summarized the value of bone marrow studies under four headings: to aid in the diagnosis of obscure conditions, to confirm diagnoses, to further the understanding of disease processes and as a method of bioassay of certain drugs.

Falconer and Leonard (1941) used marrow technic as a means of gaining additional information and suggested its interpretation in the light of other data. They recommended brucellosis and bacterial endocarditis as a field for further study. Beizer, Hall and Giffin (1942) confirmed suspected myeloma by finding myeloma cells in the bone marrow. Dameshek (1942) considered bone marrow biopsy an important tool in the differential diagnosis of refractory anemia and pancytopenia. He also ruled out leukemia in cases of thrombopenic purpura and hemolytic anemia by this method. Kienle (1942) distinguished erythroblastosis and erythroleukemia by bone marrow studies. In addition to those hemopoietic disorders already mentioned Turkel and Bethel (1943) claimed marrow aspiration from the sternum to be an almost universal practice in disturbances of the reticulo-endothelial system, certain infectious diseases and neoplasms of the marrow cavity.

Williams (1943) found the megakaryocyte count in primary pneumonia to be more than 5000 cells per cubic millimeter and suggested a definite relationship between pneumonia and hyperplasia of megakaryocytes.

Bloom (1945) is pioneering in the veterinary field in studying bone marrow in various diseased conditions in the dog. Some of his findings may be noted here: increased myeloid-erythroid ratio in pyometra, and aregenerative anemia; neutrophilic hyperplasia in marrow in pyometra and streptothrichosis; decreased myeloid-erythroid ratio and hyperplasia of the erythroid series in advanced filariasis, and the occurrence of lymphoma cells in the

bone marrow in malignant lymphoma.

If future bone marrow studies should lead to an earlier diagnosis of obscure blood dyscrasias in our farm animals it would be of considerable economic value. Mitchell (1943) suggested that an early diagnosis of lymphocytoma in cattle would result in a saving in feed and care.

In concluding, the fact may be emphasized that this is only a fragment of the studies that could and need to be made. Further work may reveal a more refined technic of obtaining marrow from these animals. Schleicher (1944) has completed a study on the volumetric pattern of sternal marrow in man including the fat, plasma, myeloid-erythroid ratio, and erythrocyte content per cubic centimeter. Such a study would be valuable in animals. Wirth (1938) investigated the reactions of the hematopoietic system of the various domestic animals in response to bleeding. He found that it varied with the species. Could this variation have been due to inherent differences in the bone marrow? No bone marrow studies were made. The gross changes associated with age and variations with sex are yet to be worked out for the various species of animals. Are lymph nodules present in the marrow of any of our domestic animals? There is a difference of opinion regarding their presence in man. Jaffé (1936) gave the weight of human marrow in percent of the body weight, 3.4 to 5.9, and found five/ninths of a gram of marrow per gram of blood. He further stated that half of the bone marrow of an adult person is active and concluded that the weight of the red marrow equaled that of the liver. How would such figures for domestic animals compare? The effect of drugs upon bone marrow of our domestic animals is yet to be determined. In the future bone marrow may become as important a diagnostic agent in the field of veterinary medicine as it now is in the realm of human medicine.

SUMMARY

This study was undertaken to determine the normal cytological picture of the bone marrow for the horse and cow. Samples were obtained from fourteen head of cattle and seven horses. The ribs were chosen as the site for securing the marrow in both species. The samples were obtained by drilling into the rib, inserting a cannula into the drill hole and aspirating 1-2 cc. of marrow. Peripheral blood samples were taken at the same time. Blood and marrow smears were made and stained with Osgood's (1937) modification of Wright's blood stain.

A sampling study was made at two different levels on each of five ribs (8th-12th) in one cow and the cell counts recorded. An analysis of variance of the means showed a significant variation from the mean for the erythroblasts, promyelocytes and lymphocytes in the 12th rib. A significant positive trend was observed from the 8th to 12th rib in the total erythroid cells, and the neutrophils showed a negative trend in the same direction. Since only one animal was used, more work along the same line is needed to confirm these data.

A study was made of the healing process of the drill hole in horse ribs and photomicrographs made to illustrate the progress of repair. Repair of the bone was almost complete in 7 weeks and all external indications had disappeared long before that.

Cell counts were made to establish a "normal" myelogram for the cow and horse. Three hundred cells were counted in the differential leucocyte count on the blood smears and 500 cells were enumerated in the differential count on the marrow smears. The mitotic figures encountered per 500 cells were also recorded. The megakaryocytes were counted in a 300 square milli-

meter area. The myeloid-erythroid ratio was determined. Cytological studies were made of the marrow cells and color comparisons were made with colors in the Munsell (1929) Book of Color. Colored photomicrographs were made of the bone marrow smears to illustrate the various types of cells and cclored drawings of the various cells were incorporated into a plate.

The myelogram for the cow (range and mean in percent): stem cell: 0.0 - 5.0, 2.14; erythroblast: 11.8 - 42.8, 30.26; normoblast: 7.2 - 39.2, 21.69; total erythroid cells (E): 21.0 - 72.2, 52.66; promyelocyte: 0.0 - 6.8, 1.51; neutrophilic myelocyte: 10.4 - 32.0, 19.39; neutrophil: 1.2 - 12.2, 5.73; eosinophilic myelocyte: 1.8 - 10.4, 6.69; eosinophil: 0.0 - 7.6, 1.92; all basophils: 0.0 - 1.0, 0.34; total myeloid cells (M): 19.6 - 60.4, 35.59; monocyte: 0.0 - 7.6, 2.64; plasma cell: 0.2 - 2.0, 0.79; lymphocyte: 1.4 - 16.8, 6.68; megakaryocytes in 300 sq. mm.: 0 - 121, 25.14; mitoses per 500 cells: 0 - 11, 4.9; myeloid-erythroid ratio (M/E): 0.27 - 2.59, 0.676.

The myelogram for the horse (range and mean in percent): stem cell: 0.4 - 3.4, 1.6; erythroblast: 8.0 - 32.0, 20.94; normoblast: 5.0 - 24.2, 13.71; total erythroid cells (E): 19.0 - 47.6, 34.66; promyelocyte: 0.0 - 5.0, 1.83; neutrophilic myelocyte: 26.2 - 56.0, 38.06; neutrophil: 1.8 - 20.2, 13.31; eosinophilic myelocyte: 0.4 - 3.6, 2.34; eosinophil: 0.2 - 1.2, 0.60; all basophils: 0.0 - 1.0, 0.60; total myeloid cells (M): 45.0 - 71.6, 56.74; monocyte: 1.2 - 4.8, 2.46; plasma cell: 0.0 - 0.8, 0.63; lymphocyte: 2.0 - 5.6, 3.91; megakaryocyte in 300 sq. mm.: 0 - 8, 1.71; mitoses per 500 cells: 0 - 8, 2.71; myeloid-erythroid ratio: 0.94 - 3.76, 1.64. (A table summary of these data may be found on page 59, Table 13).

Graphs indicated a positive correlation between the marrow neutrophilic myelocyte and the adult neutrophil in the blood but no correlation be-

tween the marrow eosinophilic myelocyte and the eosinophil in the circulating blood. Similarly, graphs comparing the marrow red blood cell series to the erythrocytes in the peripheral blood suggested some correlation though not as striking as the neutrophil or eosinophil. Individual neutrophil curves for all the animals were similar. Figures were not available for the horse but the neutrophil curve of the cow agreed favorably with those of other investigators in the field.

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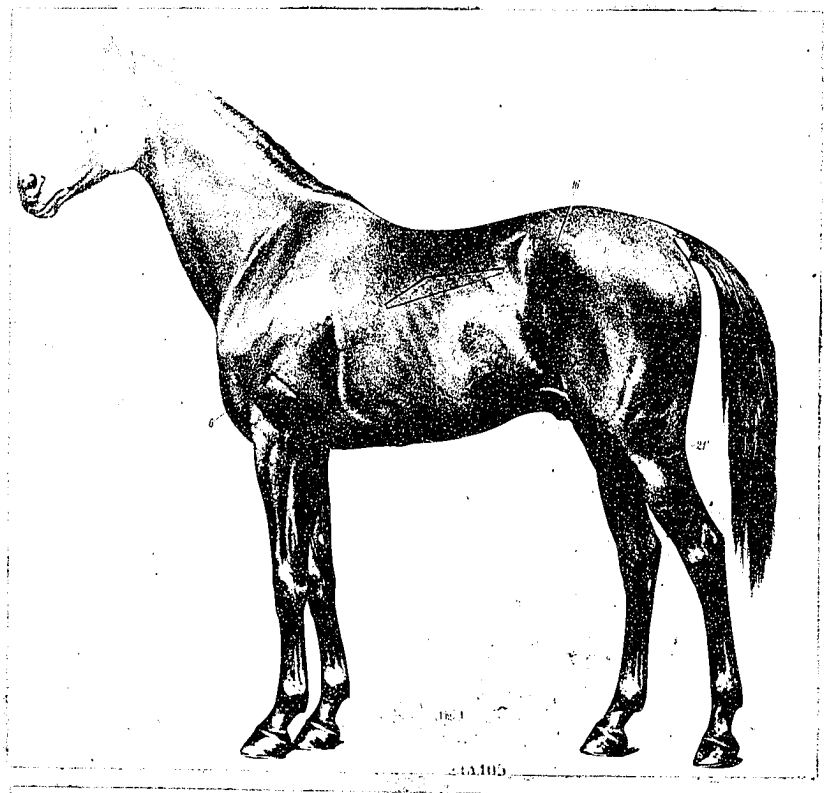
Plate I

Plates I and II are photographs of plates in Volume I and II of Ellenberger, Baum and Dittrich's (1932) "Handbuch der Anatomie der Tiere".

Fig. 1. Lateral view of the horse showing the general area in which to drill into the ribs for marrow samples.

Fig. 2. Lateral view of the horse with skin and superficial fascia removed to show what portion of the lateral surface of the ribs is relatively exposed.

Plate I



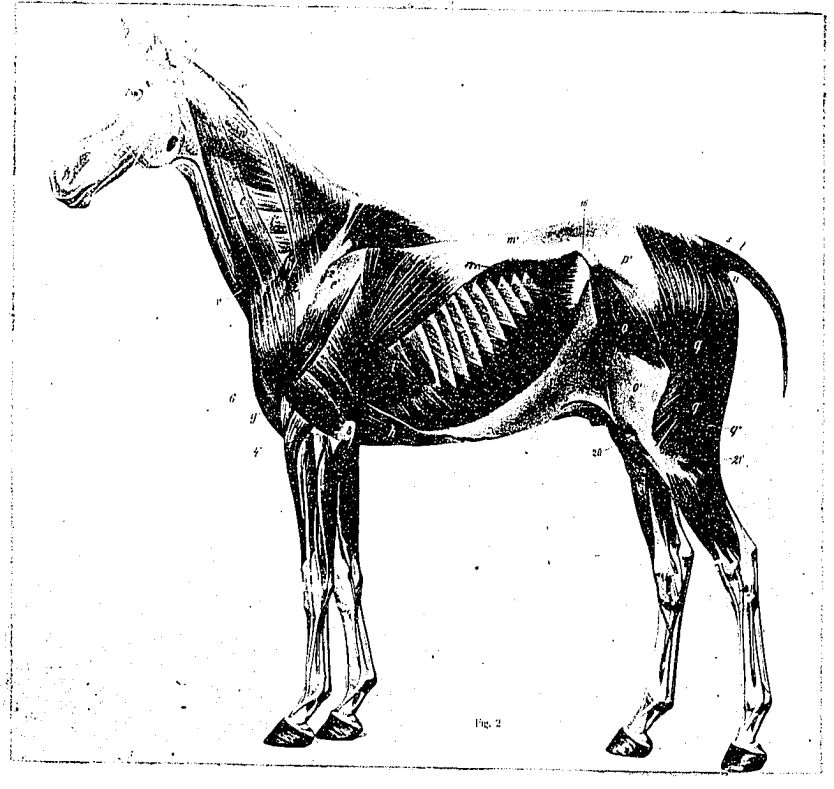


Plate II

Fig. 1. Lateral view of the cow showing the general area in which to drill into the ribs for marrow samples.

Fig. 2. Lateral view of the cow with skin and superficial fascia removed to show what portion of the lateral surface of the ribs is relatively exposed.

Plate II

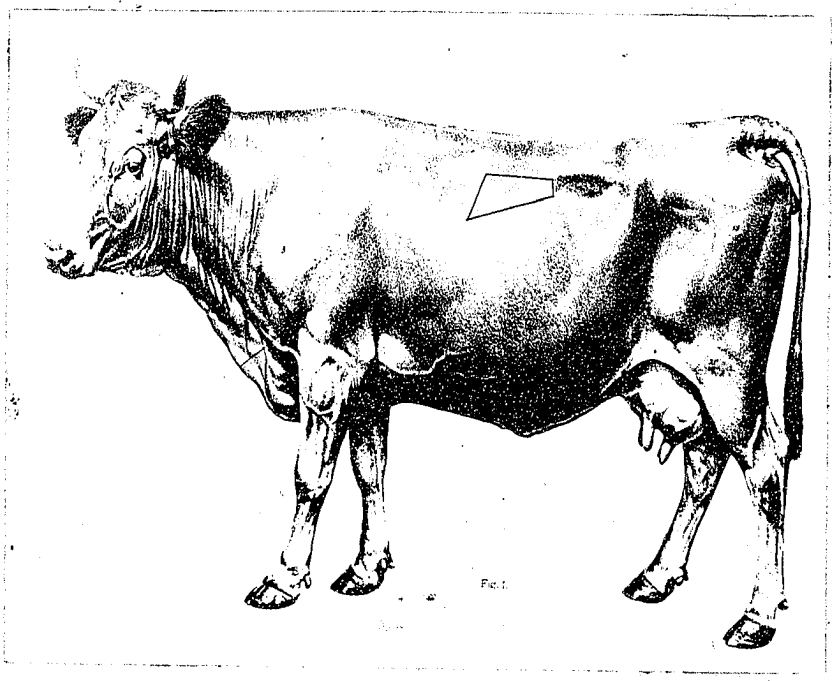


Fig. 1.

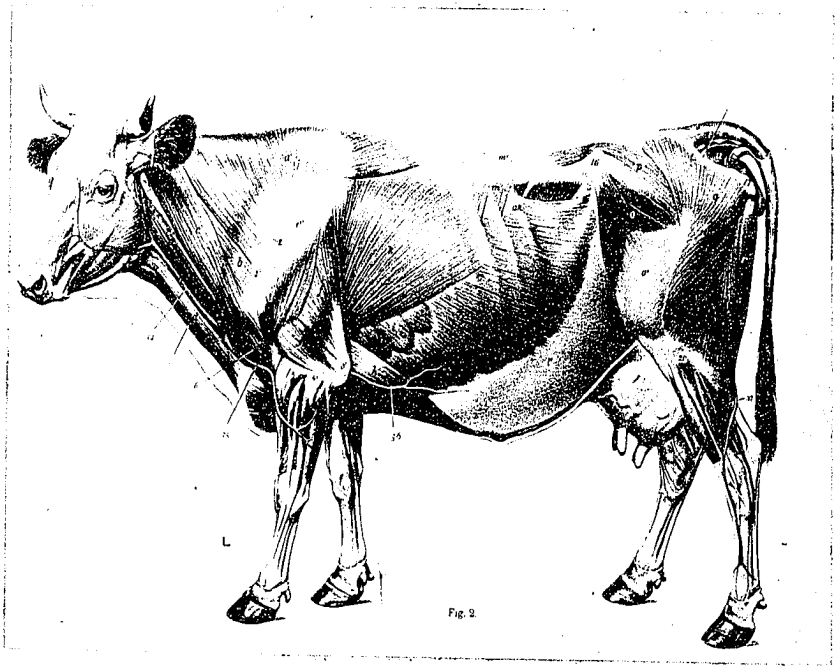


Fig. 2.

Plate III

Materials used in drilling for bone marrow.

Plate III

Plate IV

Plates IV, V, VI, VII, VIII, and IX are Kodacolor prints made from Eastman Kodacolor roll film. The pictures were taken with a Voightlander camera supported over a research microscope. A 10x ocular and an oil immersion objective were used. By measurement of the prints the magnification of the cells was determined to be approximately 700x. Since Kodacolor is designed for use in daylight and it was used here with artificial light, the color balance was not what it should have been. This resulted in the general blue-green color of the pictures and the failure of the reds to show too well. Plate X more nearly shows the true color.

Several prints from each animal are included to get as wide a variety of cells as possible.

Fig. 1. Cow bone marrow

1. Stem cells
2. Erythroblasts
3. Normoblast shedding its nucleus
4. Neutrophilic myelocytes
5. Neutrophils
6. Eosinophilic myelocytes
7. Monocyte
8. Erythroblast in mitosis
9. Degenerated or smear cell.

Fig. 2. Cow bone marrow

1. Stem cells
2. Erythroblasts
3. Normoblast shedding its nucleus
4. Neutrophilic myelocytes
5. Neutrophils
6. Eosinophilic myelocytes
7. Monocyte
8. Plasma cell

Plate IV

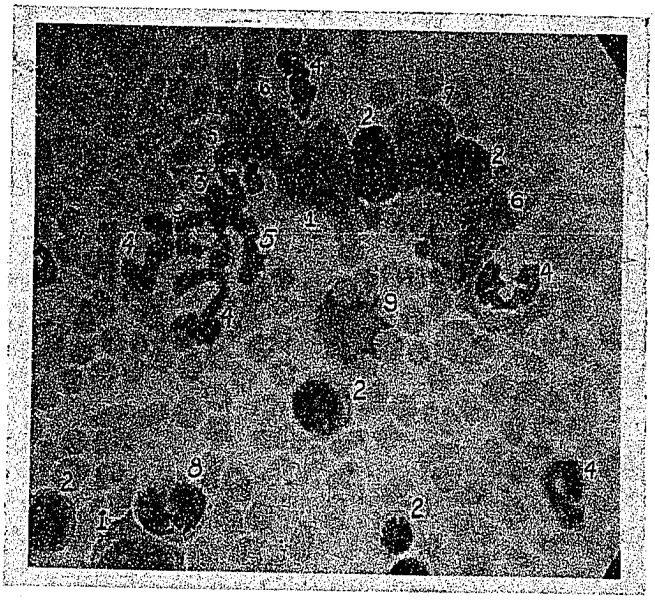


Fig. 1

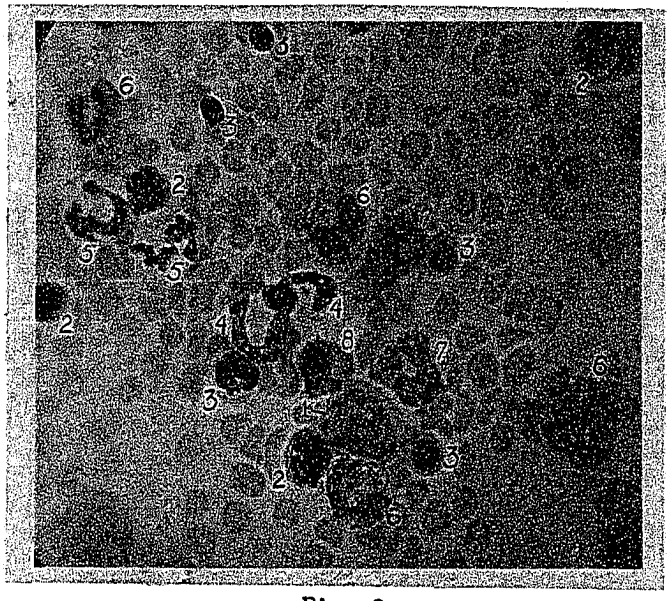


Fig. 2

Plate V

Fig. 1. Cow bone marrow

1. Erythroblasts
2. Normoblasts
3. Neutrophilic myelocytes
4. Eosinophilic myelocytes
5. Megakaryocyte

Fig. 2. Cow bone marrow

1. Erythroblasts
2. Normoblast
3. Neutrophilic myelocyte
4. Monocyte
5. Degenerated cell

Plate V

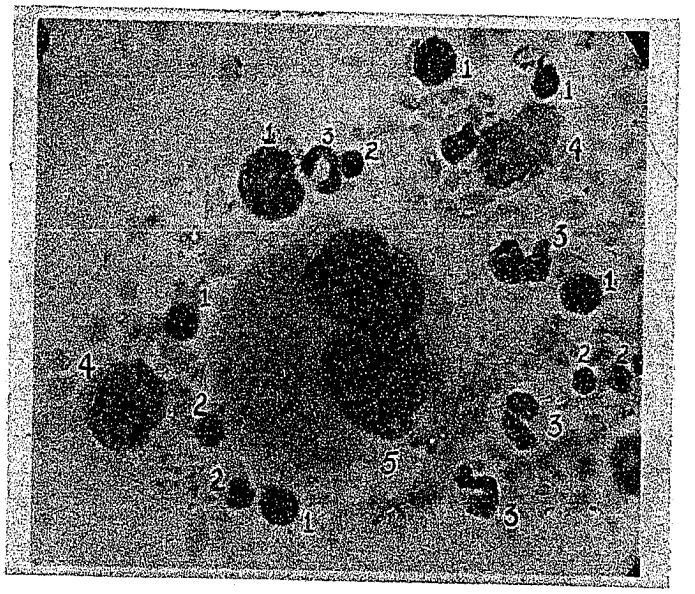


Fig. 1

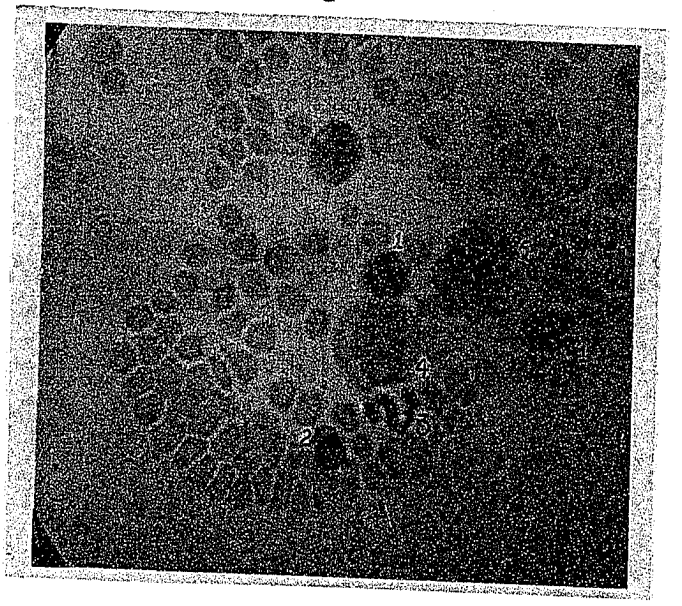


Fig. 2

Plate VI

Fig. 1. Cow bone marrow

1. Promyelocyte
2. Neutrophilic myelocyte
3. Erythroblast
4. Normoblast
5. Lymphocyte

Fig. 2. Horse bone marrow

1. Successive stages of erythroblasts
2. Normoblast
3. Neutrophil

Plate VI

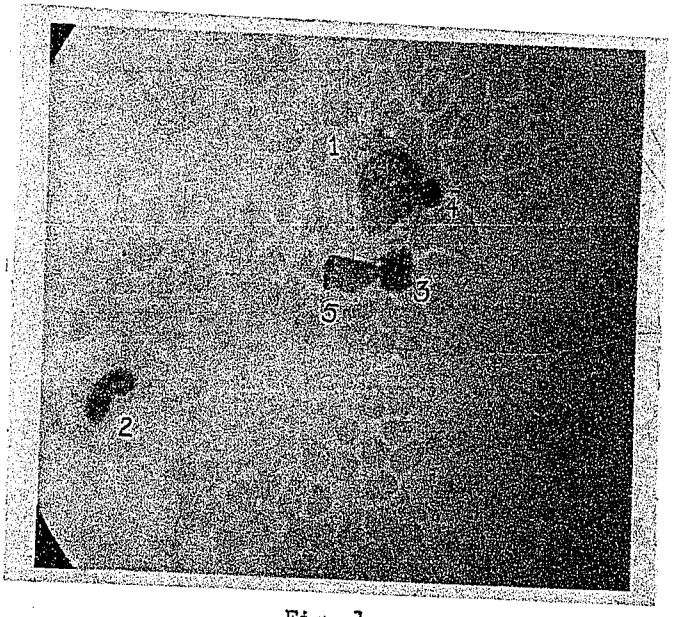


Fig. 1

Fig. 2

Plate VII

Cow bone marrow

1. Eosinophilic myelocytes
2. Nucleus of a normoblast
3. Neutrophilic myelocyte
4. Plasma cell
5. Degenerating eosinophilic myelocyte

Plate VII

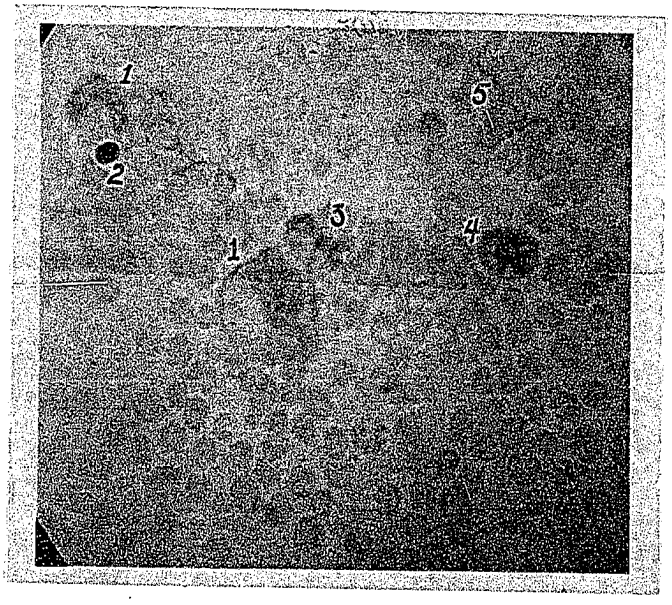


Plate VIII

Fig. 1. Horse bone marrow

1. Promyelocyte
2. Promyelocyte
3. Eosinophil
4. Erythroblast
5. Neutrophilic myelocyte
6. Lymphocyte (nucleus showing bizarre clover leaf form)
7. Promyelocyte (cytoplasmic granules not in focus)

Fig. 2. Horse bone marrow

1. Plasma cell (amitotic division)
2. Lymphocyte
3. Erythroblast
4. Neutrophilic myelocyte
5. Neutrophils

Plate VIII

Fig. 1

Fig. 2

Plate IX

Fig. 1. Horse bone marrow

1. Promyelocyte
2. Erythroblasts
3. Eosinophilic myelocytes
4. Basophil
5. Lymphocyte
6. Neutrophil

Fig. 2. Horse bone marrow

1. Basophil
2. Normoblast
3. Eosinophil

Plate IX

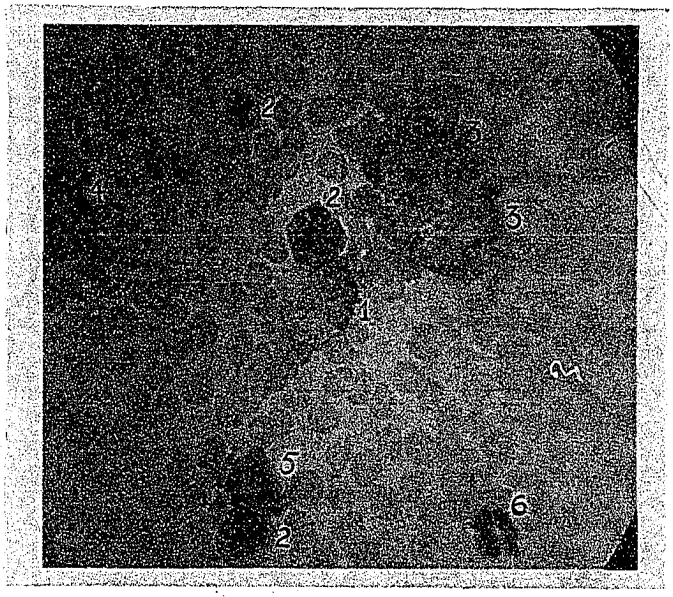


Fig. 1

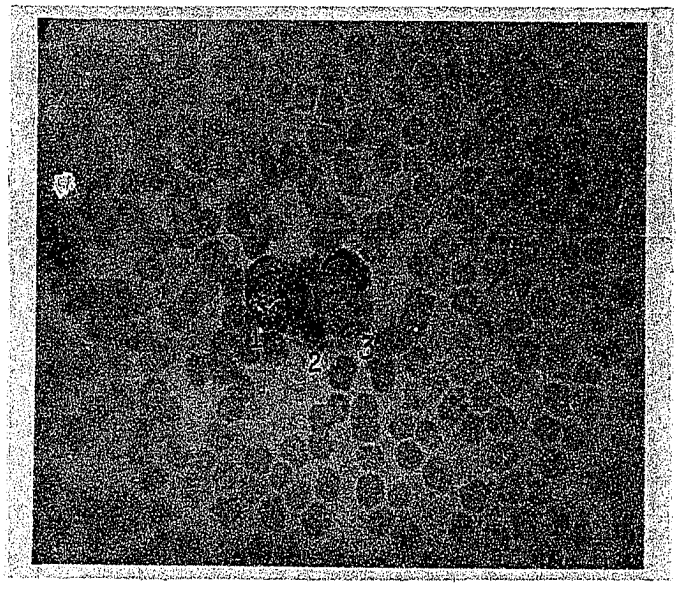


Fig. 2

Plate X

Drawings of the bone marrow cells of the horse and cow.

1. Stem cell - horse - 15 x 18 micra
2. Erythroblast, cow - 8.5 x 9.5
3. Late erythroblast, cow - 7 x 8
4. Normoblast shedding its nucleus, cow - 7.5 x 8.5
5. Plasmablast, horse - 20 x 18.5
6. Promyelocyte, horse - 16 x 17.5
7. Erythroblast in mitosis, cow - 17 x 19
8. Monoblast, horse - 15 x 16.5
9. Neutrophilic myelocyte, horse - 13 x 15
10. Eosinophilic myelocyte, cow - 22.5 x 23
11. Lymphoblast, cow - 10 x 12
12. Basophilic myelocyte, horse - 16.5 x 20
13. Eosinophil, horse - 13 x 14

Plate X

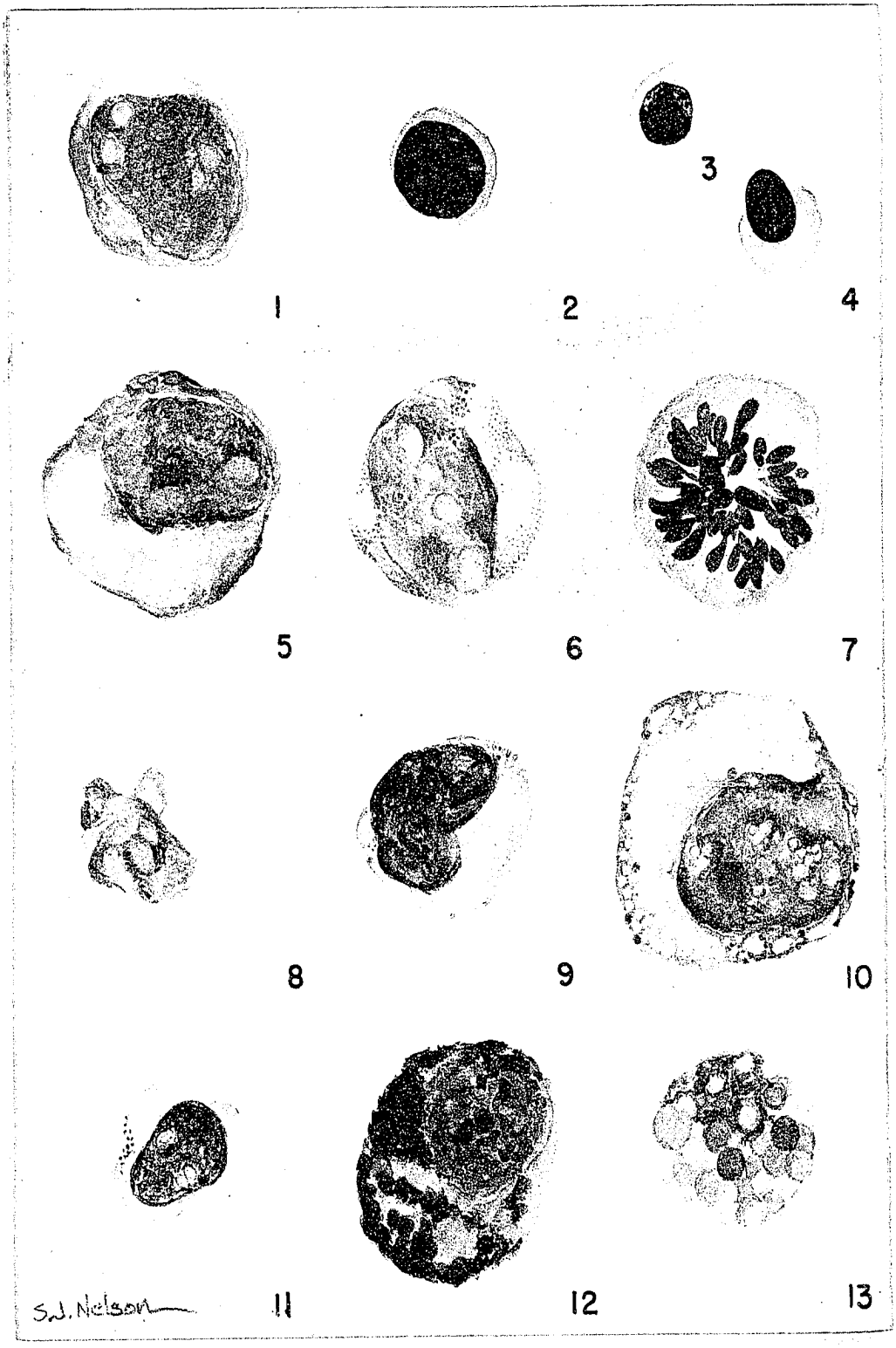


Plate XI

Fig. 1. Megakaryocyte (50 x 55 micra) x 800

Note the ragged edge with fragments (platelets) along the border.

Fig. 2. Megakaryocytes (50 x 60 micra) x 760

1. Pale-blue stained outer rim of cytoplasm.
2. Denser staining purple central mass.
3. Only apparent nuclear mass in the group.
4. Fragmenting edge.

Fig. 3. Megakaryocyte with pseudopods (55 x 100 micra) x 1200

This cell had a blue staining center with an outer rim of fine azure granules.

Fig. 4. Megakaryocyte with pseudopods (52 x 115 micra) x 800

Plate XI

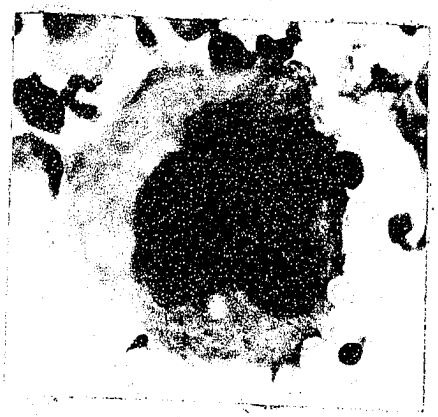


Fig. 1

Fig. 2

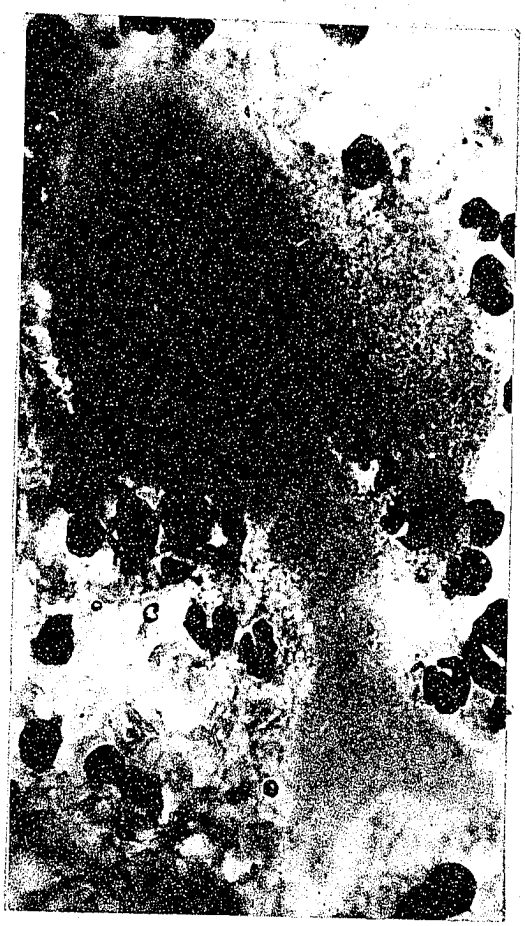


Fig. 3

Fig. 4

Plate XII

Plates XII to XV illustrate the healing process of the drill hole in the rib of the horse.

Fig. 1. Rib drilled 2 hours before killing the animal.

Ca 30 x. Fibrin in the blood clot.

Fig. 2. Rib drilled 6½ hours before killing the animal.

Ca 30 x Fibroblasts were beginning to appear in the mass of blood and fibrin.

Plate XII

Fig. 1

Fig. 2

Plate XIII

Fig. 1. Rib drilled 2 days before the animal was killed. Ca 30 x. Numbers of fibroblasts were increased.

Fig. 2. Rib drilled 4 days before the animal was killed. Ca 30 x. Fibroblasts still increasing and clot beginning to be resorbed.

Plate XIII

Fig. 1

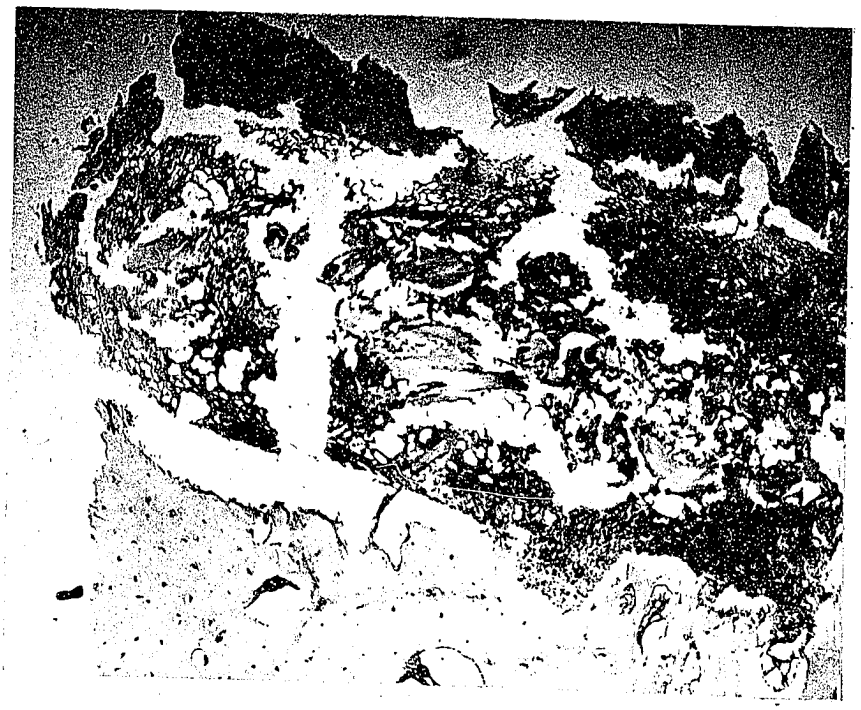


Fig. 2

Plate XIV

Fig. 1. Rib drilled 2 weeks and 1 day before killing the animal. Ca 30 x Provisional callus formed.

Fig. 2. Rib drilled 5 weeks 2 days before the animal was killed. Ca 20 x Drill hole beginning to be filled with new bone.

Plate XIV

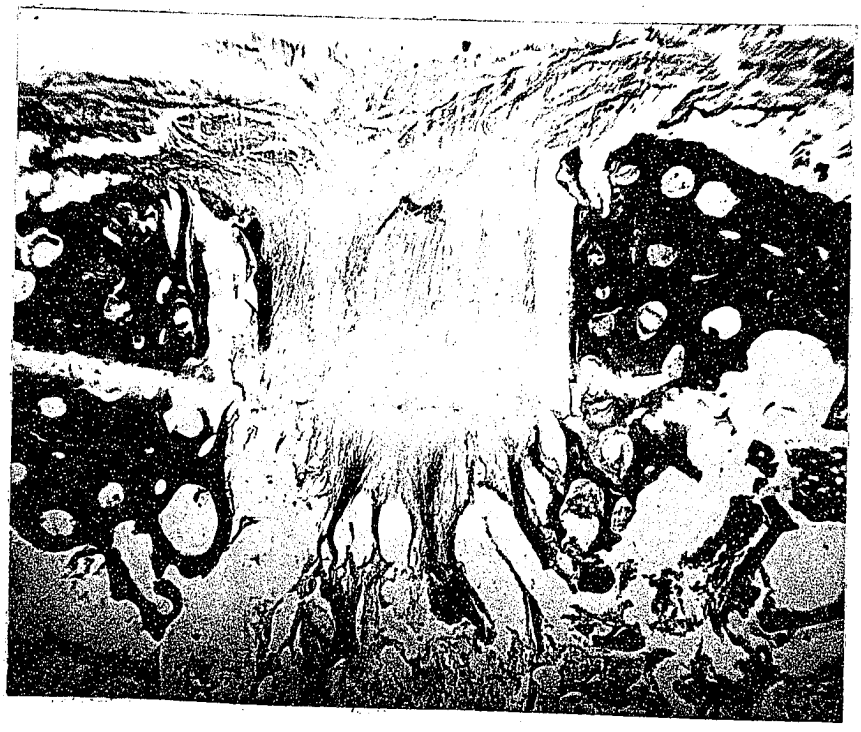


Fig. 1

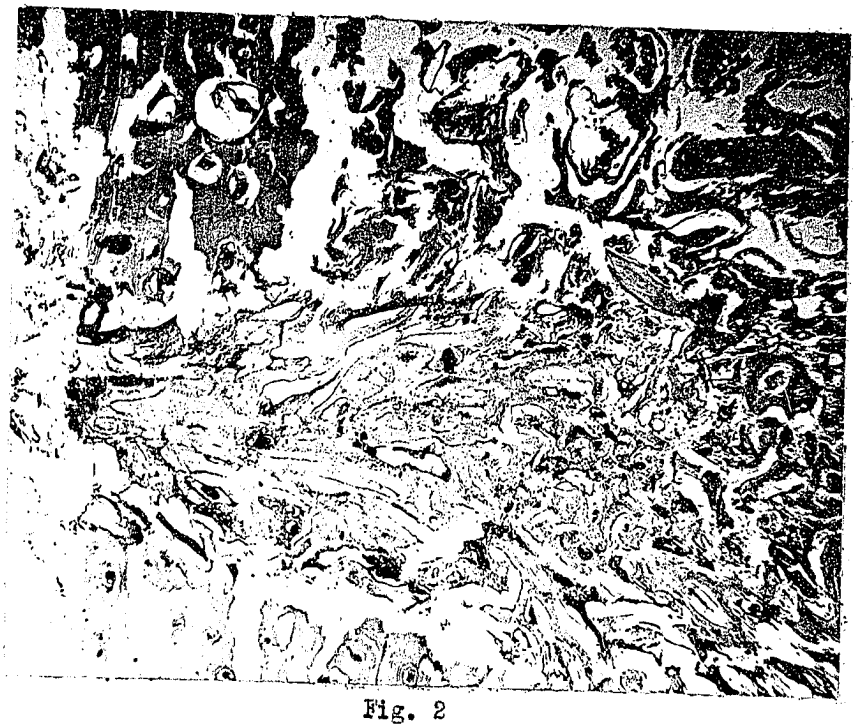


Fig. 2

Plate XV

Fig. 1. Rib drilled 7 weeks 2 days before the animal was killed. Ca 30 x The periosteal surface of the drill hole practically healed over.

Fig. 2. A longitudinal section of a rib which had not been subjected to the drill. Ca 20 x

Plate XV

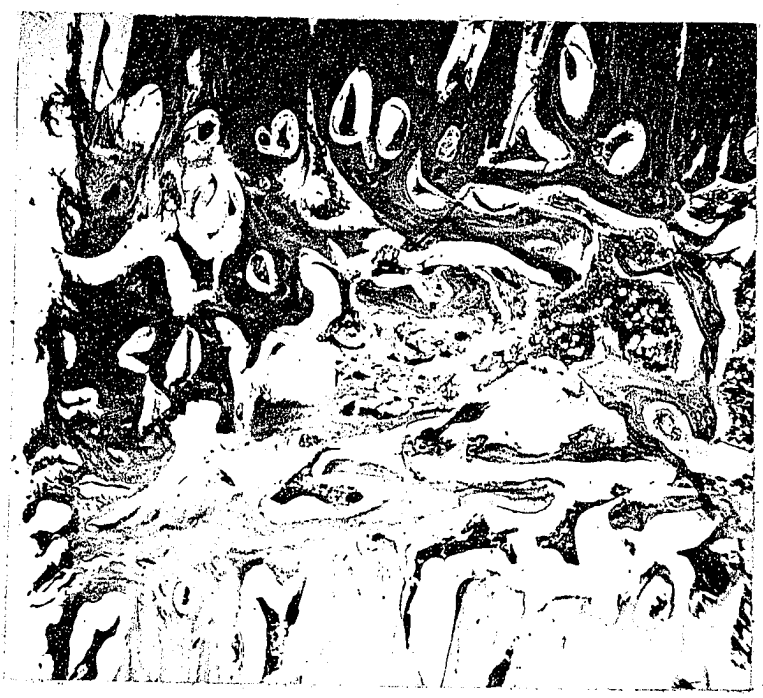


Fig. 1

Fig. 2