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M. Lois Calhoun
Michigan State College

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Technic For Obtaining Bone Marrow From the Horse and Cow

M. Lois Calhoun, D.V.M.*

IN THE light of recent advances in human hematology employing the study of bone marrow, it seemed that similar technics might be employed in veterinary medicine. In human medicine bone marrow studies have been found to be valuable in the diagnosis of obscure conditions, particularly anemias, leukemias, myelomas and cancer, to confirm diagnoses, as an aid in the understanding of disease processes and in the bioassay of drugs. A search of the literature failed to reveal any available studies on the bone marrow of our domestic animals with the exception of the dog. Alexandrov1, Fairman and Whipple2, Stasney and Higgins3, Mulligan4, Van Loon and Clark 6, Bloom7, and Bloom and Meyer5 have contributed to the knowledge of the bone marrow of the latter. Foreign papers in which the bone marrow of the horse or cow was described were written by Ackernecht9, Varicak10, Hjärre and Berthelsen11, Tkachenko12, Hjärre13, Hölzel14, and Marcato15, Hrestak16.

Operative Site

The marrow cavity of the young of all species is filled with red marrow. With increasing age the red marrow of the appendicular skeleton is gradually replaced by a labile, fatty, yellow marrow until in the adult, active, red marrow is confined largely to the axial skeleton. Investigations on the horse and cow had previously determined that the dorsal end of the ribs always retained red marrow while in some instances the sternal end of the ribs became a reservoir for fat. Hjärre and Berthelsen11 and Hjärre13 utilized the sternum as a source of bone marrow in both the horse and cow. Since such a heavy musculature covers the sternum and to enter the sternum requires that the animal be in a prone position, the rib is a more accessible site for securing marrow. The best place in the rib area is at as high a level as possible and still miss the long muscles of the back and as far forward as possible and still stay posterior to the latissimus dorsi muscle. In the cow such a location is limited to the eleventh rib. In the horse more latitude is possible because of the anatomical variation and any rib between the eleventh and fifteenth may be used. (Compare Figs. 1 and 2.)

Technic

The side and back of the animal are brushed and wiped down with a damp cloth. The operative site is shaved or the hair closely clipped, washed with soap solution and iodine applied. About 100 cc. of a 2 per cent procaine hydrochloride solution is injected, first anesthetizing the skin, next the underlying fascia and finally the periosteum.

A short incision is made in the skin through which the fascia and periosteum are incised. Such an incision will heal without complications leaving no visible scar once the hair grows out again. Use a No. 487 Goodell-Pratt hand drill equipped with a straight shank 3/32" jobber's drill to bore into the marrow cavity. Enter the rib midway between the anterior and posterior borders as there is danger of penetrating the thoracic cavity if the medullary cavity is missed. The drill "gives" when it hits the marrow cavity. The drill is then removed and replaced.

* Assistant Professor, Anatomy Department, Michigan State College, East Lansing, Michigan.
with a needle trocar (cannula and stilet) with the same outside diameter as the drill. Withdraw the stilet and attach an air tight 10 cc. syringe to the cannula. One cc. or less of marrow is aspirated into the syringe. Marrow is more viscous than blood and greyish red in color. The sample will be more or less diluted with blood depending on the amount of blood aspirated with the sample.

The sample is then ejected into an oxalated Dunham fermentation tube which has been previously prepared by evaporating to dryness 0.1 cc. of a 2 per cent potassium oxalate solution for each cubic centimeter of marrow (2 mg. of dry potassium oxalate per cc.). According to Maurer and Jones\textsuperscript{17} the oxalate should be evaporated below 80°C as a higher temperature will convert some of the oxalate to carbonate and coagulation would not be inhibited. Blood samples should be checked concurrently and should be obtained prior to securing the marrow sample. The emotional upset due to being confined and operated on might alter the blood picture. Smears of both blood and marrow may be made at the time of securing the sample if preferred. Materials needed to carry out the above technic are shown in Figure 3.

Make the blood and marrow smears as soon as possible, at the latest within an hour, as cytological changes in the oxylated samples take place very soon. Hemoglobin readings and total red and nucleated cell counts may be made within twenty-four hours. The latter procedures do not seem to give sufficient information on the bone marrow due to the fact that the dilution with blood is so variable. The smears are stained with Wright’s blood stain following the technic of Osgood and Ashworth\textsuperscript{18}. One-tenth gm. of Wright’s dry stain is mixed with 20 cc. of acetone-free methyl alcohol and left over night before use. A buffer solution with a pH of 6.4 is prepared by dissolving 6.63 gms. of monopotassium phosphate and 2.56 gms. of anhydrous disodium phosphate in a liter of distilled water. One cc. of chloroform is added. Six to 12 drops of the dye, depending on the size of the smear, are left on the slide for two minutes, then diluted with an equal number of drops of buffer and left 7 minutes longer. With the slide in a horizontal position rinse off the staining solution and wash the slide for 30 seconds with a brisk stream of running tap water. The slides are air dried. No cover slip is applied. Instead, cover the smear with a thin film of immersion oil at the time of examination.

It is advisable to count at least 500 cells for a differential marrow count because there are so many kinds of cells. Fewer cells would not often constitute a representative sample.

Young cells in bone marrow may be identified by their large size, the uniformly

\textbf{Fig. 1}

Left lateral view of the equine showing the superficial layers of muscle and underlying structures. (skin and cutaneous fascia removed).

\textbf{Fig. 2}

Left lateral view of the bovine showing the superficial layers of muscle and underlying structures. (skin and cutaneous fascia removed.) Notice difference between the costal area of the cow and that of the horse in Fig. 1.
fine granular protoplasm of the nucleus, the presence of nucleoli, the basic staining of the cytoplasm and the absence of cytoplasmic granules in the granulocytes. With increasing age the cells become smaller (megakaryocytes excepted) nuclear chromatin becomes clumped, the nucleoli disappear, and the cytoplasmic staining and granules, and the entire cell morphology approach that of the adult cell.

**Terminology**

The following twelve classifications of marrow cells were chosen:

“Stem cell”—All 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 nonspecific 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.

**Megakaryocytes**

The largest cells of the marrow, the megakaryocytes, are enumerated by low power in an area 15 × 20 mm. Schmid found an increased number of megakaryocytes in primary pneumonia in man.

**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 is determined by adding the percentages of erythroblasts and normoblasts and comparing the figure with a similar sum of the developing granulocytes.

**Normal Myelogram**

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.: 0.00-121.00, 25.14; mitoses per 600 cells: 0.00-11.00, 4.9; myeloid-erythroid ratio (M/E): 0.27-2.59, 0.676.

**Myelogram for Horses**

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.

Bloom is pioneering in the veterinary field in studying bone marrow in various diseased conditions in the dog. Some of his findings include an increased myeloid-
erythroid ratio in pyometra and a regenerative anemia; neutrophilic hyperplasia in marrow in pyometra and streptothricosis, and a decreased myeloid-erythroid ratio and hyperplasia of the erythroid

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Fig. 3

Instruments used in obtaining bone marrow series in advanced filariasis. Similar data need to be compiled for other animals.

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.

REFERENCES


A new chemical more powerful than quinine or atabrine which is successful in arresting relapsing malaria in sixteen hours, instead of six to seven days, has been announced by the National Naval Medical Center, Bethesda, Maryland.

In a study of single and multiple bull breeding units at the U. S. Range Live Stock Experiment Station in Montana it was found that the average number of cows in single bull herds was 28 and the average number of cows per bull in herds having two or more bulls was 19. Notwithstanding the higher ratio of cows per bull in the small herds their breeding record was 6 per cent better than that of the larger herds.

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