Medullary Bone of Piglets

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ABSTRACT. The bone marrow is commonly present in the medullary cavity of long bones in most mammals at birth. In the present study, the persistence of the osseous tissue was examined in the medullary cavities of piglet bones at and after birth. Immediately after birth, medullary cavities of long bones were almost completely filled with the spongy bone. The persisted spongy bone, i.e., medullary bone was prominent in younger pigs but decreased with advance in age. By 90 days of age, the medullary bone almost disappeared to form the extended medullary cavity. During the disappearing process of the medullary bone, osteoclasts showed significant increase in size and number ($p < 0.05$). In irregular and flat bones, osseous trabeculae of the medullary bone became slender with age. The extramedullary hematopoiesis in the liver and spleen was very active till 30 days of age. This may suggest that the persistence of the medullary bone narrows the medullary cavity to cause poor medullary hematopoiesis and is compensated by extramedullary hematopoiesis. The relationship among the medullary bone, extramedullary hematopoiesis and piglet anemia was discussed.—**KEY WORDS:** anemia, medullary bone, piglet.

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The presence of the spongy bone in the medullary cavities of long bones of normal animals has been reported only in the fowl [1] and 2 living families of aquatic placental mammals, manatees and dugongs, of the order *Sirenia* [2]. In the fowl, the spongy bone is called “medullary bone” and known to function for the calcium storage in the egg shell production [1, 12]. In the *Sirenia*, the bone filled its medullary cavity with persisted spongy bone is called “amedullary bone” [2]. The presence of the spongy bone might increase the total specific gravity of the body and has been considered to be advantageous for these marine mammals because they spend an excessive energy to submerge on account of the buoyancy imparted by their lung volume [2].

Through the post-mortem examination of piglets slaughtered due to unfavorable prognoses, we frequently encountered the persisting spongy bone in the medullary cavities of long bones. In the present study, therefore, the persistence of the spongy bone was examined in piglets to discuss the influence of the spongy bone on the piglet anemia.

MATERIALS AND METHODS

Animals: One hundred and sixty-nine cross feeder pigs were used in the present study. They ranged in age from 1 day to 1 year and from 400 g to 65 kg in weight. They were slaughtered during January 1987 to October 1988 due to unfavorable signs such as

weakness, locomotor disorders and intestinal hernia. They all came from local pig farms in Tottori Prefecture and were euthanatized for use in a part of another project.

Routine gross and histological examinations: After complete necropsy, tissue samples were taken from the central nervous system, lungs, heart, liver, spleen, kidneys, lymph nodes, pancreas, intestines, bone marrow and endocrine glands, including the thyroid, parathyroid, pituitary, gonads and adrenal. They were then fixed in 10% neutral buffered formalin, dehydrated in a graded series of ethanol, embedded in paraffin, sectioned at 5 μ m and stained with hematoxylin and eosin (H&E).

Gross observation of the bone: A pair of femoral bones were taken from each animal. One bone was cut sagittally to remove the bone marrow from one half of the cut bone by brush washing for the examination of the persistent medullary bone filling the medullary cavity completely (Fig. 1a) or incompletely. In the latter case, the medullary bone was separated into 2 triangular wedges with bases at proximal and distal ends of the diaphysis (Fig. 1b). The length of both diaphysis and medullary cavity was measured to determine the ratio of the length of medullary cavity to that of diaphysis. The data obtained were statistically analyzed by Duncan multiple-range test. The other bone was used for the histological examination.

Histological examination of the bone: Long

Fig. 1. Sagittal sections of femoral bones from piglets 10 (a), 40 (b) and 90 (c) days of age. The medullary bone completely fills the medullary cavity at 10 days of age, but diminishes with advance in age, and shows the wedge-shaped appearance in b. The medullary cavities in b and c are indicated by arrows.

(femoral bone and humerus), irregular (vertebral bone) and flat (rib and cranium) bones were fixed in 10% neutral buffered formalin to obtain cross slabs 2–4 mm in length from these bones with a band saw. Bone slabs were decalcified in 5% formic acid. Histological sections 3–5 μm in thickness were prepared through routine processing, and stained with H&E or toluidine blue for light microscopy.

Histomorphometry was performed on the randomly selected animals in each age group. Paraffin-embedded sections were cut at the level of mid-diaphysis of a bone whose medullary cavity was completely filled with the medullary bone (Fig. 1a), or cut at the level of the tip of the wedge-shaped

medullary bone of a bone whose medullary cavity was incompletely filled with the medullary bone (Fig. 1b). The sections were analyzed with a light microscope at a magnification of $\times 400$ using a camera lucida method to interface a data tablet to an NEC PC9801 computer. One hundred osteoclasts were randomly selected from 4 or 5 animals in each age group to trace their contours on the tablet for the calculation of the projected area and the maximum diameter of each osteoclast from coordinates in the computer. The number of osteoclasts attaching to the osseous trabeculae of the medullary bone was counted in 4 or 5 animals from each age group per 1 mm length of the perimeter of the osseous trabeculae traced by the camera lucida. Mean and standard deviations of size and number of osteoclasts were calculated among the age groups. When *F* test showed a significant difference ($P < 0.05$) among the age groups, Duncan multiple-range test was applied to compare mean values among the age groups.

RESULTS

Routine gross and histological examinations: Histological examinations revealed the very active extramedullary hematopoiesis in the liver and spleen at birth. It became gradually inactive until 30 days of age and disappeared thereafter in these organs. Neither gross nor histological changes were observed with advance in age in the endocrine glands such as thyroid, parathyroid, pituitary, gonads and adrenal.

Gross observation of the bone: The medullary bone almost completely filled the medullary cavities

Table 1. Changes of the percentage of animals with the medullary bone and in the ratio of the length of medullary cavity to that of diaphysis

Age (days)	No. of animals examined	Percentage of animals with the medullary bone (%)	Ratio of the length of medullary cavity to that of diaphysis* (%)
0-10	45	100.0	3.1 ± 6.4^a
11-20	22	100.0	4.4 ± 7.5^a
21-30	32	73.1	20.5 ± 19.4^b
31-45	17	76.9	18.8 ± 5.6^b
46-60	18	62.5	26.1 ± 16.6^b
61-90	15	45.4	42.5 ± 17.1^c
	20	0.0	60.3 ± 6.0^d

a), b), c), d) = Means with different superscripts are significantly ($P < 0.05$) different by the Duncan multiple-range test.

*Data are expressed as mean $\pm$ SD.

Fig. 2. Part of transverse section of a femoral bone from a piglet 2 days of age. Note the osseous trabeculae of the medullary bone on the right-half side. H&E. Bar=500 μ m.

Fig. 3. Part of transverse section of a femoral bone from the same piglet as in Fig 2. Note the medullary bone containing numerous cores of cartilage. Toluidine blue. Bar=500 μ m.

Table 2. Changes in the size of osteoclasts in the medullary bone

Age (days)	No. of osteoclasts examined	Size of osteoclasts (Projected area) (μ m ²)
2	100	160 $\pm$ 90 ^{a)b)}
10	100	170 $\pm$ 130 ^{c)}
20	100	180 $\pm$ 80 ^{b)}
30	100	200 $\pm$ 120 ^{b)}
45	100	180 $\pm$ 100 ^{b)}
60	100	230 $\pm$ 100 ^{b)}
90	100	190 $\pm$ 100 ^{b)}
120	100	140 $\pm$ 100 ^{a)}

a), b), c)=Means with different superscripts are significantly ($P<0.05$) different by the Duncan multiple-range test.

Data are expressed as mean $\pm$ SD.

Table 3. Changes in the number of osteoclasts in the medullary bone

Age (days)	No. of animals examined	No. of osteoclasts/1-mm length of the perimeter of the osseous trabeculae of the medullary bone
2	5	0.24 $\pm$ 0.15 ^{a)}
10	5	1.25 $\pm$ 0.79 ^{b)}
20	5	1.51 $\pm$ 0.85 ^{b)}
30	5	1.52 $\pm$ 0.67 ^{b)}
45	4	1.61 $\pm$ 0.71 ^{b)}
60	5	0.40 $\pm$ 0.13 ^{a)}
90	5	0.42 $\pm$ 0.30 ^{a)}
120	5	0.19 $\pm$ 0.26 ^{a)}

a), b)= Means with different superscripts are significantly ($P<0.05$) different by the Duncan multiple-range test. Data are expressed as mean $\pm$ SD.

of long bones immediately after birth, but diminished gradually with advance in age and disappeared by 90 days of age (Fig. 1, Table 1). No changes were observed in the compact bone, articular cartilage and epiphysial spongy bone up to this age.

Histology of the bone: Osseous trabeculae of the

medullary bone contained numerous cores of calcified cartilage surrounded by lamellar osseous tissue, were same in structure as secondary spongiosa (Fig. 2) and easily distinguished from the compact bone in toluidine blue-stained sections (Fig. 3). In irregular and flat bones, the medullary

bone became thinner with age, and showed the similar appearance to that of the bones of adult animals by 30 days of age. During the diminishing process of the volume of the medullary bone, the osteoclasts increased significantly in size and number ($P < 0.05$) (Tables 2 and 3).

DISCUSSION

In the osteopetrosis, the medullary cavity of the long bone is filled by the osseous tissue [4-7, 9, 10]. This disease is usually inherited and has been attributed to the defective osteoclastic resorption of the chondro-osseous complex [5, 8]. Therefore, the osseous tissue may persist in the medullary cavities as long as the animals survive. These findings in the osteopetrosis are not compatible to those in the present study.

Animals used in this study were slaughtered due to unfavorable signs. These animals varied in severity of the signs from mild to severe, and included apparently healthy piglets except for minimal physical handicaps. The medullary bone diminished in the medullary cavity of long bones with advance in age in the animals regardless of severity of the clinical signs. The piglets showed no gross or histological changes in the endocrine glands nor abnormal osteoclast function in the present examination. In addition, the animals came from pig farms of standard husbandry condition without history of lack or imbalance of specific nutrients in the diet supplied. Therefore, it is likely that the persistence of the medullary bone observed in these animals may be rather a physiological event peculiar to certain developmental stages of normal piglets than a pathological event.

"Medullary bone" in the fowl is known to function for the calcium storage in the egg shell production [1, 12]. On the other hand, rickets is frequently observed in fast-growing young pigs [5, 11], which indicates high requirement of calcium and/or phosphorus during the nursing period. Therefore, it appears that the medullary bone in piglets may be of advantage to pigs in providing minerals during their fast-growing period.

Piglet anemia has been attributed to 2 distinct mechanisms and classified into physiologic anemia and iron deficiency anemia. The former results from the hemodilution due to absorption of a large amount of maternal colostrum. Iron deficiency anemia occurs because of an unbalanced supply-

demand relationship caused by an increased iron requirement to meet the rapid growth during the nursing period [3]. Animals affected with osteopetrosis show anemia because normal medullary cavities are occupied with the osseous tissue [6]. In the present study, the extramedullary hematopoiesis was observed in the presence of the medullary bone and seemed to be a compensatory phenomenon to the insufficient hematopoiesis in the bone marrow. Therefore, the persistence of the medullary bone in the medullary cavities may be, to a certain extent, involved in the pathogenesis of piglet anemia. Further studies will be required to assess whether morphological changes in the medullary cavity correlate with clinicopathologic changes of anemia in individual piglets.

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