INTRAOSSEOUS ARCHITECTURE AND ITS POSSIBLE RELATION TO ACID-BASE HOMEOSTASIS IN MARINE MAMMALS

A thesis submitted to the faculty of California State University, San Francisco in partial fulfillment of the requirements for the degree Master of Arts

by

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In bone adaptation, the biological side of bone reaction must be considered as well as mechanical stimulation... changes in the form, function, elementary composition, nutritional, and vascular conditions of bone produce changes in its interior architecture and trajectorial qualities.

(Benninghoff, 1925)

INTRODUCTION

Amphibious secondarily marine mammals afford an interesting compromise between two modes of existence. They are neither totally aquatic nor terrestrial; but, like all secondarily marine mammals, they depend on the sea for their sustenance and ultimate survival. Thus, by studying the interrelationship between skeletal structure and amphibious life, an insight may be gained into the function of bone in aquatic adaptation.

The purpose of this paper is to present an objective comparison of the gross internal architecture of humeri and femora from adults of the following amphibious marine mammals: the Bearded seal (Erignathus barbatus, Phocidae), Northern elephant seal (Mirounga angustirostris, Phocidae), Harbor or Common seal (Phoca vitulina, Phocidae), Steller sea lion (Eumetopias jubata, Otariidae), California sea lion (Zalophus californianus, Otariidae),
Pacific walrus (*Odobenus rosmarus*, Odobenidae), and the California or southern sea otter (*Enhydra lutris*, Mustelidae).

The comparative approach to skeletal form and function was taken in order to distinguish species specific from more general structural characteristics in secondarily adapted marine mammals.

Very little is known about the internal skeletal structure of amphibious marine mammals. This study is believed to be the first description of the gross internal architecture of the pinniped and sea otter humerus and femur. Kaiser (1972) briefly discusses the bone structure of the sea otter (*Enhydra lutris*) and emphasizes the fragile internal structure found throughout the skeleton.

The gross skeletal structure of other secondarily adapted marine forms is better known. Felts & Spurrell (1965a, b, 1966) investigated the structural orientation, density, and development of amedullar (absence of marrow cavity), cetacean humeri and radii in relation to secondary marine adaptation. Fawcett (1942) described the extremely dense amedullary bones of the Florida manatee (*Trichechus latirostris*) and concluded that their dense skeletal structure reflected an inherent hypothyroid condition. Meister (1962) described the gross structure and histology of the long bones of the King penguin (*Aptenodytes patagonica*), Emperor penguin (*Aptenodytes forsteri*), and Adelie penguin (*Pygoscelis adeliae*) in relation to their amedullarity and high bone density (pachyostosis).
Amedullary pachyostosis also occurs in the extinct reptilian nothosaurs (Zangerl, 1935) and sirenians (Fawcett, 1942). The significance of this increased bone density and absence of a marrow cavity is usually attributed to a necessity for reducing buoyancy, but in some marine vertebrates (ichthyosaurs, plesiosaurs, mosasaurs, and cetaceans) the skeleton has gone to the opposite extreme and the amedullar bones are highly vascularized and spongy (Zangerl, 1935; Nopsca, 1923; Felts & Spurrell, 1965a, b, 1966). Zangerl (1935) has suggested that the spongy structure of the ichthyosaurs and whales is "post-pachyostotic" and that there is an evolutionary trend away from increased bone density (pachyostosis) toward skeletal rarefaction.
MATERIALS AND METHODS

One humerus and femur each from male adults of the following species constitute the materials used:

1. Erignathus barbatus
2. Eumetopias jubata
3. Enhydra lutris
4. Mirounga angustirostris
5. Odobenus rosmarus
6. Phoca vitulina and
7. Zalophus californianus

With the exception of museum loaned specimens, humeri and femora were collected in the field from animals that had recently died.

Bone dissection was not selective to either the right or left side.

Prior to preliminary maceration, defatting, and subsequent radiography, each animal was aged by either one or more of the following criteria:

1. Total length (nose to tail)

   a) Phoca vitulina: Bigg (1969) showed a strong correlation of length to age, ± 1 yr. to physical maturity at about 10 yrs.

   b) Mirounga angustirostris: Bryden (1969) showed
a strong correlation of length to age for Mirounga leonina, ± 1 yr. to physical maturity at about 18 yrs. Both of these animals are relatively close in size range (King, 1964).

c) Eumetopias jubata: Bryden (1972) using growth data from Fiscus (1961) showed a good correlation of length to age, ± 2 yrs. to physical maturity at approximately 10 yrs.

2. Teeth and cranial characters

a) Enhydra lutris: An approximate age estimate (± 2 yrs. depending on age class) can be made by noting cranial suture fusion and dental condition (Morejohn, personal communication).

b) Zalophus californianus: Height of sagittal crest and cranial suture index of males can denote relative age ± 1 yr. to approximately 10 yrs. (Orr et al., 1970).

3. Presence or absence of epiphyseal fusion to indicate physical maturity, i.e., cessation of longitudinal bone growth. All of the animals had a certain amount of humeral and femoral epiphyseal fusion.

Table 1 presents the approximate age, source, and collection locality of each specimen.

With the exception of museum loaned specimens, humeri and femora were dissected and macerated for 24-72 hours in a constant temperature hot water bath kept just under the boiling point.
TABLE 1
Approximate age, source, and collection locality of each adult specimen examined.

<table>
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<td><em>Mirounga angustirostris</em></td>
<td>12-14</td>
<td>California Academy of Sciences, San Francisco, Ca.</td>
<td>Ano Nuevo Is., California</td>
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<td><em>Phoca vitulina</em></td>
<td>6-7</td>
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<td><strong>OTARIIDAE</strong></td>
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<tr>
<td><em>Eumetopias jubata</em></td>
<td>&gt;10</td>
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<td>Ano Nuevo Is., California</td>
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<tr>
<td><em>Zalophus californianus</em></td>
<td>10-14</td>
<td>Beach cast carcass</td>
<td>Monterey Bay, California</td>
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<tr>
<td><strong>ODOBENIDAE</strong></td>
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<tr>
<td><strong>MUSTELIDAE</strong></td>
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<td>13-16</td>
<td>California Dept. of Fish &amp; Game, Monterey, Calif.</td>
<td>Monterey Bay, California</td>
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(96°C) to prevent any significant loss of bone matrix or mineral. Cleaned and air-dried bones (including museum loaned specimens) were then defatted by immersion in boiling carbon tetrachloride for 6 hours. Specimens were then dried in a 70°C oven for 24-48 hours.

Anterior-posterior and medial-lateral whole bone radiographs were taken to describe the gross internal architecture of humeri and femora for each species. Mid-diaphyseal transverse sections from the humeri and femora of M. angustirostris, Z. californianus, and P. vitulina were also radiographed.

Radiography was carried out with a Picker 300 MA machine at various exposures by the Santa Cruz Veterinary Hospital, Santa Cruz, California.
RESULTS

Gross internal architectures of humeri and femora show similar structural features for all of the species. However, the humerus and femur from M. angustirostris show certain deviations from the norm. Thus, the following general commentary should be applicable to all of the species, with the exception of M. angustirostris (described later), as exemplified by whole bone anterior-posterior and medial-lateral radiographs of humeri and femora from adults of the following representative species:

1. Z. californianus (Otariidae)
2. E. barbatus (Phocidae) and
3. E. lutris (Mustelidae)

Interspecific and interfamilial differences will be discussed as they apply.

THE HUMERUS

The humerus consists of an inner core of fine cancellous bone surrounded by a thin cortex of compact bone (Figs. 1 - 5). The cortex of dense compacta increases in thickness from each end toward the mid-shaft, while the inner core of cancellous bone becomes correspondingly narrower just past the region of the mid-shaft. Thus there is a consistent lack of a marrow cavity in all of the species.
Fig. 1. Anterior aspect of left humerus from Z. californianus.

Fig. 2. Lateral aspect of left humerus from Z. californianus.

Fig. 3A. Anterior aspect of left humerus from E. barbatus.

Fig. 3B. Medial aspect of left humerus from E. barbatus.

Fig. 4A. (top) Posterior aspect of right humerus from E. lutris.

Fig. 4A. (bottom) Medial aspect of right humerus from E. lutris.

Fig. 4B. (top) Anterior aspect of right femur from E. lutris.

Fig. 4B. (bottom) Medial aspect of right femur from E. lutris.
studied (including *M. angustirostris*). This important characteristic will be discussed later.

The anterior mid-shaft portion corresponding to the deltoid crest in cross section is composed of very fine cancellous bone with a thin cortex of compacta; while the cancellous bone in the posterior portion of the mid-shaft is coarser and surrounded with a thick cortex of compact bone (Fig. 5A).

**Trabeculations in the Upper End**

According to Culmann's Trajectorial Theory of bone architecture (published by Meyer, 1867), the trabeculae of spongy bone follow lines of maximum internal stress (trajectories) in the bone, cross each other at right angles, and arise perpendicularly from the surface of the bone or articular cartilage. Certain of these trajectories are considered to be compressive and other tensile resistant. The Trajectorial Theory of bone architecture received its best expression when Wolff (1892) published his famous monograph entitled *The Law of Bone Transformation*. As translated by Evans (1957), every change in the form and function, or in the function alone, of a bone produces changes, in accordance with mathematical laws, in its trabecular architecture and external form. Tobin (1955) objectively presented the internal architecture of the human femur (upper end) without regard to a mathematical analysis, and concluded
Fig. 5A. Cross section at mid-shaft of right humerus from *Z. californianus*.

Fig. 5B. Cross section at mid-shaft of right humerus from *M. angustirostris*.

Fig. 5C. Same as Fig. 5B, but viewed medial-laterally.
that bone formation occurs along the direction of the principal stresses of the combined-stress theory. Most investigators are in agreement that function is the trophic stimulus for bone growth, but there is marked disagreement as to the exact nature of mechanical forces presumably involved (Evans, 1957).

Trabeculations in the humerus and femur will be considered in view of the combined-stress theory without regard to a mathematical analysis.

Trabecular patterns can be traced from the medial and lateral walls of the upper mid-shaft superiorly into the head, lesser and greater tuberosities of the humerus. The trabeculae forming these patterns vary in size and thickness according to species, and probably reflect structural demands in terms of body size, degree of amphibiousness (i.e., the amount of gravitational weight-bearing), skeleto-musculature dynamics of locomotion, and mineral requirements for homeostasis.

Trabecular lines from the medial-lateral walls of the deltoid crest in the otariid (e.g., Z. californianus) pass directly into the greater tuberosity (Fig. 2). These same lines curve to follow the slightly different shape of the deltoid crest in the phocid (e.g., E. barbatus, Fig. 3B). Note also the radio-translucent area at the mid-shaft posterior to the deltoid crest in the otariids (as well as in the odobenid, O. rosmarus, Fig. 2) but lacking in the phocid (Fig. 3B).
Subtle trabecular lines from the upper medial and lateral sides of the main shaft curve superiorly to intersect each other at right angles just below the head of the humerus (Fig. 1). Trabecular lines in the head of the humerus intersect the articular surface at right angles. Trabeculae in the humerus (upper end) of *P. vitulina* (Phocidae) and *O. rosmarus* (Odobenidae) are relatively coarse while those of *E. lutris* (Mustelidae) are much finer and homogeneous. Trabecular trajectories in the humerus (upper end) of *E. lutris* were difficult to trace. This may be a reflection of the southern sea otter’s largely total aquatic nature (Daugherty, 1972), and the fact that it does not use its forelimbs for aquatic locomotion (Tarasoff, 1972; Howell, 1930).

**Trabeculations in the Lower End**

Distal to the nutrient canal, subtle trabecular lines from the lower medial and lateral sides of the main shaft (corresponding to the medial and lateral epicondyles) curve superiorly and intersect each other at right angles and the central axis at forty-five degrees (Fig. 1). This same medullary area lies within the region of greatest radio-density of the humerus. Hence, it seems that the greatest structural support is found in this region in response to muscle tension, compression, and static loading (Koch 1917, Evans 1957). The above trabecular description is much less distinct in the sea
otter (*E. lutris*, Fig. 4A (top)). In view of this animal's near total aquatic nature, it follows that the greatest structural support found in the above region is in response to muscle tension and compression and not to any forms of terrestrial (i.e., static) loading.

All of the humeri (including *M. angustirostris*) have an area of very high radio-translucency between the lateral and medial epicondyles.

**THE FEMUR**

The femur shows many of the same internal features described in the humerus. It consists of an inner core of fine cancellous bone surrounded by a cortex of dense compacta variable in thickness. Again, note the persistent lack of a marrow cavity. Viewed from the anterior-posterior aspect, the cortex increases in thickness medially and laterally toward the mid-shaft while the cancellous core becomes correspondingly narrower (Figs. 6A and 7A). If the femur is viewed in the medio-lateral plane, the areas of highest radio-density correspond to the thick anterior and posterior sides along the femoral shaft. There is also a concomitant thickening of these sides at the mid-shaft (Figs. 6B and 7B).

**Trabeculations in the Femur**

Trabecular patterns in the femur were found to be generally similar to those in the humerus. Again, the trabeculae comprising these patterns varied in thickness according to species.
Fig. 6A. Anterior aspect of right femur from *Z. californianus*.

Fig. 6B. Medial aspect of right femur from *Z. californianus*.

Fig. 7A. Posterior aspect of right femur from *E. barbatus*.

Fig. 7B. Lateral aspect of right femur from *E. barbatus*. 
Trabecular lines from the medial and lateral walls of the femoral shaft rise superiorly and obliquely to intersect at right angles (Figs. 6A and 7A). Those from the upper lateral walls (corresponding to the thickened region of the cortex) lead to the femoral head; while those from the upper medial walls lead to the greater trochanter. Those two major trabecular patterns are connected with a third system at the upper end between the head and greater trochanter forming an area of lessened density. The relationship of these trabecular lines as they intersect each other and the central axis is similar to the classic proximal end of the human femur as described by Koch (1917), Tobin (1955) and others (see Evans, 1957 for a concise review). Additional sets of trabecular lines are also seen extending from the lower end into the medial and lateral condyles. Trabecular patterns were very fine and close together in the otariid E. jubata (not shown), and somewhat difficult to trace in E. lutris (Fig. 4B, top and bottom). The difficulty in tracing trabeculae in the femur of E. lutris may be an artifact of poor or inadequate radiographic exposure and not necessarily reflect architectural deficiencies.

UNUSUAL INTERNAL ARCHITECTURE OF THE HUMERUS AND FEMUR OF THE ELEPHANT SEAL (Mirounga angustirostris, Phocidae)

Lyon (1937), and Allen (1870) have described the bones of the elephant seal as being extremely light and porous in appearance.
I have determined the density (weight of the dry, defatted bone per unit volume of the surface area; unpublished data) of a humerus from an adult animal and found it to be quite low: 0.37 g/cm³. For comparison, Felts & Spurrell (1965a) determined the humeral densities of the Finback whale (*Balaenoptera physalus*), the Pilot whale (*Globicephala malaena*), and the Beluga (*Delphinapterus leucas*) and found them to be 0.52, 0.67, and 0.69 g/cm³ respectively. The penguin humerus, in contrast, has a density of 0.98 gm/cm³ (Meister, 1963) and that of the manatee, though not recorded, apparently is still more dense. Thus, the elephant seal's bone density may be the lowest of any secondarily adapted marine mammal as well as the penguin.

The internal architecture of the humerus and femur of *M. angustirostris* indeed reflect their remarkable light weight and porous appearance. While the architecture of the humerus and femur share certain structural features in common with the others examined, the following commentary will emphasize major deviations from the norm.

**THE HUMERUS**

The most striking and unique feature of this bone (as well as the femur) is the lack of distinct compact tissue (Figs. 8 and 9). The entire bone is essentially comprised of cancellous bone sur-
Fig. 8. Posterior aspect of right humerus from *M. angustirostris*.

Fig. 9. Medial aspect of right humerus from *M. angustirostris*.
rounded by an extremely thin layer of cortical bone. Figures 5B and 5C present two views of part of a transverse segment from the mid-shaft. The anterior mid-shaft, corresponding to the deltoid crest, is composed of extremely fine cancellous tissue with a negligible cortex (Fig. 5B). The cancellous bone in the posterior portion of the mid-shaft is surrounded with a thin cortex comprised of two layers. Closer examination suggests an outer periosteal layer surrounding a second layer of dense cancellous components, possibly endosteal in origin. In addition to the lack of an open medullary cavity, the central region is less dense than the periphery. The least dense regions are made up of interspersed trabeculae and perforated platelets and spicules while more dense spongy bone consists of tubes and short cells or chambers with fenestrated walls. The boundaries of such spaces appear as trabeculae in long section.

The transverse section also reveals an irregular vascular labyrinth near the center of the medullary axis (Fig. 5B). Channels of variable diameter (2-4 mm) can be traced through the proximal and distal third of the humerus by viewing each cut end of the bone to a bright light.

Felts & Spurrell (1965a) have described structural orientation and density of cetacean humeri. The cetacean humerus in cross section shows similar "endo-ectad" gradients of spongy tissue surrounding an irregular vascular labyrinth. In general, there is much
evidence to show that the humeral and femoral bone structure of *M. angustirostris* is remarkably similar to that of the cetaceans that Felts & Spurrell describe. An implicit convergence is suggested here and will be discussed later.

Although apparently lacking a nutrient canal and corresponding foramen, an unusually high degree of vascularization is shown in the humerus (and femur) of the elephant seal. Many foramina are visible on the surface of the bone giving it a porous appearance. Foramina and the nutrient canal with various out-branchings were visible in humeri and femora of the other species examined.

**Trabeculations in the Upper and Lower End**

Although the trabecular patterns as seen in the humerus are similar in kind and direction to the other species, they are very delicate and much finer in size, thickness, and distribution (Fig. 8). The trabecular trajectories are relatively well defined and close together.

Trabecular lines leading superiorly from the medial and lateral sides of the condyles become more compact and radio-opaque at the narrowest portion of the humeral shaft (Fig. 8). This region corresponds to the dense compacta found in the medial and lateral sides of the humeri of the other species examined (Figs. 1-4). Inframedial and infralateral trabecular lines (corresponding
to the medial and lateral epicondyles) curve superiorly and intersect each other at right angles and the central axis at forty-five degrees. Since the elephant seal uses its forelimbs for terrestrial support when it hauls out during the breeding season (LeBoeuf, 1972; King, 1964), the greatest structural support in the above region is found in response to muscle tension, compression, and static loading.

**THE FEMUR**

The femur is also lacking in compact tissue and is essentially comprised of extremely fine cancellous bone surrounded by a thin cortex (Figs. 10A and 10B). A transverse segment from the mid-shaft (not shown) revealed similar structural features found in the humerus. The anterior and posterior portions of the cortex were slightly more compacted and thicker than the medial and lateral walls. Close examination revealed the cortex to be double-layered as described for the humerus. An endo-ectad gradient of cancellous bone from a central vascular labyrinth to the periphery and numerous intramedullary channels of variable diameter (1-3 mm) were also observed. Thus, the femur is also richly vascularized.

Although the bone shown in Figure 10 is from an adult male (approximately 12-14 years old)*, the epiphyses of the head,

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*Physical maturity in males of *M. angustirostris* is reached at 12-14 years. This is also considered to be near their maximum life span (Briggs & LeBoeuf, ms.).
Fig. 10A. Posterior aspect of right femur from *M. angustirostris*.

Fig. 10B. Lateral aspect of right femur from *M. angustirostris*. 
greater trochanter, and condyles show evidence of recent fusion suggesting delayed epiphyseal fusion. Although not as pronounced, a vestige of these same junctions is apparent in radiographs of the other species examined.

Lyon (1937), Allen (1870), and Howell (1930) have commented on the juvenile appearance of adult bones of the elephant seal. Turner (1888) observed that there were very few fused epiphyses in most of the skeletal elements of adult elephant seals. Flower (1881) noted that there were persistent unfused cranial sutures in seemingly aged adults of the elephant seal. More recently, Briggs & Morejohn (ms.) and King (1972) have shown late cranial suture fusion and absence of suture fusion in the facial region of the skulls of the northern (M. angustirostris) and the southern (M. leonina) elephant seals. Thus, there seems to be direct evidence for depressed skeletal maturity in many of the skeletal elements of both species of Mirounga. Felts & Spurrell (1965a, b, 1966) have also described retarded epiphyseal fusion and longitudinal growth in cetacean humeri, radii, and phalangeal elements. Depressed skeletal maturity is well known in many fossil and extant cetacea (Howell, 1930), sirenia (Fawcett, 1942) and early marine reptiles (Zangerl, 1935).
Trabeculations in the Femur

Viewed from the anterior-posterior aspect, trabecular lines along the medial and lateral sides of the mid-shaft are slightly more compacted at the narrowest part (Fig. 10A). These same trabeculations are seen to be more radio-opaque in the medial-lateral aspect (Fig. 10B) and are reminiscent of the dense compacta found in this same area from the other femora examined (Figs. 4B, 6 and 7). Thus, it can be said that the anterior-posterior plane of the flattened femur is structurally more dense and subject to more stressful tension and compression, especially at the mid-shaft region.

Very subtle trabecular patterns from the upper medial and lateral walls of the femoral shaft rise superiorly and obliquely to intersect at right angles just above the mid-shaft region (Fig. 10A). These two major trabecular patterns are connected with a third system at the upper end between the head and greater trochanter forming the area of lessened radio-density previously described for the other femora. Since members of the family Phocidae do not use their hindlimbs for terrestrial locomotion (Howell, 1930), it follows that the greatest structural support found in the above region is in response to muscle tension and compression and not static loading.

With the exception of the medial and lateral walls, a fairly homogeneous area of trabeculae is seen between the mid-shaft region
and the distal diaphyseal-epiphyseal junction. This area is afforded a better view in the medial-lateral aspect (Fig. 10B).

**SUMMARY OF STRUCTURAL FEATURES**

The following structural features were interpreted from radiographs of the humerus and femur of the pinnipeds and sea otter:

1. The persistent lack of a marrow cavity (i.e., suppressed central resorption) with corresponding areas of compact cortex and cancellous core is exhibited.

2. A depressed metaphyseal growth rate and delayed skeletal maturation is apparent based on the varied remnants of the diaphyseal-epiphyseal sutures on the head and condyles.

3. Trabecular trajectories and areas of increased radio-density correspond to the effects of muscle tension, compression, and static loading. Trabecular relationships compromise the combined-stress theory, and may reflect relative degree of limb usage in amphibious secondarily marine mammals.

4. A well vascularized shaft is evident.

The internal structure of the humerus and femur of *M. angustirostris* deviates from the above in being more specialized; yet, retaining similar gross architectural features. These deviations from the norm include:

1. Virtual absence of compact tissue and a concomitant
increase of extremely porous cancellous bone resulting in extreme radio-translucency.

2. An extremely thin cortex comprised of an outer layer (probably periosteal), and a slightly thicker layer of compacted cancellous tissue underneath.

3. A highly vascularized external porous surface and medullary region.

4. An apparent absence of the nutrient canal.

5. Delayed skeletal maturation is exhibited based on the relative absence of internal remodeling at the diaphyseal-epiphyseal junctions of the femoral head and condyles. A vestige of the diaphyseal-epiphyseal junction on the humeral head was also present but not as pronounced.

The over-all radiographic appearance of the humerus and femur of the adult elephant seal resembles that of the post-natal growing condition.
DISCUSSION

We have seen that the humerus and femur of the pinnipeds and sea otter are characteristically comprised of an endo-ectad cancellous core surrounded by a cortex of compacta variable in thickness. Compacta is virtually absent in the extreme condition found in the humerus and femur of the elephant seal. With the exception of the elephant seal, the cortex of the humerus was most thick and dense at the junction of the lower and middle thirds of the shaft rather than at the mid-shaft: while that of the femur increased in density and thickness medial-laterally toward the mid-shaft. Although lacking discrete compact tissue, the radio-density of the humerus and femur of the elephant seal was distributed in the same manner described above. In spite of varied cortical thickness, density, and differences in external form, the structural descriptions of the long bones of penguins (Meister, 1962), sirenia (Fawcett, 1942), early marine reptiles (Zangerl, 1935), and cetaceans (Felts & Spurrell, 1965a, b, 1966), are practically identical to this over-all description.

In their description of the cetacean humerus, Felts & Spurrell (1965a, b, 1966) found this structure to be analogous to the
engineered box-beam main spar commonly used in aircraft wing construction. The modified box-beam main spar has thicker top and bottom than side walls, and a hollow center interspersed with plates of uniform material and calculated thickness. These plates are oriented at prescribed distances from the neutral axis of the hollow center to produce an overall structure that is resistant to considerable torque as well as superior-inferior compressive bending.

Like the cetacean humerus and radius, the trabeculations, as defined by Culmann's Trajectorial Theory (combined-stress theory), of the pinniped and sea otter humerus and femur complement the engineered box-beam.

The works of Meister (1942), Fawcett (1962), and Felts & Spurrell (1965a, b) have shown that the persistent lack of a marrow cavity is primarily due to the absence of endochondral resorption. Therefore, cartilaginous matrix persists and there is also a paucity of osteoclasts. Most of the internal histology indicates primary Haversian with some signs of secondary Haversian systems. There are few signs of reconstruction or reorganizational activity. Meister found evidence of hemopoiesis in the adult penguin humerus, while Felts & Spurrell saw none in the humerus of the fetal cetacean.

The periosteum has no osteogenic function in adult mammals under normal conditions (Enlow, 1961). Fawcett (1942) demon-
strated that the periosteum of the manatee humerus continues to deposit bone long after the animal has reached adult size and physical maturity. With the absence of internal resorption, one can see how and why the bones of this animal are pachyostotic.

Despite the absence of histological and histogenetic material in this study, one may expect relatively similar histological features in the pinnipeds and sea otter humerus and femur. For example, the lack of a marrow cavity is most likely due to repressed endochondral resorption. Nevertheless, a comparative histological investigation would be most productive.

In general, the histological and structural organization of the humerus of cetacea, penguins, and sirenia suggests a very young skeletal age even though bones were from adult animals. Most of the authors make this point very clear. As we have seen, depressed skeletal maturity is also suggested in the humerus and femur of pinnipeds and the sea otter. The condition is so pronounced in the elephant seal that one could label this phenomenon "skeletal neoteny," in the sense that there is a retention of postnatal characters well into adulthood. The significance of skeletal neoteny will be discussed in a later section.

Zangerl (1935) has shown that amedullary pachyostosis occurs in early marine reptiles but later fossil examples from the same lines possessed amedullary spongy bone. He suggests that
the medullary spongy bone structure of the extant whales is "post-pachyostotic." Although I have not measured and compared absolute densities in this study, one can see that absolute density may be expected to vary within the pinniped and sea otter group based on relative radio-density. The implication here is that a similar post-pachyostotic trend may be apparent in extant amphibious marine mammals as evidenced by the extreme spongy structure of the humerus and femur of the elephant seal (M. angustirostris). The absence of compacta, extreme skeletal immaturity, and very low density of the elephant seal may further corroborate this point. Thus, based on the results of this study and those of Meister (1962), Fawcett (1942), Felts & Spurrell (1965a, b), and Zangerl (1935), a reconsideration of the evolution of medullary bones in secondarily marine forms is warranted.

Felts & Spurrell (1965a, b) and Fawcett (1942) have entertained the theory first stated by Zangerl (1935) who suggested that increased bone density in early secondarily marine mammals was a means of compensating for large lung volume and overall buoyancy. Nevertheless, Felts & Spurrell (1966) have commented that this theory can hardly be argued in the absence of knowledge on the composition of early marine mammal forms.

Nopcsa (1923) postulated that the dense medullary bones of the Sirenia were due to chronic hypoxia. Since hypoxia ordinarily
increases hemopoiesis, he reasoned that chronic stimulation of the bone marrow may have brought about, first hyperplasia (increase in marrow size and hemopoietic activity), aplasia (hemopoietic failure), and finally replacement by bone. Fawcett (1942) disagreed with Nopcsa's theory because there is no resemblance between Sirenian bone and the osteocleroses (a condition of increased hardness and density) which occurs in man secondary to metastatic neoplasia, leukemia, or aplastic anemia.

Nopcsa (1923) also suggested that the amedullary dense bones of the manatee may be due to hypothyroidism caused by a high content of iodine in the algae that this animal is known to eat.

Sickenberg (1931) further elaborated upon this endocrine theory and believed that hypoxia was a primary factor in the evolution of amedullary bones, but, contrary to Nopcsa, he maintained that the effect of hypoxia was directly upon the thyroid gland and not upon bone marrow. His beliefs were based on the works of Deucher (1925), and Reich and Blauel (1913) who showed that surgical constriction of the trachea in experimental animals produced hypofunctioning of the thyroid. Fawcett (1942) examined the manatee thyroid and, indeed, found strong evidence for inherent hypofunction in this gland. McLean and Urist (1968) later confirmed Fawcett's observations. Hence, Fawcett concluded that the retardation of osteogenesis, lack of internal bone remodeling, low metabolic rate,
and histology of the thyroid, together with diet, relate to an inherent hypofunctioning of the thyroid, in the manatee. Therefore, amedullary pachyostosis may have been secondary to an inherent hypothyroidism which arose in an early period of imperfect adaptation to the aquatic environment.

Felts & Spurrell (1965b) agree that hypoxia and its hypofunctioning effect on the thyroid, may have been an important factor in the evolution of amedullary pachyostosis in early cetacea and other secondarily marine forms. They also suggest that hypothyroidism, as exemplified in the manatee condition, may no longer be a factor in extant cetacea because whales are known to have high metabolic rates (Slijper, 1962).

On the basis of evidence presented in this paper, hypoxia and hypothyroidism may have also been involved in the evolution of the amedullary "box-beam" bones of amphibious marine mammals. Since Irving (1939) has shown that both cetacea and pinnipeds exhibit a high basal metabolic rate, and assuming that the latter are also undergoing selective post-pachyostosis, classic hypothyroidism may no longer be a factor in extant pinnipeds. Due to the active behavioral spectrum of the sea otter (*Enhydra lutris*; Kenyon, 1969), hypothyroidism may also be ruled out for this animal.

Felts & Spurrell (1965a, b) have studied osteogenesis in the cetacean humerus and radius, and suggest that the structural
evolution of this bone, and probably the limb bones of other secondarily marine forms, originally involved a tubular terrestrial form such as now exists in most land animals. This was then succeeded by a similar form made continuous and dense (pachyostotic) by suppressed internal resorption "...for whatever reason... (Felts & Spurrell, 1965a)." They further suggest that the lower density and spongy internal structure of the living adapted cetacean humerus was effected by the opening up of the dense bone's vascular channels (post-pachyostosis) and that this process was selective for the mechanical environment of the flipper. Their detailed analysis of humeral structure attempts to show that its amedullary "box-beam" character is an evolutionary, i.e., selective, product of transverse medial-lateral loading forces from the distal flipper caused by high-speed hydroplaning. "The strongest indication that this (amedullary "box-beam" condition) somehow is related to full (my underlining) aquatic adaptation is the fact that the bones of the partly-terrestrial sea-lions, seals, and walruses have some internal cavitation (Felts & Spurrell, 1965a)." The results of this study disagree with Felts & Spurrell on this last point. I have shown that the humerus and femur of six species of pinnipeds and the sea otter, indeed, lack internal cavitation. This implies that the amedullary "box-beam" condition may not necessarily be related to full aquatic existence, nor does it involve only extrinsic, i.e., mechanical stimuli in its
structural development and evolution. Furthermore, the amedullary "box-beam" condition of the manatee humerus can hardly be considered an effect of medial-lateral hydrodynamic loading from the distal flipper in a sluggish animal that spends much of its time feeding and not actively pursuing a meal at high speeds. Hence, on-going intrinsic factors must also be responsible for the characteristic internal bone structure of secondarily marine forms. As Benninghoff (1925) aptly emphasized, "...the biological side of bone reaction must be considered as well as mechanical stimulation..." 

THE POSSIBLE HYPOXIC EFFECTS OF CHRONIC PROLONGED APNEA IN EXTANT MARINE MAMMALS

Irrespective of the origins of amedullarity, and the extrinsic effects of mechanical stimulation on internal bone structure, the following question still remains: What are the intrinsic selective advantages of skeletal neoteny and the persistence of well vascularized cancellous bone in extant marine mammals as exemplified in the marked skeletal immaturity of the elephant seal? Consideration of the above question warrants close examination to the possible interplay of diving and its physiological effects on skeletal growth, mineralization, bone turnover, and maintenance of the integrity of skeletal tissue.

In view of the foregoing, the following discussion will attempt
to construct a reasonable hypothesis relating skeletal neoteny to the prolonged apnea of diving and its possible consequences in the diving physiology of extant marine mammals. I will first try to show that chronic hypoxia may selectively effect thyroid function to cause skeletal neoteny. Second, I will endeavor to show that skeletal neoteny has a profound physiological effect on the whole blood buffering capacity of the efficient aquatic diver.

**Chronic Hypoxia and Thyroid Function in Marine Mammals**

I have already cited examples relating hypoxia and its effects on the thyroid: namely that it is known to depress thyroid function in experimental animals and that based on the hypothyroid condition of the manatee, hypoxia may have been an important factor in the evolution of amedullary pachyostosis in early cetacea and other secondarily marine forms (Sickenberg, 1931; Deucher, 1925; Reich & Blauel, 1913; Fawcett, 1942; Felts & Spurrell, 1965a, b, 1966).

Since marine mammals usually incur hypoxia during prolonged dives (Elsner, 1969), and that decreased thyroxine is normally responsible for retardation of bone growth and epiphyseal centers of ossification (Sisson, 1956), let us direct our attention to the possibility that delayed skeletal maturation is hormonally involved with the thyroid gland.
Harrison (1969), Harrison & Kooymans (1968) have reviewed the extensive literature on endocrine (hypophysis, thyroid, and adrenal) characters in marine mammals. In contrast to the manatee, the thyroids of pinnipeds and cetacea appear to be highly active. Slijper (1958) and Crile (1937) have claimed that the thyroid gland is significantly larger in marine mammals than their terrestrial relatives when compared on a thyroid weight to body weight basis. No doubt, this reflects the high rate of metabolism some smaller odontocetes are known to have (Scholander, 1940). Even though marine mammals may generally have a high degree of basal metabolism (Irving, 1939), chronic hypoxia may, nevertheless, have a selective effect on the thyroid to cause skeletal neoteny.

Since diving efficiency (I will use this term to denote diving depth, duration of dive, and the amount of surface recovery time between dives) varies among marine mammals, one might expect a direct correlation with increased diving efficiency and depressed thyroid activity. For example, one might perform an interspecific comparison of the ratio between thyroid weight to body weight and note any strong correlation with diving efficiency. Unfortunately, such a comparison would be difficult to corroborate because it appears that the growth rate of the thyroid is allometric with body weight and there is insufficient data on growth series in the literature for statistical comparison (Harrison, 1969). Nevertheless, some
intraspecific age comparisons may prove to be more productive in this regard.

Studies by Slijper (1958), Harrison (1969), and Scheffer (1960) with the odontocete Phocoena phocoena, and the pinnipeds Phoca vitulina, and Callorhinus ursinus indicate that thyroid weight and possibly activity decreases with age. This may be suggestive of the effects of chronic hypoxia on the thyroid with age or it may merely reflect a normal decreased growth rate for this gland.

Another investigation by Harrison et al. (1962) on thyroid activity in the young growing Common seal (P. vitulina) showed that there is a marked repression of thyroid secretion coincident with the age period of nutritional independence and increased diving performance (8 mos. of age) up to sexual maturity (3-6 yrs.). He noted that even 2 and 3 year olds had thyroids that resembled those of yearlings. He later discusses the fact that estrogens and androgens stimulate thyroid activity in pinnipeds during the onset of puberty which generally coincides with the reproductive season (Harrison, 1969).

Based on the results of the foregoing investigation, three important implications can be acertained:

1. the results may be interpreted to show that chronic hypoxia may effect hypofunctioning of the thyroid;

2. the thyroid stimulating affects of the gonadal hormones are antagonistic to the thyroid depressive effects of chronic hypoxia;
3. If so, skeletal growth rate and rate of epiphyseal ossification may be accelerated during the breeding season due to thyroid stimulation by the gonads, and conversely depressed during the non-breeding season when gonadal activity should be quiescent.

It has been the tacit assumption by many researchers that the dentine and cementum growth layers of many cetacean and pinniped teeth are the result of fasting during the breeding season (Jonsgaard, 1969). Since dentinal ossification may also be retarded by the thyroid depressive affects of hypoxia, the growth layers of dentine and cementum would then be affected during the breeding season when metabolism is highest and not during the non-breeding period when the animal is diving and undergoing chronic hypoxia.

In general, the effects of chronic hypoxia would be directly proportional to the diving efficiency of the animal. With this in mind, let us look at some of the biological aspects of the elephant seal since the skeletal structure of the humerus and femur of this animal exhibited such a high degree of neoteny.

The elephant seal (both *M. angustirostris* and *M. leonina*) is considered by many to be a highly efficient diver based on its diving proficiency, diving physiology, and benthic food preferences (Elsner, 1969; Bryden & Lim, 1969; King, 1964).

Bryden (1969) has demonstrated that there is a rapid growth of the skeletal components of the elephant seal (*M. leonina*) during
and up to the time of nutritional independence and incipient diving proficiency corresponding to about 6 to 8 months of age. Skeletal growth then decreases until the onset of sexual maturity (3 to 4 years for females, and 5 to 7 years for males) at which time skeletal growth increases but not as much when compared to the post-natal period. Bryden explains that the increased skeletal growth rate at the time of sexual maturity is associated with the greater need for structural bone in a sexually mature animal during the breeding season when activity is at its peak. However, he does not explain why there is a decrease in skeletal growth rate between the post-natal period and puberty and not for the other soft tissues of the body. Therefore, this decreased period of skeletal growth may be associated with the depressive affects of chronic hypoxia on the thyroid as the animal becomes nutritionally independent and proficient at diving. This last point is very important: it may indicate that chronic hypoxia may selectively affect thyroid function to cause depressed skeletal maturity and not depress the growth rates of other tissues and organs of the body.

In general, rapid skeletal growth is accompanied by poor skeletal mineralization (McLean & Urist, 1968). Thus, the elephant seal's low humeral density (0.37 g/cm$^3$) may be the result of a high perinatal skeletal growth rate that is abruptly depressed between post-natal and pubertal periods when the animal is nutri-
tionally independent, i.e., diving, and incurring the highly depressive effects of hypoxia on skeletal maturation. Note then, that the neotenous effects of hypoxia on bone structure also depend on the skeletal growing condition prior to the onset of diving activity in the life cycle of the animal. This is extremely important if, indeed, skeletal neoteny is selectively advantageous to an efficient diver. Allow me to leave this point for further discussion in a later section.

The elephant seal is a highly social and polygynous species that only hauls out to breed and molt once a year (LeBoeuf, 1972). A problem that has intrigued many investigators is why elephant seals do not feed during the reproductive season (LeBoeuf et al., 1972). One would think that at this time more than any other, energy demands for social interaction, parturition, and lactation would be at their peak. As was implicated earlier, the thyroid stimulating effects of the gonadal hormones are antagonistic to the thyroid depressive effects of chronic hypoxia. This may, therefore, explain why both sexes of the elephant seal do not feed during the reproductive season. Galactopoiesis is stimulated by the hormone prolactin which increases thyroxine to regulate the production of milk (Hoar, 1966). Thus, if the female were to dive during the lactation period, milk production could easily stop due to the depressive effects of chronic hypoxia on thyroxine. Male aggresive-
ness is controlled and affected in part by testosterone levels (Bartholemew, 1970). Testosterone would correspondingly raise thyroxine levels to increase the amount of energy needed for successful fighting and copulation by the large dominant bulls. Thus, if males were to dive during their needs for high energy and increased metabolism, the depressive effects of chronic hypoxia on thyroxine could prevent reproductive success and ultimately extinction. Consequently, both sexes spend most of the year accumulating high energy fats and proteins to later be metabolized during the extended fast of the reproductive season (Bryden, 1969).

Thus I have attempted to show by implication that chronic hypoxia may selectively affect thyroid function to cause skeletal neoteny and that this process may even affect behavior. Accordingly, the hypoxic effects on skeletal maturity and behavior would vary in degree relative to the diving efficiency of the marine mammal.

Let us now consider a possible selective advantage of skeletal neoteny and its effect on the internal physiology of the diving mammal.

A minimum framework for interpreting the physiological affects of skeletal neoteny

If, in time, the foregoing discussion on the neotenous effects of deep-diving on skeletal structure proves to be false, there is, nevertheless, direct evidence for depressed skeletal maturity in many cetacea and (as I have indicated) some pinnipeds. The
salient features of this condition are:

1. a depression of endochondral resorption so that cartilaginous matrix and primary tissues persist into adulthood with little remodeling or reorganization; and

2. a concomitant delay in epiphyseal fusion.

Although many authors have commented on the neotenous skeletal condition of cetacea and the elephant seal, no one has attempted to explain the intrinsic biological function, assuming there is one, of this peculiar condition.

Prefatory to a discussion on the physiological ramifications of skeletal neoteny, it is essential that we understand the physiological aspects of diving in marine mammals and some basic characteristics of bone.

**Physiological Aspects of Diving in Marine Mammals**

Lenfant (1969) and Elsner (1969) have reviewed the unique anatomical and physiological aspects of diving in marine mammals. They discuss the physiological responses to diving and explain the methods by which marine mammals (including the penguins) avoid asphyxia (i.e., decrease in oxygen with corresponding increase in carbon dioxide) during prolonged apnea (i.e., cessation of breathing). These physiological abilities are based on several interacting mechanisms that involve:
1. marked slowing of the heartbeat (bradycardia);
2. massive peripheral vasoconstriction during the dive to reduce blood flow to muscles so that the vital organs receive oxygen, while the muscles respire anaerobically;
3. venous reservoirs and a caval sphincter probably associated with massive redistribution of the blood;
4. the combination of a relatively high oxygen capacity and a large blood volume facilitating a considerable increase in blood oxygen storage;
5. relative respiratory center insensitivity to dissolved carbon dioxide (respiratory acidosis) and lactic acid (metabolic acidosis).
6. high blood buffering capacity to extremes of hydrogen ion concentrations.

Scholander (1940) and others have shown that the acid-base equilibrium of the blood in marine mammals is considerably altered during prolonged dives. The diving animal incurs acidemia during and after the dive resulting in an increased hydrogen ion concentration (pH) of the blood. During the dive respiratory acidosis occurs from the production of carbon dioxide in cell metabolism and is expressed by the following combined equilibrium equations:

\[
\text{carbonic anhydrase} \\
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad (1)
\]
These reactions occur primarily in blood cells because they contain large amounts of the enzyme carbonic anhydrase. Thus, the addition of carbon dioxide to the blood results ultimately in bicarbonate and hydrogen ions. A smaller fraction of carbon dioxide also combines directly with hemoglobin:

\[
\text{CO}_2 + \text{Hb} \rightleftharpoons \text{HbCO}_2
\] (2)

Upon surfacing, muscle vasodilation effects an increase in blood lactic acid (metabolic acidosis) that was produced via anaerobic glycolysis intracellularly during the dive. This results in an increased hydrogen ion concentration expressed by the following equilibrium equation:

\[
\text{lactic acid} \rightleftharpoons \text{H}^+ + \text{lactate}^-
\] (3)

At the surface, a period of recovery ensues whereby the reactions in the above equations proceed to the left. Hence, carbon dioxide is eliminated via the lungs and lactic acid is further oxidized to pyruvic acid to yield energy (ATP), carbon dioxide, and water. Recovery time depends on the animal's rapid ability to efficiently buffer the acid metabolites accrued from respiratory and metabolic acidosis, and how quickly it can expire \(\text{CO}_2\). The end result is thus acid-base equilibrium of the blood.

Whole blood oxygen capacity and oxygen-combining capacity
of hemoglobin are directly influenced by the partial pressure of carbon dioxide \(P_{CO_2}\) on the blood (Vander et al., 1970). Any increase in plasma \(P_{CO_2}\) is also accompanied by an increase in plasma hydrogen ion concentration. If \(P_{CO_2}\) is high during a dive, the whole blood oxygen capacity and the hemoglobin-dissociation (i.e., the per cent saturation of hemoglobin with oxygen at a given partial pressure) is considerably lowered because it prevents the diffusion of oxygen from the plasma to hemoglobin and from hemoglobin to the vital tissues. A high hydrogen ion concentration also decreases the hemoglobin-dissociation because hemoglobin has less affinity for oxygen when blood acidity is high (Vander et al., 1970). Thus, the efficiency of a blood buffering system during and after a dive lies in its capacity to decrease the partial pressure of carbon dioxide by:

1. binding the hydrogen ions given off from carbonic acid (equation No. 1) and lactic acid (equation No. 3).

2. directly binding carbon dioxide (equation No. 2).

The most important blood buffers are hemoglobin, carbonate \((-CO_2)\), large anions such as plasma proteins, and intracellular phosphate complexes (Vander et al., 1970). Another buffer which standard physiology texts ignore is the fact that the labile fraction of the skeleton (localized in the new and incompletely mineralized Haversian systems of bone) acts as a non-regulatory buffer to
changes in the electrolyte composition of the body fluids (Neuman and Neuman, 1958). But its rapid reaction to high concentrations of hydrogen ion are age dependent since most of the dissolution of stable bone mineral (hydroxyapatite crystals) can only be mobilized via the parathyroid secretion of the hormone parathormone. Thus, the younger the bone the more reactive it is to local hydrogen ion concentrations without the mediation of parathormone (Neuman and Neuman, 1958).

The most important buffers to a marine mammal are those that can rapidly effect blood buffering during the high hydrogen insults of respiratory and metabolic acidosis, i.e. during and immediately after a prolonged dive. Intracellular phosphate complexes (HPO$_4^{2-}$) and carbonates are involved with renal buffering. Let us assume that the kidney buffering function alone cannot be that effective in terms of rapid buffering needs. Likewise the plasma proteins because of their large molecular size and relative small number (in the human there are approximately 70 grams per liter; Vander et al., 1970) may by themselves be disregarded as effective rapid buffers of the blood. However, Lenfant (1969) has shown a relatively high plasma protein concentration in the killer whale, Orcinus orca (average for four animals, 85 grams per liter). Nevertheless, by themselves plasma proteins are only partial contributors to blood buffering. I have already pointed out the limi-
tions of the bone buffering system, but keep in mind that bone can be most reactive when it is immature, i.e., when primary tissues predominate with incompletely mineralized calcium phosphate.

The most important single effector of blood buffering is hemoglobin for all mammals including aquatic ones. Its suitability for this role depends upon a remarkable characteristic of the hemoglobin molecule: reduced hemoglobin has a much greater affinity for hydrogen ions than oxyhemoglobin does. As blood flows through the tissues, a fraction of oxyhemoglobin loses its oxygen and is transformed into reduced hemoglobin. Simultaneously, a large quantity of carbon dioxide enters the blood and undergoes (primarily in the red blood cells) the reactions (equation No. 1) which ultimately generate $\text{HCO}_3^-$ and $\text{H}^+$. Because reduced hemoglobin has a strong affinity for hydrogen ion, most of these hydrogen ions become bound to hemoglobin (Vander et al., 1970).

Coming back to marine mammals, most investigators agree that, with the exception of buffering capacity, no single physiological or anatomical adjustment explains the resistance that marine mammals have to asphyxia. Indeed, the most noticeable difference between marine mammals and terrestrial mammals is undoubtedly the buffering capacity which is consistently higher in the former (Lenfant, 1969). However, Lenfant emphasizes the following:
It...appears that the differences between various species of marine mammals and the differences between marine mammals and other mammals are not ascribable to the differences in hemoglobin concentrations. Thus, it appears that either the buffer value of each gram of hemoglobin is greater in the marine mammals or that their blood contains other buffer systems. (My underlining.)

I will return to this "other buffer system" shortly. But for the moment, let us get a brief understanding of the basic characteristics of bone.

Basic Characteristics of Bone

Bone has two major functions: 1) as a structure it gives support to the body and provides surfaces for the attachment of muscle; and 2) as a labile tissue or organ, it acts as a mineral reserve for calcium, phosphorus, sodium, magnesium, and other mineral elements, as well as metabolically important compounds. It is with this latter function that we will be concerned with.

For purposes of the present discussion, the following biochemical characteristics of bone will be treated as axiomatic (McLean & Urist, 1968; Neuman and Neuman, 1958; Bourne, 1956; Hoar, 1966):

1. Hydroxyapatite, $\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2$ is the stable maturation end point of bone crystal in the adult animal with a calcium to phosphate ratio of 1.67:1.
2. A high degree of immature and partially amorphous bone mineral called octacalcium phosphate (sometimes called tetracalcium hydrogen triphosphate), \(2\text{Ca}_4\text{H(PO}_4\text{)}_3 \cdot 2.5\text{H}_2\text{O}\) may be present in the formative stages of bone mineral especially in young individuals. It has a calcium to phosphate ratio of 1.3 : 1.

3. The circulating fluids of the blood of all vertebrates are largely supersaturated with respect to calcium and phosphate, but below the level of spontaneous precipitation, i.e., metastable.

4. Transport of calcium and phosphate within the animal is a function of the circulating blood and intercellular fluid.

5. The age of bone is the primary determinant of its chemical reactivity. Young bone is more vascular and has a higher water content, permitting faster diffusion rates.

6. Availability of the skeletal mineral is inversely proportional to age. At early ages bone turnover is high, but because of the continuing maturation of the structural elements of bone during normal growth, bone mineral becomes decreasingly available with age.

7. The labile fraction of the skeleton acts as a non-regulatory buffer to changes in the electrolyte composition of the body fluids relative to bone age.
8. The skeleton can serve as a buffer reservoir only for those ions which tend to concentrate at the bone crystal surfaces or exchange with lattice ions. These are usually \( \text{Ca}^{++}, \text{PO}_4^{\text{2-}}, \text{H}_2\text{O}^+, \text{Na}^+, \text{Mg}^{++}, \text{CO}_3^{\text{2-}} \) and citrate\( ^{\text{2-}} \).

9. Homeostatic regulation of calcium ion concentration in the blood is actively regulated by the parathyroid glands. The negative feedback of \( \text{Ca}^{++} \) (and also \( \text{Mg}^{++} \)) controls the secretion of parathormone, the calcium activating hormone.

A POSSIBLE MECHANISM FOR RAPID ACID-BASE HOMEOSTASIS IN MARINE MAMMALS

At this point the reader may be wondering what the foregoing discussion has to do with skeletal neoteny and its physiological significance in the marine mammal. Let us now return to that "other buffering system" in the marine mammal and see how skeletal neoteny may be associated with a possible mechanism for rapid acid-base homeostasis in the efficient diver. In view of this possibility, I propose the following hypothesis:

In marine mammals the internal skeleton, by virtue of its mineral content, acts as an essential non-regulatory buffer to the extreme hydrogen ion insult of prolonged diving. For the efficient diver, the intrinsic advantage of skeletal neoteny lies in its high
phosphate to calcium ratio due to the abundance of octacalcium phosphate in immature bone tissue. In undergoing dissolution, the immature partially amorphous bone mineral (octacalcium phosphate) ionizes to form calcium cations and phosphate anions. In this process the phosphate ion of the bone mineral changes from the trivalent state \((\text{PO}_4^{3-})\) to a mixture of divalent \((\text{HPO}_4^{2-})\) and monovalent \((\text{H}_2\text{PO}_4^-)\) anions. To do this, the phosphate group must acquire hydrogen ions from the circulating blood medium. An increase in hydrogen ion beyond the buffering capacities of hemoglobin, serum proteins, and the metastable plasma-bone solution (axiom No. 3) dissolves more immature bone mineral (octacalcium phosphate) for further mobilization and utilization as a buffering agent. As the hydrogen ion is metabolized and dissipated during post-dive recovery, calcium and phosphate ions recrystallize to their original stability at various sites within the cancellous bone tissue. At the same time hemoglobin, serum proteins, and the metastable plasma-bone solution return to their normal function. With respect to the high phosphate to calcium ratio of neotenous bone, the efficient diving marine mammal is, therefore, inherently endowed with another extremely rapid and effectual buffering system. Thus the blood buffering capacity of the marine mammal, depending on the neotenous state of its skeleton, differs from its terrestrial relatives in that it has this "other buffer system" to augment and complement the buffering
capacities of blood cells (hemoglobin), the kidneys (carbonates and intracellular phosphates), the plasma proteins, and the metastable plasma-bone solution of the blood.

The most salient aspect of the above hypothesis is that skeletal neoteny may be the **sine qua non** of the most successful marine mammal. If we measure biological success by the food getting ability of an animal, the biological and evolutionary success of a marine mammal is therefore intimately associated with its rate of skeletal maturity. But such a unique specialization does have its liabilities. The skeletal rate of maturity must be depressed otherwise the diver loses his blood buffering advantage and, unable to exploit his feeding potential, becomes extinct in time. Thus, there would have to be selective pressures for a depressed rate of skeletal maturity if the marine mammal is to survive. The fossil record seems to corroborate this point. If you recall (p. 3) it was Zangerl (1935) who noted that there is a trend in early marine mammals away from dense bone (pachyostosis) to spongy or porous bone, i.e., post-pachyostosis and skeletal neoteny.

As I attempted to show earlier, skeletal neoteny is affected by the thyroid depressive affects of chronic hypoxia. Thus, chronic hypoxia, thyroid hypofunction, skeletal neoteny, and diving proficiency are intimately associated both on a temporal and evolutionary scale. The final question remains: What is the stimulus that ultimately
leads to the specialization of skeletal neoteny and its effect on acid-base homeostasis? The answer has to do with the same reason that secondarily placed mammals in a marine environment in the first place: the answer is FOOD! Figure 11 presents a hypothetical model summarizing the major afore-mentioned points of this thesis. The problem of food availability and intra-inter-specific competition constitutes the major stimulus for diving which ultimately leads to the solution of this problem by evolving another rapid and efficient blood buffering system to facilitate diving efficiency. Hence, diving efficiency counteracts the problem of food availability and competition. The temporal components to this solution constitute: 1) the effects that chronic hypoxia may have on the thyroid to depress thyroxine which in turn may cause and maintain skeletal neoteny; and 2) the high phosphate to calcium nature of neotenous bone augments the use of inorganic phosphate buffers in acid-base homeostasis to afford more efficient diving.

Since the actions of the thyroid with the pituitary and the hypothalamus are inextricable, the latter two glandular actions are depicted in Fig. 11. Any decrease in thyroxine from the thyroid causes the pituitary via the hypothalamus to secrete Thyroid Stimulating Hormone (TSH) within a negative feedback mechanism. The affects of hypoxia on the pituitary are not postulated but can be expected to affect its target organs as well.
Fig. 11. A hypothetical model for the evolution of skeletal neoteny and its dynamic relation to acid-base homeostasis and diving efficiency. Dotted arrows represent temporal relationships while solid arrows represent evolutionary.
In the past, most of the tests for carbon dioxide absorption, oxygen capacity, and whole-blood buffering capacity of marine mammals have been done in vitro under simulated conditions of oxygen and carbon dioxide tension, temperature and pH (Lenfant, 1969; Elsner, 1969). In those studies where blood analyses were performed in vivo, the investigator chose to study one blood property and ignoring others for the same sample, e.g., studying oxygen capacity but not determining the whole-blood buffering capacity at the same time. There are major problems and drawbacks with performing blood reactive tests in vitro. In general, one can never be sure that the results you have are representative of a total blood-body interaction. Since the blood is not circulating, the investigator fails to describe the dynamic aspects of blood-body interaction that may occur during an actual dive. In this regard, all of the buffering capacity determinations by various investigators described by Lenfant (1969), were done in vitro and cannot be considered to demonstrate the total buffering capacity of an animal if it indeed uses the augmentative phosphate buffer system that I have postulated. Thus, it is not surprising to see that no relationship has been established between the absolute value of whole blood buffering capacity and diving ability in the various marine mammals. Consequently, I am left with only one example to support my contentions with regards to the possibility of augmentative bone buffering.
It appears that the elephant seal holds the record for maximum oxygen capacity of the total blood. Scholander (1940) determined the whole blood oxygen capacity of the southern elephant seal, *M. leonina* and found it to be 40 vol. % (*in vivo*). Elsner et al. (1964) performed similar experiments with *M. angustirostris* and found a range of 28 to 39 vol. % (*in vivo*) indicating that both species have a very high oxygen capacity. Bryden & Lim (1969) determined the blood volume of elephant seals (*M. leonina*) ranging in age from birth to physical maturity and found that their blood volumes were over 3-1/2 times larger than man (207/ml kg empty live weight), or about an average of 11% of body weight. Wasserman & Mackenzie (1957) and Harrison & Tomlinson (1956) determined the blood volume of young harbor seals, *Phoca vitulina* and found the blood volume to range from 11 to as high as 18% of body weight. Since both the elephant and harbor seals have similar blood volumes in per cent of body weight, one would expect them to both have similar whole blood oxygen capacities. Yet the whole blood oxygen capacity of the harbor seal is considerably lower, 22.8 - 35.7 vol. % (*in vitro*) (Irving et al., 1935). The disparity may be explained by the fact that the experiments by Scholander (1940) and Elsner et al., (1964) were performed *in vivo*, i.e., the animal's circulating blood was drawn during a simulated dive, whereas the experiments by Irving et al., (1935) were based on simulated conditions of temperature,
pressure and pH on drawn blood only. Yet, had Elsner and his associates performed in vivo determinations, one would expect the elephant seal's oxygen capacity to still be higher by virtue of the fact that this animal is known to regularly stay submerged during a dive for over 30 minutes (Matthews, 1952). Of the Pinnipedia, the elephant seal is possible exceeded only by the Weddell seal, *Leptonychotes weddelli*, in its adaptation for prolonged diving (Bryden & Lim, 1969). The implication here is that the diving efficiency of the elephant seal is much higher than the harbor seal. Consequently, even though their blood volumes are the same, the expected disparities in whole blood oxygen capacities could be attributable to a more advanced blood buffering system since the more carbon dioxide and hydrogen ion buffered, the more partial pressure oxygen you get into solution.

Logic is indeed a powerful tool, but it is no real substitute for experimentation. An interspecific comparison of inorganic phosphate serum levels before, during, and after a dive correlated with determinations of whole blood oxygen capacity and whole blood buffering capacity could serve to test the foregoing hypothesis.

If subsequent experimentation proves the existence of an augmentative bone-derived phosphate buffering system, the profound importance of rapid and efficient acid-base homeostasis in marine mammals cannot be overemphasized.
Vertebrate evolution depends upon the homeostatic ability of the organism. Fitness in the neo-Darwinism sense is more than reproductive capacity and physical vigor: it is a complex expression of homeostatic ability. Homeostasis, therefore, is the arcanum of life....

(McClean and Urist, 1968)
SUMMARY AND CONCLUSIONS

The humerus and femur from six species of pinnipeds and the sea otter exhibited remarkable structural and possibly developmental features common to the bones of penguins, sirenia, cetacea and early marine reptiles. These include the persistent lack of a marrow cavity, depressed skeletal maturation ("skeletal neoteny"), and a characteristic "box-beam" structure. These findings suggest that the bone structure of secondarily marine forms exhibits an interf phyletic group-specific pattern.

A possible mechanism for acid-base balance of the blood utilizing bone-derived phosphate buffers and its relation to diving ability is discussed. Evolutionary implications of this theory suggest that the availability of phosphorous in relation to diving efficiency may be an important selective factor in secondarily marine animals.

Whereas previous authors have emphasized bone development and structure in terms of mechanical function, this paper concentrates on the dynamic interrelationship between bone structure and the physiological demands of an aquatic existence. Internal architecture is considered to be an expression of both homeostatic ability and skeleto-muscular mechanics in the secondarily marine animal.
LITERATURE CITED


