CHAPTER I

INTRODUCTION

INTRODUCTION

The importance of the skeleton in higher animals is well known. The skeletal system has many functions; it gives support to the soft tissues of the body; it forms the basis for movement and gives protection to many of the vital organs. Haemopoesis takes place in the red bone marrow. The skeleton also acts as a mineral storehouse of many ions mainly calcium and enables the constant adjustment of calcium level in blood and body fluids. The skeleton may also be considered an important reservoir of water, as that of an adult man weighing 70 kg contains 4-8 litres of water and almost all of this is exchangeable in about 4 hours (Edelman et al., 1954).

The growth of the skeleton may well determine the growth of many of the soft tissues. These may be prevented from attaining their correct proportions relative to each other, and to the body as a whole if skeletal growth becomes abnormal.

The skeletal system includes the bones and related cartilages including the joints or articulations. Throughout life a two way chemical traffic occurs between the blood stream, the cells and the bone matrix and surface as a result of which the composition and structure of the bone is always changing both at the molecular and at the macroscopic level

with a well-regulated balance between the bone-forming activities of osteoblasts and the eroding activities of osteoclasts (Amprino and Bairati, 1936).

The 206 bones of the human skeletal system may be broadly placed under two categories based on their location, namely, the axial skeleton (80) and appendicular skeleton. They are designated as long, short, flat or irregular according to morphological characteristics. They are also classified according to the mode of ossification, namely, endochondral or intramembranous.

Bone is a dense material whose matrix is composed of collagen fibres, embedded in a cement composed of mucopolysaccharides, glycoprotein and lipids, on which are deposited hydroxy-apatite crystals of calcium-phosphate, although other salts of calcium and other mineral components are also present (Pritchard, 1972). In addition, bone contains water in various forms ranging from bulk water in the Haversian canals to tightly bound water in the organic matrix (Timmins and Wall, 1977).

About a third of the total protein in the mammalian organism is collagen. The collagen fibres are resolvable into fibrils ranging from 400 to 1200A in diameter. The nature and structure of collagen are important in inducing crystallization. It constitutes a rigid frame for a carbo-

hydrate-protein complex which serves as a mineralizing matrix and is progressively removed as calcification proceeds to completion.

Collagen consists of a triple helix polypeptide chain in which glycine forms about one third of the amino acids present. The hydroxylated derivatives of lysine and proline are important for the cross-linking of collagen fibres and the hydroxylation is brought about by enzymes after the basic structure is formed. A minimum of 35% hydroxylation is considered essential for the collagen molecule to aggregate into a triple helix configuration (Rosenbloom et al., 1978). Ascorbic acid acts as a cofactor in the hydroxylation of proline (Peterkofsky and Udenfriend, 1965) by activating the enzyme prolyhydroxylase. In addition, it stimulates the secretion of collagen from the cells in which it is made (Peterkofsky, 1972).

The mucopolysaccharides (glycosaminoglycans) in the ground substance are long, straight-chain carbohydrates which contain many acidic (carboxyl or sulphate) groups. They exist as proteoglycans in which many of the chains are linked to a protein molecule at the terminal reducing sugar residue. The initial stages in the biosynthesis of proteoglycans are similar to those in glycoprotein synthesis; the polysaccharide chains are synthesized by the progressive addition of monosaccharide units, the first step being the

addition of specific sugar residues to the protein core.

The major sulphated glycosaminoglycan in bone is chondroitin sulphate (Vejlens, 1971). Of the two isomers, chondroitin-4-sulphate and chondroitin-6-sulphate, the latter is specifically mobilized during the mineralization of osteoid as inferred by Dorey and Bick (1977) based on histochemical studies. Similar observations are made during the mineralization of predentin to dentin (Engfeldt and Hjerpe, 1973) and during the transformation of non-calcified cartilage to calcified cartilage (Lohmander and Hjerpe, 1975).

Glycoprotein in bone is closely associated with collagen. As for the formation of glycoprotein in bone tissue, limited information is available as a result of work with tritium-labelled fucose. It is also possible that some of it is derived from the plasma. Preliminary studies (Andrews et al., 1967) show that osteoblasts synthesize and secrete a glycoprotein along the osteoid where the combination of glycoprotein with collagen provides a suitable substrate for mineral deposition.

The total content of lipid in cortical bone is very low, amounting to 0.0673% by weight of dry femoral bone (Leach, 1958) in which about 80% is triglycerides (Table-1). The acidic phospholipids could be extracted completely only after the demineralization of the tissue followed by the use of acid solvents. Shapiro (1970) found that phospholipids made

Table-1: The Lipids of Bovine Cortical Bone[φ]

Class of lipids	Lipid content	
	% dry weight	% total lipid
Triglycerides	0.0535	79.2
Free cholesterol	0.0089	13.3
Cholesterol esters	0.0011	1.7
Phospholipids	0.0015	2.2

φ - Data from Leach (1958)

up about 0.08% of the organic matrix in mature bovine bone with sphingomyelin, lecithin and phosphatidyl-ethanolamine as the major fractions and that the bound phosphatidylserine, phosphatidyl inositol and phosphatidic acid were obtained from bone only after demineralization.

The mineral in bone occurs in the form of closely arranged crystals of 30-50A in width and upto 600A in length (Fernandez-Moran, 1957). The crystals may be needle-shaped or tubular (Robinson, 1951). Bone mineral is present mainly in two forms, namely the amorphous phase (a hydrated tri-calcium phosphate) and the crystalline phase (hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$).

Bone contains calcium and phosphorus approximately in the ratio 2:1, that in the whole body being 1.8:1. The Ca/P ratio in rat femur epiphyses was found to be 1.0 at birth and to increase sharply to 1.9 at 21 days when the animals were weaned. The ratio continued to rise slowly to a value of 2.2 at 158 days (Dickerson, 1962). In the epiphyses of the human femur the ratio rose from 1.84 at birth to 2.29 at 12-24 months of age.

As mentioned earlier the water in bone may occur as 'free' or 'bound' water (Figure-1). The former is contained in the different water-containing pores and capillaries, while the latter is that absorbed mainly by collagen and by hydroxyapatite, the chief water absorbing matter, and to

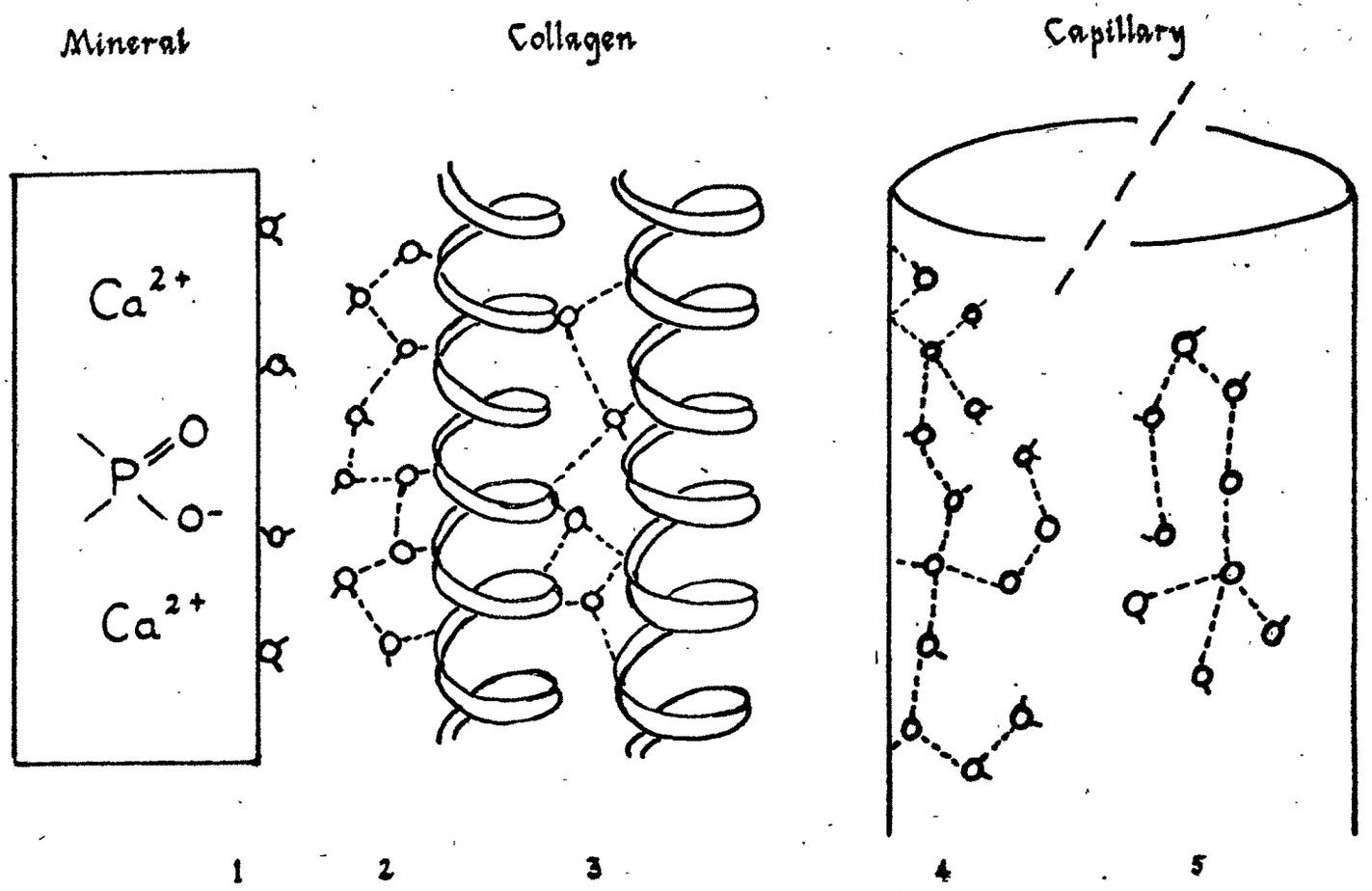


Figure 1. Schematic diagram of possible locations for bone water: (1) Water associated with mineral phase; (2) Water on collagen fibril surface ~ inter-fibrillar water; (3) Intra-fibrillar water; (4) Water on surface capillaries; (5) Water at some distance from capillary surface ~ i.e. essentially bulk water; O Water molecules. Intra cellular and intra-membranous water also present are not illustrated. (Timmins and Wall, 1977.)

some extent by mucopolysaccharides (Marino et al., 1967). Very little is known about bound water which is important as a structural element of bone (Timmins and Wall, 1977). The nature and dimensions of water-containing pores are given below:

Pore	Dimension (μm)	Number/mm^3
Haversian canal	20 - 100	
Lacunae	10 x 15 x 25	20,000
Canaliculi	0.3	10^6 (5-100/lacuna)

About 27% of the water in the rat tibia is believed to be in the extrafibrillar spaces and the remainder in the intrafibrillar spaces (Katz and Li, 1973).

If the whole bone is considered, the marrow also contributes to water content. The red, non-fatty marrow has a water content of about 88% by weight and 91% by volume.

The three major components of bone, namely, the collagen fibres, the crystals and the ground substance have an intimate relationship, approaching the molecular level of integration and bound by physical as well as chemical bonds.

Histologically, bone is a well-knit tissue having functionally-varied cells acting in concert. Osteoblasts

are found wherever bone is being formed. They are located along the inner layer of the periosteum and the endosteal surface of diaphysis and the trabecular surface of metaphysis. They increase the thickness of these surfaces by appositional bone growth, i.e., by direct formation of bone on pre-existing bone surface. After that, the osteoblasts themselves get caught in the calcified matrix and are transformed gradually into osteocytes. The osteoclasts have an independent origin and are found all over the bone surfaces where they are involved in the removal of the calcified matrix.

The location of these cells on the bone surface of young and adult animals is shown diagrammatically (Figure-2).

The cartilage cells form an essential part of the whole bone in the adult and in the embryo. The cells at the epiphysis regulate bone growth and during the embryonic stage construct the model of endochondral bone.

Cartilage is a necessity in embryonic life because it is a tough, rigid tissue which stands pressure well and can grow by interstitial expansion. Moreover, during the embryonic stage it serves as the model of the endochondral bone and during growth the cartilage cells of the epiphysis regulate bone growth. In the adult skeleton cartilage is found at points of pressure.

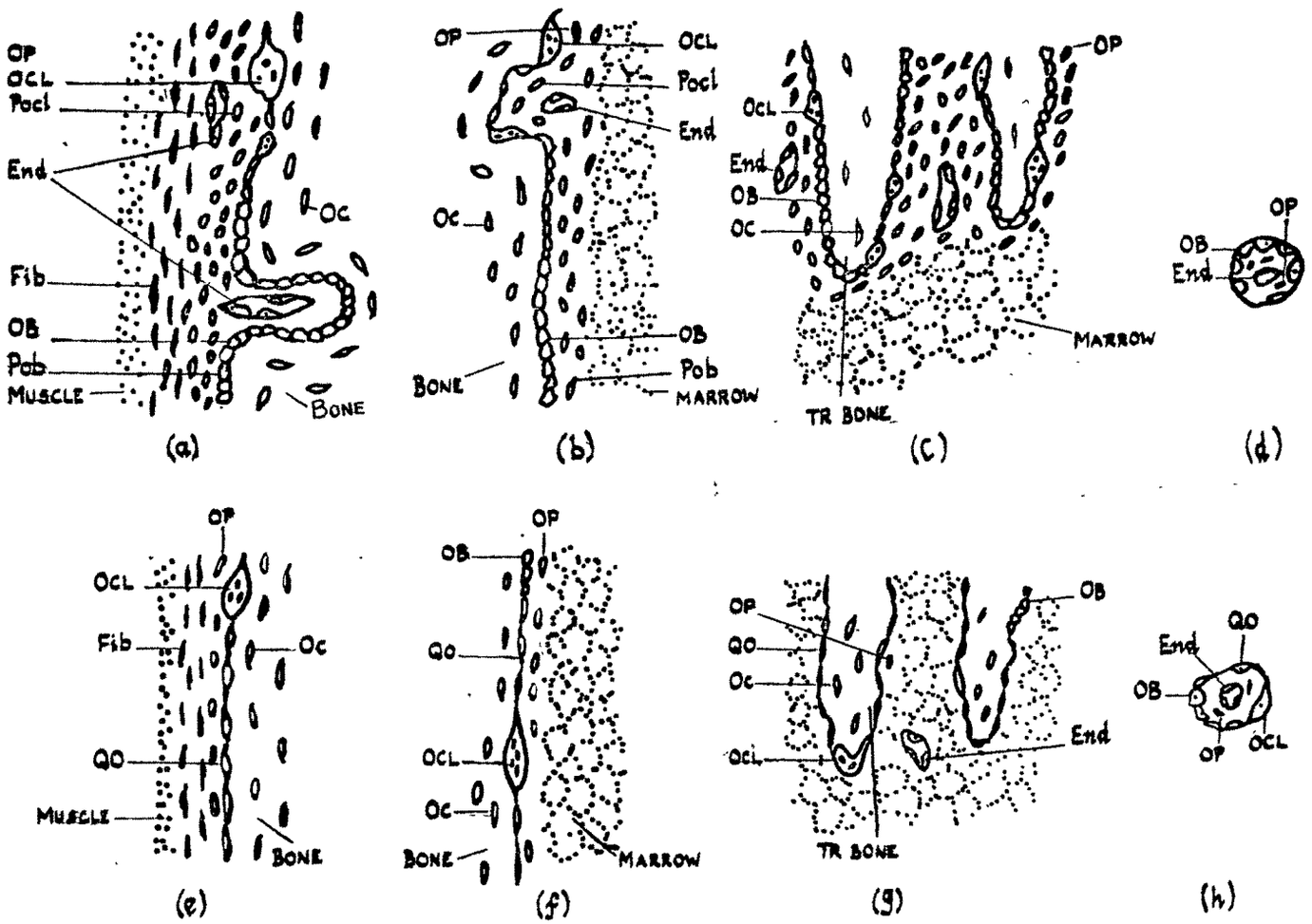


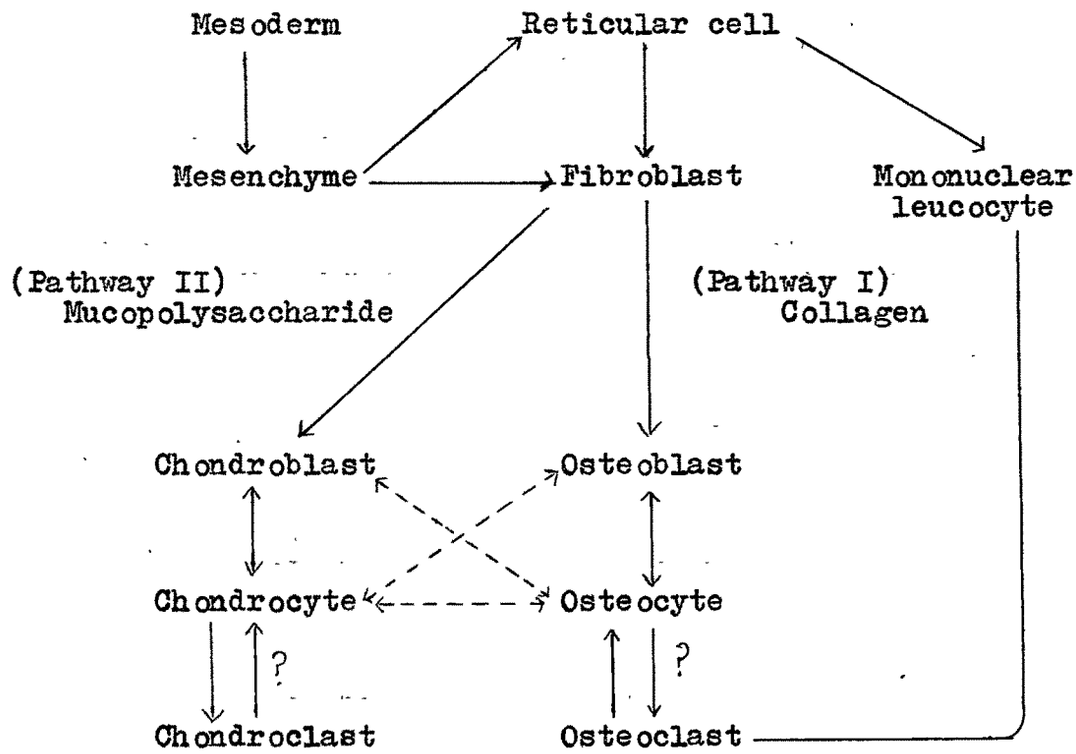
Figure 2. Diagrammatic representation of the cells associated with bone surfaces in young animals (a)~(d), and in adult animals (e)~(h); (a) and (e) periosteal surface, (b) and (f) endosteal surface, (c) and (g) trabecular surfaces, (d) and (h) Haversian canal. In young animals the layers of cells are thick and the majority of them are actively metabolizing. In adult animals most of the surface is covered by flattened, inactive cells. Osteoprogenitor cells are usually easily distinguishable in young animals but rarely so in adults. OCL, osteoclast; OP, osteoprogenitor cell, Pocl, preosteoclasts; End, endothelial cell; Fib, Fibroblast; OB, osteoblast; Pob, preosteoblast; Oc, osteocyte; and QO quiescent osteogenic cell.

Information on the histogenesis of bone cells is mainly obtained from studies of marrow tissue. It has been shown that marrow contains two main cellular systems, the stroma and the haemopoietic system. These are derived from histogenetically distinct cell lines in the postnatal organism. The stromal cells of the marrow and the monocytes which are the components of the haemopoietic system appear to differentiate under appropriate conditions into osteoblasts and osteoclasts respectively (Owen, 1978).

In endochondral ossification, the spindle shaped cells covering the transformed perichondrium of the cartilage model are the main precursors of osteoblasts.

Osteoclasts are also reported to have their origin in cells of the vascular wall (Rewink and Vermeiden, 1977) and in the monocytes (Mundy et al., 1978). The attraction of monocytes to bone resorbing surface has been suggested to be chemotactic (Mundy et al., 1978).

The different types of cells in the skeletal tissue seem to form an inter-related sequence. Although this has been a subject of controversy for many years, the transformations of cells within skeletal tissues may be diagrammatically expressed as follows, as pictured by Hall (1970).



———— = Differentiation

-------- = Metaplasia

Pathway I is believed to be activated by:

(1) predetermined information (membrane bone formation);
(2) chemical induction (endochondral bone);
(3) hyperoxia, pressure, tension, good blood supply (fracture repair).

Pathway II is believed to be activated by:

(1) predetermined information (endochondral ossification);
(2) chemical induction (vertebral cartilage);
(3) hypoxia, pressure, tension, ischaemia (fracture repair).

Though cartilage and bone cells show morphological and histological differences, they seem to be very similar chemically and their cells are readily intertransferable (Hall, 1970). An analysis of the enzymes in cartilage and bone show only quantitative differences between the two and no one particular enzyme is localized either only in cartilage or bone (Hall, 1968). Collagen synthesis is predominant during osteogenesis whereas acid mucopolysaccharides synthesis is predominant during chondrogenesis. Selective enzyme enhancement is probably responsible for these differences. The external factors which initiate osteo-or chondrogenesis include mechanical factors, degrees of vascularity, oxygen levels, ionic environment, vitamins and hormones. Such factors may determine the proportion in which collagen and mucopolysaccharides are produced in skeletal cells.

Osteoblasts are considered transitional cells which temporarily display certain distinctive morphological features as they engage for a limited period in certain specialized metabolic and secretory tasks connected with bone formation. They do not show any mitotic activity, but their precursors show a high rate of mitosis.

The morphology of the osteoblasts is varied. The length varies between 15 and 80 μ in man (Kollicker, 1889). They also vary in shape and may be ovoid, rectangular,

columnar, cuboidal or pyriform forming a pseudoepithelium with lateral cell contact.

The osteoblasts effect the synthesis of collagen, mucopolysaccharides, glycoproteins and their elaboration into the matrix. Certain peptides and lipids which are seen in the newly formed bone matrix are probably manufactured by these cells (Nylen et al., 1960; Bassett, 1962). The cytological structure of these cells and the localization of enzymes in the same also bear witness to the fact that they have the requisite cell machinery and energy supply.

The Golgi apparatus of the osteoblasts closely reflects the functional state of the cells in relation to bone matrix production (Pritchard, 1952). The mitochondria become more numerous, longer and thinner as the preosteoblasts are transformed to osteoblasts. After the secretory activity is completed, the osteoblasts either revert to the inactive state or turn into osteocytes.

Glycogen is present in large quantities in the precursors of osteoblasts in prospective centres of ossification in the fetus (Gendre, 1938; Glock, 1940), but in fully differentiated osteoblasts there is little or no glycogen (Horowitz, 1942). A similar phenomenon is observed in intramembranal bone formation (Bevelander and Johnson, 1950).

Alkaline phosphatase detected in calcifying tissues (Robison, 1923) was assumed to be mainly manufactured by osteoblasts and hypertrophic cartilage cells (Martland and Robison, 1924; Fell and Robison, 1930, 1933). Considerable phosphatase activity was found to be present in the mesenchymal cells in presumptive sites of ossification, increasing with their differentiation into osteoblasts, and falling abruptly as matrix production and calcification get under way (Bevelander and Johnson, 1950; Pritchard, 1952).

The supersaturation of serum and the extracellular fluid with calcium and phosphorus should result in abnormal calcification in soft tissues and bone. This is prevented by the presence of pyrophosphate. One of the triggers for calcification in bone is the break-down of pyrophosphate by pyrophosphatase (Russel and Fleisch, 1976).

In mineralized bone, pyrophosphate might influence the rates of calcium and phosphate movements in and out of bone by enzymatic control, with alkaline phosphatase regulating deposition and an acid lysosomal phosphatase, possessing pyrophosphatase activity, regulating resorption (Figure-3). The conditions around the site, e.g. pH and type of cell, may determine whether deposition or resorption occurs (Russel and Fleisch, 1976). Some of the other enzymes

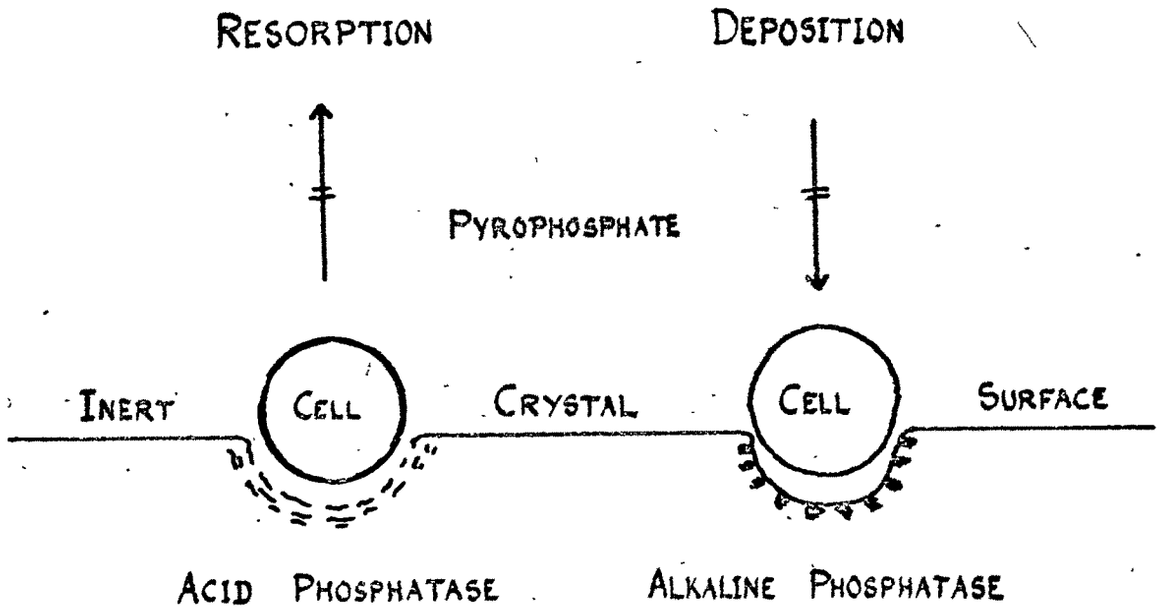


FIGURE 3.

SCHEMATIC MODEL TO SHOW THE ROLE OF PYROPHOSPHATE IN MINERAL DEPOSITION AND RESORPTION AT CRYSTAL SURFACES IN BONE.

detected in osteoblasts are indicated below:

Acid phosphatase	.. Wergedal and Baylink	(1969)
	Radden and Fullmer	(1969)
Cytochrome oxidase (abundant)	.. Follis	(1948)
	Follis and Berthrong	(1949)
Phosphorylase	.. Townsend and Gibson	(1970)
Glycogen synthetase	.. Fullmer and Lazarus	(1967)
Collagenase	Rodan and Anbar	(1967)
Pyrophosphatase	.. Fleisch and Neuman	(1961)
	.. Cox and Griffin	(1965)
	Eaton and Moss	(1966)
Citric acid cycle and Glycolytic enzymes	.. Radden and Fullmer	(1969)

The appositional growth rate of the osteogenic surfaces depends upon the productivity of osteoblasts and thus on (a) the number of osteoblasts per unit of the osteogenic front, and (b) their size and "secretory territory" (Marotti et al., 1976). The appreciable variation in appositional growth rate and matrix production can be seen from the following studies:

Reference	Site	Appositional rate per site µm/day	Daily matrix production μm^3
1. Owen (1963)	Rabbit diaphysis	-	2860
2. Marotti et al (1976)	Large Haversian canal	1.8	180
	Small Haversian canal	0.4	90
3. Schen et al (1974)	Osteoblast Human rib cortical bone	1.0	220
4. Jones (1974)	Rat Parietal bone	3.12	470
5. Hammond and Storey (1970)	Sprague Dawley - male periosteal	3.2	-
6. Baylink et al (1970)	Sprague Dawley - male tibia periosteal	10.3	-
7. Stauffer et al (1972)	Sprague Dawley - male tibia periosteal	6.0	-
	Endosteal	2.9	-
8. Hammond and Storey (1974)	Wistar male caudal vertebrae - periosteal	10.0	-

Osteocytes which are derived from osteoblasts resemble them in their cytological components as may be expected. They remain buried in the calcified matrix, some filling the lacunae in which they lie whereas others are separated from the surrounding walls by a zone of amorphous material and

loose collagen fibrils. Histologically, the osteocyte space includes canaliculi, lacunae and the surrounding space (Lipp, 1954).

A few short cytoplasmic processes extend from the cytoplasm of bone cells and freely anastomose with those of neighbouring cells, the cell membranes forming tight junctions where two cell processes meet (Weinger and Holtrop, 1974). Such junctions are found between osteocytes and osteoblasts and between osteoblasts on the cell surface.

On the whole the osteocytes do not have a well-developed endoplasmic reticulum except for a few dilated cisternae (Dudley and Spiro, 1961). Golgi bodies may be as extensive as in osteoblasts but with a tendency for the sacs to be collapsed.

Many functions have been assigned to the osteocytes. They are believed to facilitate exchange of materials between tissue fluids and matrix, to resist the leaching of the matrix and to preside over rejuvenation and remodelling (Pritchard, 1956). The presence of mucopolysaccharides around these cells suggests a role in their synthesis. The generous amount of water surrounding it may be concerned with transport of materials between the blood and the bone surfaces. Osteocytes are also believed to inhibit mineralization in their immediate environment.

"Osteolytic" activity has also been assigned to the osteocytes by Belanger and Migicovsky (1963) and it was suggested that actively osteolytic osteocytes in normal trabeculae eventually perish by autolysis (Jande and Belanger, 1969).

The functional life of osteocytes varies and may extend between the osteoblastic phase and osteoclastic phase (Jande and Belanger, 1973).

The osteoclasts in bone are 'aggressive' cells engaged in the removal of the bone. They are large, multinucleated cells of variable shape and are in fact the largest of living cells. The cell body shows processes with the nuclei generally held together. They are characterised by the presence of erosion pits or lacunae or the appearance of a striking looking "brush" border or "ruffled" border on the cell surface in contact with bone, composed of a collection of folds and fingerlike protrusions (Cameron, 1972).

The number of mitochondria per unit area of cell is very high in osteoclasts (Cameron, 1972) and they are distributed throughout the cytoplasm except near the area of the brush border. The endoplasmic reticulum is not a conspicuous component, being rudimentary in some and widely spread in others (Scott and Pease, 1956). The Golgi apparatus is found around the nuclei and the centriole (Matthews et al., 1967; Cameron, 1968). Of the many dense bodies found in

osteoclasts (Schenk et al., 1967), some may be lysosomes (Doty et al., 1968), a functional necessity.

Some of the enzymes reported to be prominently present in osteoclasts are acid phosphatase (Hancox and Boothroyd, 1963; Doty et al., 1968), leucine aminopeptidase, and β-glucuronidase (Hancox and Boothroyd, 1963).

During the embryonic period a condensation of mesenchymal cells always precedes bone formation. The ossification may be endochondral or intra-membranous depending on the bone in which it takes place.

In endochondral bone formation mesenchymal cells in the fetus condense first to form a cartilage model of the future bone. This is called the primary cartilage and it is enveloped by a layer of perichondrium. The cartilage model resembles the adult bone in shape and grows in size by appositional and interstitial growth. The midshaft is the narrowest region containing the older cells whereas the new ones are formed at the two ends. The transformation of the cartilage into bone is initiated at the narrow midshaft where a "bone collar" is formed all around. From this end the transformation proceeds towards the two ends. At the extremes of the long bone, the cartilage is not replaced, but forms the epiphyseal plate. Further elongation of the bone is caused by the multiplication of cartilage cells in the epiphyseal column followed by their replacement by new bone.

A coordinated sequence of cellular processes is observed in endochondral ossification, which is evident from the study of the cells of the epiphyseal plate. Here, the cartilage cells are arranged in longitudinal rows separated by intercellular matrix, which are calcified during the process. The cells in the column are in various stages of maturation such as multiplication, growth and degeneration. The multiplying cells lie at the top of the epiphyseal plate and the degenerating cells towards the inner end. As the cartilage cells degenerate or hypertrophy, the matrix between columns of cells calcifies. Following this, an invasion of blood vessels from the adjacent metaphysis occurs and the degenerated cartilage cells are completely destroyed, leaving the calcified cartilage or the primary spongiosa. Over this region there is immediate ossification by osteoblasts and thus the formation of bone trabeculae or secondary spongiosa. This is subjected to extensive remodelling by continued bone formation and osteoclastic resorption. Gradually, the trabecular bone of the metaphyseal region is transformed into compact bone of the diaphysis. The changes in the composition of the cartilage with this sequence of events are indicated in Table-2.

As mentioned earlier, osteoblasts secrete an osteoid which is the organic matrix consisting of collagen, water and small amounts of mucopolysaccharides. The events occurring between the synthesis of the matrix and calcification

Table-2: Organic composition of Washed Epiphyseal Cartilage and Bone[a] (Herring, 1972)

	Zone				
	Resting cartilage	Proliferating cartilage	Hypertrophic cartilage	Calcified cartilage	Cancellous bone
	Percentage of Organic matrix[b]				
Collagen[c]	60.1	39.3	23.3	22.1	1.2
Chondromucoprotein[d]	40.7	59.2	41.8	19.3	4.1
Sialoprotein[e]	3.43	4.69	4.49	4.80	2.39
Lipid	1.00	4.86	7.70	8.50	0.61
Sulphur	1.54	2.07	1.40	0.83	0.37
Nitrogen	10.8	9.8	10.3	8.9	11.8

a - From Wuthier (1969)

b - Organic matrix is freeze-dried weight minus ash content of washed tissues

c - Percent of hydroxyproline/0.141 (Eastoe, 1955)

d - Percent hexosamine - (Percent sialic acid x 0.45) / 0.264 (Luscombe and Phelps, 1967; Herring, 1964)

e - Percent of sialic acid/0.171 (Herring, 1964)

are not known. But at the calcification front, an abrupt transition of matrix occurs so that it is mineralized to 70% of its ultimate full mineral capacity (Jowsey and Gordon, 1971).

Intramembranous ossification begins with the formation of the primary centre. The first spicule of bone surrounded by osteoblasts grows in size and forms trabeculae which radiate from the centre. The pattern of growth and orientation of trabeculae differ from bone to bone. Arising from the primary trabeculae are the secondary trabeculae all of which coalesce to enclose a space containing vascular connective tissue, the forerunner of the haematopoietic tissue. The primary trabeculae advance forming new bone until it comes in contact with another bone, where it begins to form a smooth bony border. The intramembranous bone so formed is predominantly fibrous and is made of coarse bundles. This is replaced by lamellar bone after birth. Noback (1943) categorised two types according to the nature of the peripheral trabeculae, namely, the open reticular plate bone as in the cranial vault bone and the smooth bordered plate bones (as in maxilla and mandible). A secondary cartilage is formed in certain bones such as the mandible.

The ossification centres follow a definite sequence in the development of the skeleton, different centres appearing at different ages. The appearance of these centres has

been used for the assessment of skeletal development. The sequence may, however, show some variation in different individuals.

With growth and aging the proportions of organic matter, the inorganic minerals and water also vary. These variations are associated with changes in the finer structure of the bone.

Kuftinec and Miller (1973) demonstrated that the organic phase of bone growth resembles that of other tissues in that instead of just a simple linear expansion and enlargement, growth spurts are observed.

Three biochemically definable phases of bone growth occur during the early development of the rat, one near birth, the second at about 8 to 12 days of age and the third around weaning.

In the long bones, the DNA content per unit weight is high just after birth. Following an initial reduction, it again shows a sharp increase during days 4 to 6, 11 and 12 and shortly before weaning. This is associated with a slight increase in RNA content. The total protein content also shows three characteristic peaks at approximately the same ages.

A marked fall occurs in the growth rate of the bones at about the time of puberty in the rat (Hammett, 1925;

Dickerson and Widdowson, 1960) in which the chemical maturation of the bones is almost complete at 75 days, i.e., by the end of pubertal period.

During the development of the femur and humerus of the rat, the basic change in composition is an increase in mineral at the expense of water, the increment in organic matter playing a relatively insignificant role in the dehydration which occurs with age (Table-3). Neuman and Neuman (1958) found a similar relationship in bone cortex. The increase in mineral is more than is accounted for by the loss of water and the ratio of mineral to organic matter increases with age.

The other changes reported include an increase in the carbonate of rat bone (Kramer and Shear, 1928), the proportion of collagen nitrogen to total nitrogen at certain stages in man, pig, rat and fowl (Dickerson, 1962) and an increase in the Ca/Mg ratio (Hammett, 1925).

Both during ontogenesis and during regeneration of bone tissue, a gradual decrease is found in hexosamine concentration (Tables 4a and 4b). In bones derived by endochondral calcification, this is partly due to the replacement of cartilage by bone.

Table-3: Changes in chemical composition with age in the whole femur in the rat[φ]

Age (days)	Composition of fresh bone (g/100 g)		Composition of fat-free bone (g/100 g)			
	Lipid	Water	Calcium	Phosphorus	Total N	Ca/N
0	Nil	71.0	2.24	1.39	2.56	0.88
7	1.6	73.2	2.02	1.26	2.25	0.90
14	2.2	65.5	3.60	2.01	2.64	1.37
21	1.9	59.6	5.00	2.65	2.93	1.71
33	0.32	58.6	6.88	3.31	3.06	2.26
46	1.02	49.1	8.82	4.72	3.28	2.70
56	1.2	42.1	11.60	6.10	3.50	3.36
108	1.1	27.5	18.2	7.94	3.50	5.23
158	2.1	25.8	19.1	8.60	3.32	5.72

φ - Values taken from Dickerson (1962)

Table-4a:

Changes in the hexosamine content in the bone tissue of man during ontogenetic development (Tubular bones) (Kasavina and Zenkavich, 1961)

Age	Hexosamine content (mg % dry tissue)
Fetus - 14-16 weeks	185
Upto 12 months	138
13 months to 5 years	125
6 to 10 years	105
11 to 20 years	96
21 to 40 years	98
41 to 60 years	98
Over 60 years	94

Table-4b:

Changes in the hexosamine content during regeneration (Kasavina and Zenkavich, 1961)

Days after fracture	Hexosamine content (mg % dry tissue)	
	Bone tissue	Callous tissue
7	121	1760
14	104	1050
21	123	730
55	115	145

Not only total hexosamine content but also the proportion of chondroitin sulphate varies with age. In cartilage the degree of sulphation of chondroitin sulphate increases during development, as reflected by a decline in non-sulphated disaccharide units that occur in embryonic or young tissue and an increase in disulphated disaccharide units (Greiling and Baumann, 1973; Mathews, 1973). Frequently, an increase in the amounts of chondroitin-6-sulphate relative to the amount of chondroitin-4-sulphate is found (Mathews, 1973; Hjertquist and Lemperg, 1972). But in some cartilages, such as rabbit costal cartilage, the reverse occurs and this may be related to subsequent calcification and loss of proteoglycan (Mathews, 1973).

Kaplan and Meyer (1959) found that the proportion of keratan sulphate in the total acid MPS fraction of human costal cartilage increased linearly with age. The ratio of keratan sulphate to chondroitin sulphate increased with the age of the animal (bovine) (Goh and Lowther, 1966).

Though the exact role of albumin in bone is not known its presence in bone has been well documented (Lipp, 1967; Ashton, 1972). In other tissues albumin helps maintain osmotic pressure, acts as a carrier for hormones, ions and metabolites and may form a source of amino acids (Bingham et al, 1978). Owen (1973) described a "labile" pool and a

"fixed" pool. Growing bones take up albumin (Owen, 1973) and the rate of uptake seems to vary inversely with growth rate (Bingham et al., 1978).

Reference was made earlier to the increase with age in the proportion of collagen nitrogen to the non-collagenous components of the matrix. Similarly a decrease is found in the proportion of amorphous bone mineral which accounts for 69% in young animals (Termine and Posner, 1967) and 40% in the adult (Harper and Posner, 1966).

The lipid rich vesicles in the matrix are found to accumulate calcium and phosphorus (Anderson and Reynolds, 1973) and the growth of the crystals within the vesicles is believed to rupture the limiting membrane of the vesicle (Anderson, 1973) resulting in the formation of an early bone nodule. Amorphous bone mineral is deposited on or near the nodules, which grow until they coalesce at around 1 μm in diameter. The amorphous bone mineral is replaced first by randomly oriented hydroxyapatite crystals within the nodule and subsequently by crystals oriented along the collagen fibres between the nodules (Gay, 1977).

Both ATP and Mg are involved in the stabilization of amorphous mineral and its conversion to the crystalline form. The conversion of amorphous calcium phosphate to hydroxyapatite occurs only with the progressive breakdown

of ATP to near zero levels and, *in vitro*, this breakdown is prevented by Mg according to Posner *et al* (1977) who suggested that ATP prevents hydroxyapatite formation by (a) poisoning heterogenous nucleation sites in the transformation system or (b) binding to embryonic hydroxyapatite nuclei preventing their growth to the critical nuclear size which is defined as the smallest ionic cluster which will not redissolve.

Less than 1% of the total calcium of about 1200 g in the body is distributed outside the bone in the extracellular fluid and the soft tissues including the various membrane structures. The level of calcium in the serum is maintained in a narrow range around 10 mg per dl by the combined action of Parathyroid hormone (PTH), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and calcitonin. Closely involved in this homeostatic regulation are the intestine, bone and kidney.

Of the serum calcium, 60% is ionized and the rest is in a physiologically inert form. The latter form of calcium is either bound to protein (35%) or complexed with citrate, bicarbonate and phosphate (5%).

Though calcium absorption takes place throughout the small intestine, the upper part of the small intestine is found to have the highest absorptive potential and is the major effective site of net calcium absorption (Schryver *et al*., 1970).

Calcium accumulation by mitochondria, which have a high affinity for calcium (Lehninger, 1970), may play an important role in calcium transport by the small intestine (Krawitt et al., 1976).

Calcium movement across the brush border vesicles may involve an electron transport system linked to the hydrolysis (Hearn and Russel, 1977) of ATP which has been reported to inhibit calcium transport, an effect which is partly prevented by Mg^{2+} (Wrobel and Michalska, 1977).

It is well known that the absorption of calcium (DeLuca, 1978) is effected by (CaBP) which is synthesized in the intestine, the trigger for which is $1,25(OH)_2D_3$. Vitamin D_3 is hydroxylated at the number 25 carbon in the liver and undergoes a second hydroxylation in the kidney, in the α-1-position. PTH, the synthesis of which is subjected to very fine regulation and is modulated in response to serum calcium levels, acts as a trigger for the second hydroxylation.

Though supplementation with $1,25\text{-}(OH)_2D_3$ increases the level of calcium binding protein in the intestine (Friedlander et al., 1978; Ueng et al., 1978), in vivo synthesis of calcium binding protein is not a simple function of 1,25-DHCC availability, but rather is influenced both by dietary calcium and phosphorus (Morrissey et al.,

1978), and the calcium need of the animal. Intestinal calcium binding protein levels are stimulated by dietary calcium depletion (Friedlander et al., 1978) and a reduction in dietary phosphorus (Fox and Care, 1978; Fox et al., 1978).

Calcium absorption seems to be promoted in the intestine in the presence of lactose (Wasserman et al., 1956; Wasserman, 1964), xylose (Pansu et al., 1976) and glucose (Vaughan and Filer, 1960). The action of lactose is believed to be due to its hydrolysis to glucose at such a rate as to make glucose available at the site and time of calcium absorption. However, the absence of lactose does not appear to be an impediment for the absorption of calcium from the many studies available using lactose-free diets.

Wasserman et al (1956) found enhanced absorption of Ca^{45} when administered along with lysine to starved rats. The effects are presumed to be on absorption as they were not found with intraperitoneal administration of Ca^{45}. Similar effects were found for other amino acids such as L-arginine and L-tryptophan. The effects of lysine supplementation to a basal diet, however, seem to depend on the adequacy of the diet with regard to lysine. Beneficial effects were observed when a diet lacking in lysine was supplemented with the same (Likins et al., 1957) but not when

a casein diet was supplemented (Raven et al., 1960; Senior et al., 1978). Similar observations have been made in this laboratory (Prasanna Kumari, 1966; Saraswathi, 1966; Upadhyay, 1974).

Studies have shown that the efficiency with which calcium is absorbed from oxalate rich foods is not directly linked to the amount of oxalate. Perhaps, they vary in the proportion of bound and free oxalate because of differences in their chelation with calcium to form insoluble and non-absorbable salts of calcium.

Of the approximate amount of 670g phosphorus, in the body of man, about 85 to 95% are present in bone. The plasma concentration of phosphorus varies in the range 2.5 to 4.5 mg per dl and its level is mainly regulated by renal excretion.

Hypocalcemic tetany in children has been attributed to phosphorus rich cow milk with which they are fed (Krook, 1968). An excess of dietary phosphorus relative to calcium seems to have a stimulatory effect on bone resorption in man (Bell et al., 1977).

High phosphate diets induce hyperparathyroidism ultimately causing bone resorption (Krook, 1968). A similar observation has been made by Anderson and Draper (1972) in

adult rats. On the other hand, phosphate absorption may be poor in young children because of genetic or other factors resulting in phosphate deficiency (Fraser and Scriver, 1976). Such children respond to phosphate supplementation. (Lapatsanis et al., 1976).

Considerable absorption of phosphorus (and copper) seems to occur distal to the proximal duodenum (Pfeffer et al., 1970). 1,25-$(OH)_2D_3$ also activates the transfer of inorganic phosphate across intestinal ileum and jejunum (Chen et al., 1974; Harrison and Harrison, 1961; Kowarski and Schachter, 1969; Wasserman and Taylor, 1973; Walling, 1977). This process is independent of calcium transport and thus represents an entirely different function of the vitamin (Chen et al., 1974).

Magnesium is not only a component of bone but is essential for its steady state maintenance. Maternal deficiency of magnesium causes a reduction in magnesium in the maternal femur and in the fetuses (Hurley et al., 1976). In weanling rats, chronic suboptimal intake of magnesium does not result in overt signs of magnesium deficiency, but reduced levels of Mg are found in carcass, plasma and tibia associated with decreased bone strength (Heroux et al., 1975).

Manganese deficiency leads to a skeletal defect called perosis in chickens (Wilgus et al., 1936). Perosis

is characterised by enlargement and malformation of the tibio-tarsal joint, twisting and bending of the tibia, thickening and shortening of the long bones and slipping of the gastrocnemius tendon from its condyles. Manganese has also been found to prevent skeletal abnormalities in other species of animals such as the rat (Amdur et al., 1945), guinea pig (Everson et al., 1959), swine (Plumlee et al., 1956) and cattle (Rojads et al., 1965). Manganese does not seem to play a role in the calcification process (Caskey et al., 1939; Parker et al., 1955), but is found to be important in chondrogenesis (Wolbach and Hegsted, 1953). Leach et al., (1969) and Tsai and Emerson (1967) have reported a severe reduction in cartilage mucopolysaccharide content associated with manganese deficiency.

The effect of manganese on mucopolysaccharide synthesis seems to be responsible for congenital ataxia arising from a deficiency of this mineral (Norris and Caskey, 1939; Hill et al., 1950; Hurley et al., 1958).

Skeletal abnormalities are also reported in copper deficient animals (Hunt et al., 1966; Lahey et al., 1952; Teague and Carpenter, 1951). The histological changes observed include thinned cortices, broadened epiphyseal cartilage, and a low level of osteoblastic activity (Follis et al., 1955). Copper is involved in connective tissue and bone collagen synthesis (O'Dell et al., 1961; Rucker et al.,

1969). Some of the changes observed in copper deficiency may be because of defective cross-linking of collagen as copper is essential for lysyl oxidase which effects the hydroxylation of lysine.

Fluorine accumulates with age in human bones, even when intakes from both food and water are low. Excessive accumulation results in osseous lesions. The newly formed fluorotic bone is histologically similar to normal bone; but it is less well calcified and contains wide areas of osteoid; but no areas of hypermineralization occurs in any part of fluorotic bone.

Fluoride seems to increase the stability of bone mineral by increasing its resistance to the action of resorbing cells (Haddad et al., 1970) though it does not prevent the formation of osteoid in vitro (Goldhaber,1967). Fluoride appears to exert its effect on bone calcium metabolism predominantly via a reduction in mineral solubility (Messer et al., 1973). When fluoride is given therapeutically to patients with osteoporosis, an increase in new bone tissue is seen (Jowsey et al., 1968). This new tissue will be incompletely mineralized if not accompanied by generous intakes of vitamin A and calcium (Jowsey et al., 1968; Merz et al., 1970).

Silicon has been reported to be localized in active growth areas of bones and to be essential in the early stage

of bone calcification (Carlisle, 1970, 1971, 1974). In chicks silicon deficiency is associated with thinner cortices and reduced circumference of leg bones, increased proneness of femur and tibia fractures, flatter cranial bones and highly flexible beaks (Carlisle, 1972 a,b) and the skull bones with less trabeculae and calcification and a reduced collagen content (Carlisle, 1980). An impairment of mucopolysaccharide synthesis appears to be responsible for these bone abnormalities (Schwarz and Milne, 1972). In rats, hexosamine is significantly reduced in the metatarsus and tibial epiphyses and metaphysis. It is assumed that silicon is essential for linking either portions of the same polysaccharides to each other, or acid mucopolysaccharides to protein (Schwarz, 1973, 1974).

The role of 1,25 $(OH)_2D_3$ in bone metabolism has already been referred to. The regulatory mechanism for its synthesis is related to the variation in the activity of intramitochondrial 25-hydroxycholecalciferol-1-hydroxylase.

The other prominent metabolite, 24,25-dihydroxycholecalciferol (24,25-$(OH)_2D_3$) seems to be produced when the formation of 1,25-$(OH)_2D_3$) is depressed (Omdahl and DeLuca, 1973), suggesting a reciprocal regulation in the production of the two (Fraser, 1977). As mentioned earlier the production of 1,25-$(OH)_2D_3$ is promoted by PTH (Garabedian

et al., 1972; Fraser and Kodicek, 1973) and is inhibited by calcitonin (Rasmussen et al., 1972).

Vitamin D and its hydroxylated metabolites are transported in plasma bound to a specific binding protein, called the vitamin D binding protein (DBP) (Kawakami et al., 1978; Bouillon et al., 1978). $1,25\text{-}(OH)_2D_3$ and $25\text{-}(OH)D_3$ are bound to different proteins localized in the rat intestinal cytosol (Kream and DeLuca, 1977).

Vitamin D facilitates bone mineralization by promoting the availability of calcium and phosphorus through influencing their absorption in the intestine (Zerwekh, 1979). Indirect evidences suggest a role for vitamin D in bone formation through mechanisms other than its effect on calcium absorption in the intestine. $24,25\text{-}(OH)_2D_3$ is believed to stimulate the activity of osteoid osteocytes (Rasmussen and Bordier, 1978). Also vitamin D metabolites seem to exert a directive effect on the process of mineralization.

$1,25\text{-}(OH)_2D_3$ induces bone resorption in vivo (Tanaka and DeLuca, 1971) and in vitro (Raisz et al., 1972) leading to mineral release, degradation of organic matrix and unique metabolic changes correlated with bone resorption, including decreased citrate decarboxylation and increased lactate production (Wong et al., 1977). Such resorption is essential for bone growth and renewal.

Administration of vitamin D to a vitamin D deficient animal stimulates bone formation (Baylink et al., 1970), and in man, leads to a more gradual but significant increase in the extent of the osteoblastic bone formation surface (Rasmussen and Bordier, 1974). PTH has no effect on bone in the absence of vitamin D as the previously formed mineral layer is covered by osteoid tissue. A significant increase occurs in both trabecular and cortical bone mass of the mature skeleton after administration of low doses of $1,25\text{-}(OH)_2D_3$ in rats, the higher doses being found toxic, (Larsson et al., 1977). This biphasic effect may be due to the stimulatory effect of the hormone on both osteoblasts and osteoclasts, the net result being decided by the proportions of these two cells which in turn depend on the dose of vitamin D.

Vitamin D mobilizes calcium from the bone into the extracellular fluid in the presence of parathyroid hormone (Rasmussen et al., 1963). Weisbrode et al. (1978) report that $1,25\text{-}(OH)_2D_3$ is able to elevate serum calcium independent of dietary calcium, PTH and calcitonin primarily by inducing osteoclastic activity with minimal dependence on either osteocytic osteolysis or the activation of osteocyte-osteoclast calcium pump.

In rickets and osteomalacia, an increase is found in the osteoid because the rate of bone mineralization is not

commensurate with that of osteoid formation. This situation arises from a lack of vitamin D essential for calcium mobilisation from intestine and bone renewal. Simple vitamin D deficiency responds to vitamin D, unlike the vitamin D dependency rickets, related to a specific deficiency of 1,25-hydroxycholecalciferol hydroxylase, which has to be treated with $1,25(OH)_2D_3$ (Reade *et al.*, 1975). A direct correlation appears to exist between the plasma $25(OH)D_3$ concentration and the extent of the mineralization front, especially in patients with phenobarbitol induced osteomalacia, in whom even with normal levels of $1,25(OH)_2D_3$, a rise in the plasma concentration of $25(OH)D_3$ restored normal mineralization. This metabolite increases the renal tubular reabsorption of phosphate and possibly of calcium (Rasmussen and Bordier, 1978).

The bones of the rachitic rat show a decrease in the contents of ash, phospholipid and hexosamine and an increase in hydroxyproline associated with a decrease in whole blood ATP and phosphorus. An injection of either ATP or inorganic phosphates in this condition increases serum phosphorus, blood ATP and metaphyseal ash, suggesting an alteration in ATP production and phosphate availability in rickets (Hong and Cruess, 1978).

The abnormal accumulation of lipids in the cartilage cells of the rachitic epiphysis may also interfere with

calcium transport (Carson et al., 1978).

Hypervitaminosis D in the rat increases the intestinal absorption of calcium and phosphorus leading to a higher concentration of these in blood and to an excess of calcified bone in metaphysis. The periosteal apposition of normal bone is slightly increased whereas in combined hypervitaminosis A and D, the peripheral apposition rate exceeded that induced by vitamin A or D alone and the new bone formed was very fragile (Belanger and Clark, 1967).

Vitamin A influences the position and activity of osteoblasts and osteoclasts and thereby regulates the shape of bone. In general, vitamin D deficiency promotes excessive osteoclasis on bone surfaces adjacent to the marrow, and osteoblastosis, on non-marrow surfaces whereas after excessive doses the reverse is found (Stewart, 1975).

In the vitamin A-deficient dog, deposition of bone occurs at sites where neither bone resorption nor bone cell activity was evident (Mellanby, 1947). Similar observations were made by Hayes and Cousins (1970) who also observed an increase in osteoblasts. Hypertrophy of the periosteal portions of the otic capsules was found in rats resulting in the narrowing of the internal auditory canal due to excess bone.

The abnormal bone in growing animals subjected to hypovitaminosis A is attributed to altered endochondral growth, faulty appositional growth, and interference with normal resorption and remodelling (Gallina et al., 1970).

In the vitamin A-deficient rat, calcium mobilisation from bone and its level in serum are normal (Zile et al., 1973). A decrease in the amount of calcium excreted in urine suggests an increase in calcium retention (Zile et al.,1972).

Vitamin A in excess inhibits cell proliferation and affects ribonucleic acid and protein synthesis to varying degrees (Vasan and Lash, 1975). But moderate amounts are required for the differentiation of epithelial cells into mucus secreting cells and mesenchymal cells into the "blast" stage (Hayes, 1969; Boren et al., 1974).

Vitamin A normally plays an important function in glycoprotein synthesis (DeLuca et al., 1970; 1975; Bonnani et al., 1973) and is possibly involved in the maintenance of certain cell surface glycoproteins and stimulates glycosylation (Hassell et al., 1975, 1978; Bonnani et al.,1973; Pennypacker et al., 1978).

The function of vitamin A in the synthesis of mucopolysaccharides which form the ground substance in bone matrix and which are involved in the calcification process (Bowness, 1968; Bowness and Jacob, 1968; Cuervo et al., 1973) has

been inferred to be either in the polymerization of the uridine nucleotides or the activation and/or transfer of sulphate to the polymer.

The role of ascorbic acid in the hydroxylation of prolyl and lysyl groups has been already referred to. In fibroblasts cultured in an ascorbic acid deficient medium, the amount of protein synthesized is 65% and hydroxyproline 7% of control values suggesting a drastic impairment of hydroxylation of proline. The proportion of collagen was, however, not changed, suggesting that in the absence of ascorbic acid, significant quantities of underhydroxylated collagen are produced (Schiltz et al., 1977).

No specific changes due to deficiencies of members of the vitamin B complex have been reported in the bones of animals. But it is probable that other effects brought about by vitamin B deficiencies, particularly overall growth retardation may mask any specific effects.

Thus, normal skeletal development presupposes the availability of several nutrients including calcium and phosphorus and vitamins D, A and C and the adequate availability of food energy (Dickerson and McCance, 1961; Pratt and McCance, 1965; Widdowson and McCance, 1960; Tonge and McCance, 1965; Upadhyay, 1974; Rajalakshmi and Dave, 1977) and protein (Frandsen et al., 1954; LeRoith and

and Pimstone, 1973; Jha et al., 1968; Stewart and Platt, 1961; El-Maraghi et al., 1965; Shenolikar and Rao, 1968; Florescy, 1974; Radhakrishnan, 1966; Diorio et al., 1973; Fleagle et al., 1975; Shrader and Zeman, 1973; Upadhyay, 1974; Dave, 1976).

← needs a heading here

The diets of the poor in many developing countries are either inadequate or marginally adequate with regard to many of these nutrients. Malnutrition and undernutrition start well before birth because of a low plane of maternal nutrition and persist to varying degrees after birth, being most marked in the postweaning period because of the transition from breast milk to poor weaning diets, and during adolescence, because of the inadequacy of the diet to meet the increased nutritional demands of rapid growth and development during this period. All this adds up to a picture of fetal undernutrition evidenced by a high prevalence of infants who are small for gestational age, some prevalence of growth retardation in the neonatal period, appreciable prevalence of clinical malnutrition in the form of marasmus and kwashiorkor in the postweaning period and continued growth retardation, particularly during adolescence, culminating in stunted physical stature as adults.

Malnutrition at any stage during development, has been found to affect skeletal development. Several investigators have found skeletal retardation during the fetal period due to maternal malnutrition (Scott and Usher, 1964; Pryse-Davis et al., 1974; Roord et al., 1978). Philip (1974) observed newborn infants with low birth weight showing retardation of both membrane and endochondral bone formation. The degree of skeletal growth retardation in utero is considered to be an index of the duration and severity of an adverse fetal environment (Miller and Hassanein, 1971; Roord et al., 1978).

Skeletal retardation may continue or become manifest in infancy and early childhood and persist through adolescence as can be seen from the data given in Table-5, the degree of retardation varying with the severity of malnutrition.

Malnutrition during early childhood includes a range of conditions ~~ranging from~~ including kwashiorkor to marasmus. In kwashiorkor, the bones present a general appearance of poor calcification with the long bones reduced in length and poorly developed in shape and size (Dean, 1965; Jones and Dean, 1959). The ratio of metacarpal width to length is higher than for normal children and with significantly less trabecular bone (Adams and Berridge, 1969).

Table 5 : Preliminary studies on the skeletal status of children and adolescents in different segments of the Indian Population (S.S. Shah unpublished)

Age (years)	Mean Developmental Quotient					
	Rural poor		Urban poor		Kerala poor	
	Male	Female	Male	Female	Male	Female
0 - 2	71	73	103	102	33	73
2 - 4	66	67	79	77	68	68
4 - 6	66	70	82	93	64	64
6 - 8	64	85	84	91	72	82
8 - 10	69	86	91	99	69	82
10 - 12	79	90	95	110	67	107
12 - 14	90	96	96	102	89	98
14 - 16	90	95	96	103	89	110
16 - 18	94	99	101	101	89	95

Marasmus is characterised by dehydration, stunting and wasting to the point of initial absence of subcutaneous fat. However, skin and hair changes are relatively rare. A poor skeletal status is found even at comparable bone ages, the marasmic child presenting features such as a marked deficiency in compact bone suggesting both a simple failure to gain more bone as well as actual bone loss by resorption (Behar et al., 1969; Maniar et al., 1974).

Because of the association of skeletal retardation with malnutrition in children, attempts have been made to study the effects of nutritional stress of different types at different ages on the development of the bone. Most of the studies pertain to undernutrition or protein deficiency which are the most common deficiency syndromes prevalent in children but, a few studies have been carried out on the effects of vitamins and mineral deficiencies.

In experimental animal studies undernutrition is induced by manipulating the diet in one of the several ways. During the prenatal and preweaning periods, the mother may be fed a restricted amount of food (Chow and Lee, 1964) or a low protein diet (Barnes et al., 1966). During the latter period undernutrition can also be induced by increasing the litter size (Widdowson and McCance, 1960) or allowing the pups restricted access to the mother (Eayrs and Harn, 1955).

In the rat a dietary level of protein below 5% is considered insufficient for ovulation and reproduction. An adequate supply of protein is specially needed during the earliest stages of placental and fetal development (Nelson and Evans, 1953). Severe maternal protein deficiency leads to resorption of the fetus, reduction in litter size and low weight at birth of the progeny.

Rats born of mothers restricted in food intake and rats underfed before weaning showed permanent growth impairment and reduction in skeletal dimensions (McCance and Widdowson, 1962; Toes and Lee, 1975). Although the malnourished animals showed no growth, still they continued in skeletal elongation, but at low rates (Fleagle et al., 1975).

The level of undernutrition affects both the rate of growth and the ossification of bones. The growth of secondary centres of ossification, and thus the maturation of the bone are more closely related to the length of the bone than to either chronological age or body weight (Dickerson and Widdowson, 1960).

In rats protein deficiency during the fetal and postnatal periods caused a significant delay in the time of appearance of ossification centres in the fetuses and pups (Shrader and Zeman, 1973).

In young rats subjected to protein deficiency, a reduced calcium accretion leads to an under-mineralized bone (LeRoith and Pimstone, 1973; Upadhyay, 1974; Dave, 1976). A similar reduction in bone appositional growth rate is also observed in monkeys (Jha et al., 1968).

The net biochemical effect of a low protein diet or a poor quality protein diet is a fall in their bone ash as observed in rats, dogs, chickens and lambs (Floresey, 1974).

Young rats fed a low-protein diet or normal diet in restricted amounts showed histological changes of the same type, but they were more severe in the animals given low-protein diets. The bones of these animals showed a decrease in the width of the epiphyseal plate, reduction in the number and size of cartilage cells and a relative increase in the amount of cartilaginous substance, with fewer and coarser bony trabeculae (Frandsen et al., 1954).

Variations in the calcium:collagen ratio, collagen: total N ratio and in the percentages of water, total N, collagen, calcium and phosphorus are some of the common features of undernutrition (Dickerson and McCance, 1961). Also there is a reduction in the density of the bones of the undernourished animals (Reddy et al., 1972).

Structural changes in bone are also observed in undernutrition. Adult pigs subjected to severe undernutrition showed significant structural changes in the jaws and teeth. The shape and anatomical relationships of the jaws were altered and the development of the teeth was delayed (Tonge and McCance, 1965).

The effects of undernutrition on different bones are not uniform. For example, in pig the lower jaw is less affected and is larger than the upper jaw in undernutrition. The height of the condyle of the mandible is also increased more than the length of skull (McCance and Ford, 1961).

Compared to the long bones the mandible appears to complete the period of the critical rapid growth phase earlier. Moreover, the responses of parameters indicative of cell size and function also suggest differences in the sensitivity to malnutrition of several cellular mechanisms (Nakamoto and Miller, 1977).

Due to the different response pattern of growth centres and/or ossification patterns, tibiae and mandibles were found to differ in their response to the nutritional stress, when the undernourished pups were given a protein calorie supplement (Di Orio et al., 1973).

The effects of osteomalacia are found to be most in the spine, ribs, shoulder girdle, pelvis and lower limbs,

whereas in osteoporosis the bones most prone to fracture are commonly the upper end of the femur or the lower end of the radius (Davidson et al., 1975).

Bone mass and bone activity are known to vary from bone to bone, depending on the function of the bone within the skeleton. Bone formation rate depends both on apposition rate and on the number of bone formation sites. The former does not vary significantly with skeletal locations. The number of bone formation sites and, therefore, the bone formation rate, varies widely in different parts of the skeleton and in any given bone location varying with time (Amprino and Marotti, 1964; Lee et al., 1965; Harris et al., 1968).

Individual bones may vary in their capacity to accumulate minerals, which may depend on local factors. Thus the percentage of total ash in trabecular and cortical parts in different bones are reported to show variation (Gong and Ries, 1970). Hollins et al (1973) observed that the various parts of the skeleton possessed different abilities to concentrate americium and calcium.

Earlier investigations in this department were concerned with the effects of variations in the quantity and quality of dietary protein on the inorganic components of femur (Upadhyay, 1974) and the effects of deficiency of protein,

calorie, calcium, vitamin A and vitamin D on the inorganic and organic components of femur (Dave, 1976). In a preliminary study on the composition of different bones, namely, the calvarium, mandible, femur and ribs and incisor, differences in the level of inorganic components were noted (Iyer, 1978).

In view of the variations in the pattern of maturation and chemical composition and in their response to nutritional stress at different ages in man and experimental animals (Table 6), comparative studies were made on the different bones in the skeletal system.

The bones chosen for the study are the pelvis, femur, tarsus and mandible (Figures 4a to 4d). The choice of these bones was guided by the variability in their structure, pattern of maturation, chemical composition and the possibility of differential response to nutritional stress.

The pelvic girdle is made up of two equal parts- the ossa innominata. In the os innominatus, the bone of the pelvis - ilium, ischium and pubis - are separate in the young, but completely fused in the adult. The growth of the rat has been studied with respect to the growth and histology of this bone by Harrison (1958 a and b) who observes that the pelvic growth in the rat is not subjected to specialized local control, but is regulated by general growth determinants.

Table-6: Response of different bones to nutritional variation

Name of bone	Type of bone	Nutritional stress	Age	Findings	Reference
Rat:					
Mandible and Tibia	Membranous Endochondral	Protein deficiency in maternal diet	Preweaning	- greater restrictive effect on bone growth than on molar and greater restrictive effect on tibia than on mandible in the undernourished	DiOrio et al., (1973)
Mandible and Long bones	Membranous Endochondral	Protein deficiency in maternal diet	Preweaning	- Mandible- Ca/DNA ratio not changed and fewer cells of normal size in the undernourished - Long bones - Ca/DNA ratio reduced and fewer cells of smaller size	Nakamoto and Miller (1977)
Femur, Tibia and Humerus	All Endochondral	Ca deficiency during pregnancy	4-5 months old	- Sensitivity of the bones differed to the nutritional stress; Reduction in ash highest in femur, less in tibia and least in humerus	Rasmussen et al., (1977)
Calvaria, Femur and Humerus	Membranous Endochondral	Nil	at birth	- Calvarial more calcified than the long bones at birth	Zika and Klein (1975)

Table-6 continued.... Response of different bones to nutritional stress

Name of bone	Type of bone	Nutritional stress	Age	Findings	Reference
Hen:					
Ribs, Sternum, Ischium and Pubis, Vertebrae, Fibulae, Metatarsal and Skull	All Endochondral	Low calcium diet	Laying	- greatest proportional loss of mineral in the ribs, sternum, ischium and pubis, cocygeal vertebrae and fibulae	Taylor and Moore (1954)
	Membranous			- Affected least in the skull, metatarsi and toe	

Figure: 4a Pelvis

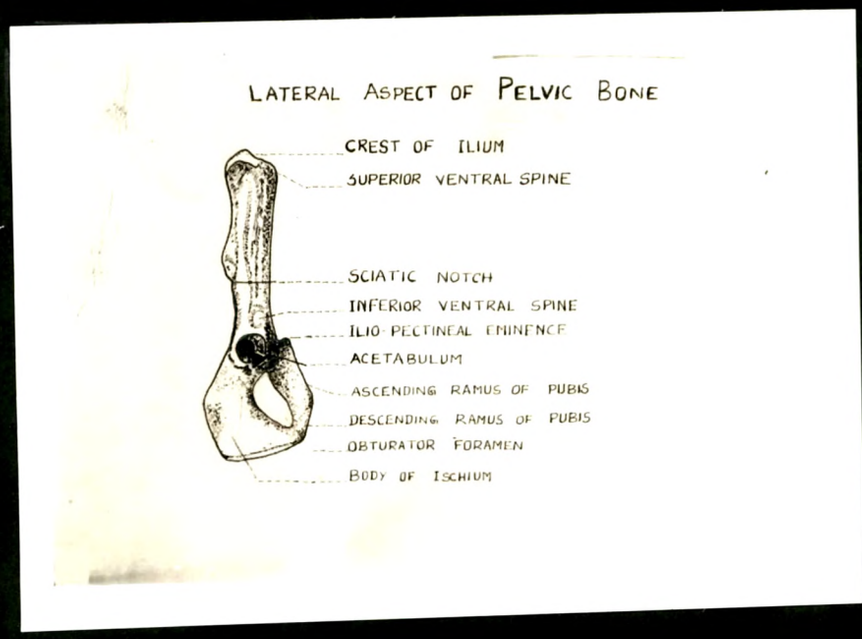
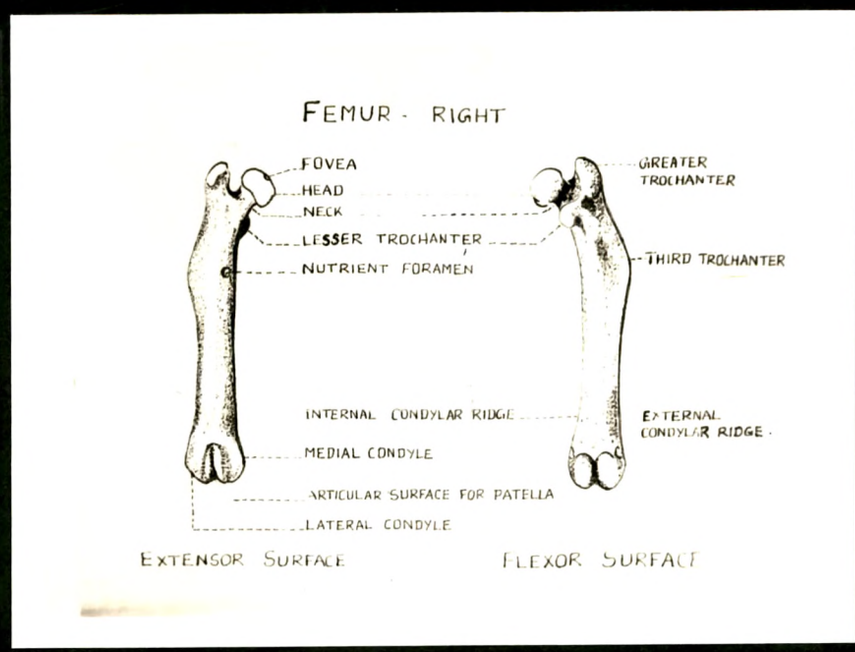


Figure: 4b Femur



The femur has a long shaft. The head is rounded and is attached to the medial side of the proximal end of the shaft by a distinct neck. Near the head there are three trochanters for the attachment of the hip muscles. The distal end of the femur has two rounded condyles on a conspicuous epiphysis. The greater trochanter is near the head and the lesser and third trochanters are below it with the lesser on the inside and the third on the outside.

The tarsus is composed of 8 bones, namely, tibiale, talus, calcaneus, navicular, 1st cuneiform, 2nd cuneiform, 3rd cuneiform and cuboid. The tarsals are arranged in two irregular rows with the navicular between them. The proximal row consists of three bones. The fibulare or calcaneum is very large and is prolonged under the intermedium or talus (or astragalus) to provide attachment for the strong muscles of the calf of the leg. The talus has a cotton-reel-shaped surface for articulation with the tibiofibula. The tibiale is small. The distal row consists of four bones, the three cuneiforms and the cuboid.

In the mandible, the body of the bone is modified to carry the dentition. In the rat this consists of one incisor tooth and three molars. On the outer surface of dentary is the Mental foramen for the exit of a branch of the fifth cranial nerve. At the posterior end of the

Figure: 4-C Tarsus

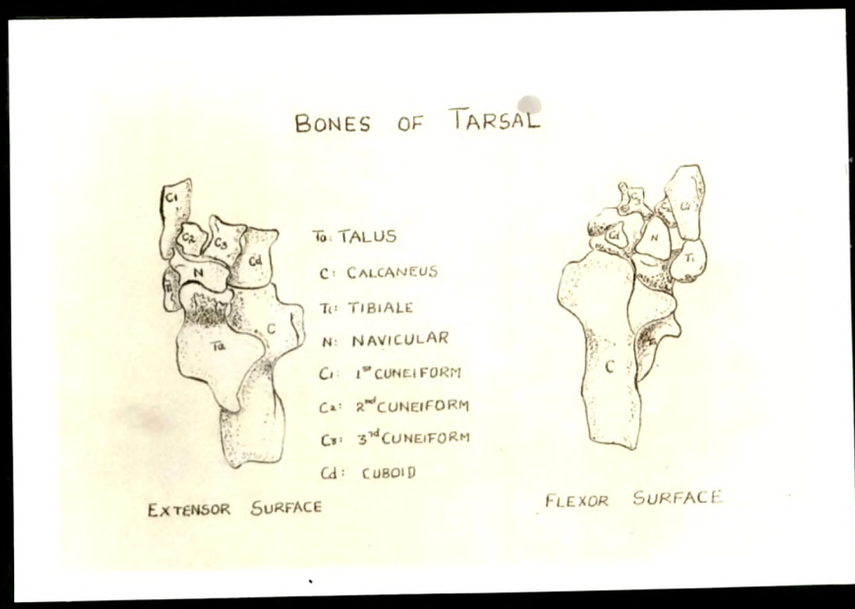
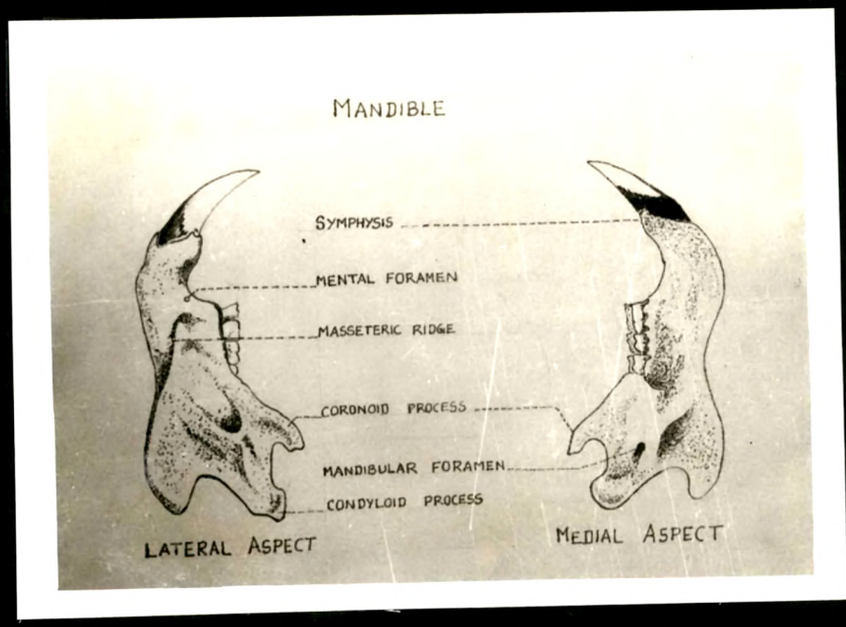


Figure: 4-d Mandible



mandible are present three processes, the coronoid process for muscular attachment to close the jaw, the angular process for muscular attachment to open it and the condyle or articular process which fits into the glenoid cavity on the ventral side of zygomatic arch (Wells, 1964; Rowett, 1960).

The early development and the number of ossification centers of the mandible have been studied in man and in pig. Strong (1925) using cleaned skeletons of albino rats, reported that the rat mandible begins to ossify at 17 days after insemination. Bhaskar (1953) investigated the growth pattern of the mandible in the rat based on a histological analysis of complete serial sections of the albino from 13 days of insemination age till 30 days postnatal. According to him the ossification of the mandible begins at 16 days after insemination.

In the present studies an attempt is made to compare the chemical pattern of development of these four bones from birth to 26 weeks of age. In addition, studies were also made of variations in the response of these bones to nutritional stress, by providing diets deficient in protein, calorie and vitamin A during the preweaning and postweaning periods of growth.