CHAPTER IV

SUMMARY AND CONCLUSION

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The skeletal system is the supporting structure of the body and is also the reservoir of many minerals, especially calcium.

Chemically, bone is composed of an organic matrix and superimposed by a mineral layer. The organic matrix contains mainly collagen along with small but critical quantities of mucopolysaccharides and lipids. The main bone mineral is deposited in the form of calcium phosphate which is amorphous in the first stage of mineralization and transformed into the crystalline form, namely hydroxyapatite crystals. Other minerals such as magnesium, manganese, silicon, zinc, copper and fluoride are also present in bone in small quantities.

For the normal formation of bone an adequate supply of nutrients including food energy, protein and vitamins such as A, D and C is found to be essential. In this laboratory and elsewhere, a few studies have been carried out on the effects of a deficiency of these nutrients during different periods of growth on the chemical composition of bones in the rat.

The position and function of the individual bones in the skeletal system vary and hence differences may be expected in the rate of their general growth, chemical maturation, composition and response to nutritional stress.

It was therefore considered worthwhile to study the developmental pattern of selected bones. The bones chosen for

the studies are the mandible, femur, pelvis and tarsus.

Pelvic and femur bones were chosen as representatives of long bones. Femur is a typical long bone whereas pelvic bone is a compound bone in the sense it is composed of ilium, ischium and pubis. Mandible differs from other bones in its early mode of development as a membraneous (flat) bone. So it was included in the studies as a representative of membrane bone. Tarsus is composed of 8 short bones which are separable in early stages but are fused in later stages. It was taken to represent the short bone.

Since the time of maturation and the period of rapid growth were found to vary in these bones, studies were further carried out on the effects of the deficiency of food energy, protein and vitamin A on these bones during the preweaning and postweaning periods.

These studies were concerned with morphological features and chemical composition. Apart from estimations of body weight and bone weight and size, bone was analysed for total ash content, calcium, phosphorus, collagen (hydroxyproline), total mucopolysaccharide (MPS) (hexosamine) and chondroitin sulphate (uronic acid).

Studies carried out on the developmental pattern of these bones showed that the pattern of growth with regard to bone as judged by weights and lengths was essentially similar to that of whole body growth. The rate of body growth was rapid during the initial stages, but declined progressively

with age. But the percentage of increments in the weights of the bones were higher during the initial stages than those in body weight, a phenomenon consistent with the expectation that skeletal growth takes precedence over that of many other tissues.

The bone values corresponding to 25% and 50% of the 26 week values were reached much earlier in the case of bone length and somewhat later in the case of other parameters. These values were reached earlier in the tarsus and mandible in that order than in the femur and pelvis. The percentage of increments were comparable in the latter two.

The maturation and chemical composition of bones may vary depending on their structure, function and ontogenetic priority. But in general the maturation of bone is associated with an increase in the concentration of ash, calcium, phosphorus and collagen and a decrease in moisture and MPS. The concentration of total lipids remains more or less the same.

This general trend is reported by other investigators (Widdowson and McCance, 1960; Dickerson, 1962; Dave, 1976). The rate of these changes however, differed in different bones. The decline in moisture was gradual in the pelvis and femur whereas it was faster in the tarsus and mandible. Also the decline was faster during the preweaning period than in the postweaning period. These changes and their differential rates were associated with complementary changes in bone mineral.

In the early stages of development the Ca:P ratio was found to be somewhat lower than in mature bone. This may be so because the immature bone contains more of amorphous mineral as well as imperfect crystals of hydroxyapatite of calcium phosphate, deficient in calcium (Eanes and Posner, 1970).

The A:R ratio (Ash/Residue) was found to increase steadily during the course of development. Mandible, the most highly mineralized bone among those studied was found to have the highest A:R ratio at any age.

The rate of percentage increments in collagen increased considerably in the first two weeks and thereafter gradually declined in all the bones studied. That is the percentage increment in collagen was greater before weaning than in the postweaning period. Of these 4 bones tarsus had the highest percentage increment in the first two weeks after birth which seems to be its period of rapid growth.

The values for femur collagen in this study were compared with those for femur N in previous studies (Dave, 1976) in rats of comparable age, body weight, bone weight and bone composition. The total N as per cent of fresh weight appeared to remain unchanged with age in previous studies, whereas in the present study the concentration of collagen (hydroxyproline) was found to increase with age. This may be due to the presence of appreciable amounts of non-collagenous proteins in the early

stages, which disappear progressively with the maturation of bone.

Both total MPS and chondroitin sulphate decline with age, the rate being rapid in chondroitin sulphate in the later stages. Both were present in high concentrations in the tarsus at birth indicating its cartilaginous state at birth.

Although a few studies have been made on the impact of undernutrition on the chemical components of bones, no studies appear to have been made in a comprehensive way on the comparative effects of different bones which differ appreciably in chemical composition and pattern of maturation with different degrees of undernutrition.

Different degrees of undernutrition were induced during the preweaning period either by feeding the mother a low protein diet after partus ($G^{+}L^{-}$) or by increasing the litter size from 8 to 16 and feeding the mother a normal diet ad libitum (LL).

The deficits in body weight and the various parameters studied in bone in the different experiments are given in Table 42.

The deficits in body weight, bone weight and length were found to be greater in $G^{+}L^{-}$ group than in LL group. They were found to be the least in the mandible and increased progressively in the order mandible, tarsus, femur and pelvis. The deficits in linear measurements in all the bones were much

Table 42: Deficits in body weight and bone parameters in the different experiments.

	Deficit as per cent control values							
	Food energy and protein deficiency				Vitamin A deficiency			
	Preweaning		Postweaning		Preweaning		Postweaning	
	LL	G^+L^-	HP-R	LP	LL	Vit.A^-	Weight matched	Vit.A^-
Body weight	44	69	65	65	44	29	65	64
Fresh weight:								
Mandible	35	49	53	50	32	14	22	12
Femur	48	72	58	59	49	32	39	31
Pelvis	50	73	64	63	51	28	48	33
Tarsus	32	59	33	30	36	26	17	23
Dry weight:								
Mandible	35	51	54	53	33	15	23	17
Femur	48	73	60	64	53	32	43	42
Pelvis	53	75	66	65	56	30	49	45
Tarsus	38	65	35	34	48	33	20	26
Fat-free dry weight:								
Femur	51	74	66	65	53	33	45	49
Pelvis	55	77	70	66	58	32	52	52
Tarsus	42	69	44	42	50	36	23	30
Bone length:								
Mandible	13	26	10	12	14	11	12	8
Femur	15	30	24	26	15	16	13	16
Pelvis	19	34	28	28	18	16	16	13
Tarsus	15	25	3	8	16	13	6	0

Table 42 continued

	Deficit as per cent control values							
	Food energy and protein deficiency				Vitamin A deficiency			
	Preweaning		Postweaning		Preweaning		Postweaning	
	LL	G^+L^-	HP-R	LP	LL	Vit.A^-	Weight matched	Vit.A^-
Bone width:								
Mandible	13	19	16	20	11	11	17	10
Femur	11	28	11	12	11	9	4	3
Pelvis	21	37	26	30	19	15	17	13
Chemical composition:								
Ash:								
Mandible	42	55	58	55	43	20	21	26
Femur	51	78	73	69	54	36	52	61
Pelvis	60	80	76	70	61	36	56	62
Tarsus	50	74	54	47	56	42	26	45
Collagen:								
Mandible	33	49	53	52	35	15	30	7
Femur	40	69	67	63	39	33	46	27
Pelvis	41	73	70	65	42	25	52	29
Tarsus	33	61	49	43	33	23	18	6
Total MPS:								
Mandible	29	48	37	37	21	11	23	0
Femur	20	60	30	30	19	20	23	0
Pelvis	21	63	40	40	19	27	30	0
Tarsus	21	42	3	8	32	21	8	0

less than those in either body weight or bone weight, an observation consistent with the smaller impact of malnutrition on linear measurements.

When expressed as mg/g of body weight, the weights of mandible and tarsus in relation to body weight from the undernourished group were higher than in controls suggesting that the growth of mandible and tarsus is protected to some extent inspite of overall growth retardation. On the other hand, the weights of femur and pelvis relative to body weight were reduced suggesting that the long bones are more susceptible to the effects of the overall growth retardation.

Among the constituents of the bone, moisture content was greater in all the bones in the undernourished groups, suggesting delayed maturation. The least difference was found in the mandible, indicating its advanced stage of calcification as compared to other bones and decreased vulnerability to postnatal nutritional stress.

The lipid content of the bones was greater in the undernourished groups, the differences being less in the LL group.

The decrease in ash is also reflected in the decrease in A:R ratio of the undernourished animals.

In the present studies the bones of undernourished animals were found to have a lower concentration of collagen (determined as hydroxyproline) whereas in previous studies

concentration of total nitrogen was increased. These observations taken together suggest either an increased proportion of underhydroxylated collagen and/or an increased amount of non-collagenous protein as in immature bone.

A higher concentration of total MPS and chondroitin sulphate was found in the undernourished animals.

All the bones thus showed morphological and chemical changes suggestive of delayed maturation with undernutrition. The mandible and tarsus showed a comparative pattern of changes which were, in general, of a smaller degree than those in femur and pelvis in spite of appreciable differences in chemical composition. This similarity is probably due to the ontogenetic pattern of the two bones, the mandible undergoing its most rapid phase of maturation before birth, and tarsus soon after birth.

In the studies on the effects of nutritional stress during the postweaning period although the bone weights were found to be affected with postweaning undernutrition, weights in relation to body weight were more than in controls.

The mandible is one of the few bones to ossify in late gestation in the rat and it undergoes membranous ossification. Mandible shows a greater biochemical maturity at birth as compared to other bones. But after birth, the growth of the mandible slows down compared to the long bones, namely pelvis and femur, which are calcified less at birth and grow faster

after birth. In the rat the appearance of ossification centres in the tarsus is postnatal. The rate of growth and mineralization of tarsus is very high in the first two postnatal weeks and slowed down in the postweaning period. Thus, in the postweaning period the pelvis and femur have a higher rate of growth when compared to the mandible and tarsus.

During the postweaning period the pelvis was found to have the highest rate of growth and the tarsus the least. Deficits varied in different bones but were found to be related to the rate of growth during the period under study on the basis of percentage increments in the control between 3 and 9 weeks of age. The same pattern applied to bone length.

The concentrations of moisture, total lipid and total MPS were greater in the experimental groups whereas that of ash was less. No significant differences were found with regard to hydroxyproline. The difference in moisture was greater in the protein deficient group whereas that in lipid as well as ash was greater in the calorie-deficient group.

The deficits in ash in the undernourished animals were found to be greater in femur and pelvis than in the mandible and tarsus.

Although the deficits in the body weights of the rats subjected to protein deficiency during the preweaning (69%)

and postweaning (65%) periods are comparable, the deficits in bone weights and ash weights of the femur, pelvis and tarsus were less with postweaning stress whereas that in the mandible remained the same (Table 42). The deficit in length was less in the postweaning period, especially in mandible and tarsus. This observation, therefore, suggests that the preweaning period is the critical period for the growth of the bones.

Vitamin A is known to play a role in the formation and renewal of cartilage and bone and skeletal changes are seen in both hypo- and hypervitaminosis A. Although a number of studies have shown that various bone components are affected when vitamin A deficiency is imposed on growing animals, due to variations in their experimental designs and experimental animals the results are not in agreement.

The present studies are concerned with the effects on selected bones of vitamin A deficiency during the suckling period and postweaning period in rats.

In the preweaning vitamin A deficiency, as in the case of the undernourished rats, bone weight and length, ash, calcium, phosphorus, collagen, total MPS and chondroitin sulphate were reduced.

The deficits in bone weights of the vitamin A deficient rats were less than those in the LL group. This could be due to differences in the degree of growth retardation.

As in undernutrition, the length of the bones was less affected than bone weight and body weight, the length of the pelvis and femur being affected relatively more than the tarsus and mandible.

Ash content and concentration were found to be reduced in vitamin A deficiency. However, in the mandible, concentration of ash was affected in undernutrition but not in vitamin A deficiency. Although the amounts of collagen were significantly decreased in the bones of the vitamin A deficient rats, its concentration was unaffected.

Although the content of total MPS was significantly reduced in the bones of the vitamin A deficient rats, its concentration was not affected. In the case of chondroitin sulphate both content and concentration, and, therefore, proportion of chondroitin sulphate to total mucopolysaccharide were significantly decreased in all the bones except the mandible.

Although the deficits with reference to some bone parameters were apparently less affected in vitamin A deficiency than in undernutrition, the former was associated with abnormalities in the shape of the bone.

In the rats fed the vitamin A deficient diet after weaning, deficiency symptoms such as corneal xerophthalmia and hind limb paralysis appeared with 7-8 weeks of treatment. Liver vitamin A levels were found to be markedly reduced at

this point.

As in other studies, the body weights in the experimental groups which were 35% of the controls were affected more than the weight and size of the bones.

In the case of linear measurements, the deficits were less in the vitamin A deficient animals except for femur length which was less than in the weight matched animals. This may be due to impaired bone renewal and the resulting abnormalities.

Higher concentrations of moisture as compared to controls were found in both groups but the differences fell short of significance in mandible and tarsus.

Similarly higher concentrations of total lipids were found in both the experimental groups, the differences being greater in the vitamin A deficient group, especially in femur and pelvis. These observations are in agreement with those of Mellanby (1950).

The content and concentration of ash were also significantly less in both the test groups, the deficits being greater in the vitamin A deficient group. The greater deficit in the vitamin A deficient group as compared to the undernourished group is probably because of decreased mineral accretion.

The significantly smaller amounts of Ca and P in both the test groups as compared to the controls and in the vitamin A

deficient group as compared to the weight matched controls are consistent with the pattern found with reference to ash. The Ca:P ratios were significantly less in the vitamin A deficient rats.

The A:R ratio was significantly less in the bones of the two test groups as compared to controls, the only exception being the mandible in the undernourished group.

Collagen content was affected in both groups but less so in vitamin A deficiency than in undernutrition. This is consistent with the impact of vitamin A deficiency on bone remodelling, resulting in relatively larger bone size and its greater osteoid content.

In spite of the bone size being smaller the amount of total mucopolysaccharide in the two test groups were not significantly different from the controls except in the femur and pelvis of the weight matched animals. The concentration of the total MPS was increased in both groups and significantly so in the case of vitamin A deficiency. The only exception to this statement was the tarsus with reference to which the difference was not significant.

From the degree of deficits in ash and A:R ratio, as well as other features it is observed that in vitamin A deficiency the least affected bone is the mandible, the impact increasing in the order tarsus, femur and pelvis.

In conclusion, it is found that vitamin A deficiency during the postweaning period leads to a higher concentration of moisture, total lipid and MPS, to a reduction in the concentration of ash and no variations in the concentration of collagen.

From the results of the vitamin A deficiency experiments during these two periods, it was noted that the deficit in the body weight was more in the postweaning period than in the preweaning period. However, the deficit in the weight of femur, pelvis and tarsus was comparable in the two groups whereas it was less in the mandible in the postweaning period. The deficit in ash content during the two periods was comparable in the mandible and tarsus, whereas it was more in the pelvis and femur with postweaning vitamin A deficiency. Thus the long bones which have a relatively more rapid growth as compared to other bones such as the mandible and tarsus in the postweaning period were affected to a greater extent with vitamin A deficiency during this period.

In the preweaning undernutrition, deficits in bone weights, ash and collagen were the highest in pelvis followed by the other bones in the order femur, tarsus and mandible. In the case of bone length and total MPS, the order of deficit was pelvis, femur, mandible and tarsus, with the tarsus having the least deficit.

In the postweaning period the deficits in all these bone parameters followed the same pattern, with pelvis having the highest value and followed by femur, mandible and tarsus.

Although the deficits in the body weights of the rats subjected to protein deficiency during the preweaning (69%) and postweaning (65%) periods are comparable, the deficits in bone weights and the contents of ash, collagen and total MPS of the femur, pelvis and tarsus were less with the postweaning nutritional stress, whereas that in the mandible remained the same. The deficit in length was less in the postweaning period, especially in the mandible and tarsus. This observation suggests that the preweaning period is the critical period for the growth of the bones.

In vitamin A deficiency, with reference to the deficits in dry and fat-free dry weights and ash, the pelvis and femur seemed to be affected more during the postweaning period than during the preweaning period.

Because of the longer and probably more severe deprivation, growth deficits as judged by body weight were greater in the animals subjected to postweaning (64%) vitamin A deficiency than in the preweaning animals (29%).

In spite of this, the deficits with reference to many bone parameters were comparable in both experiments suggesting that the effects of preweaning deficiency were more pronounced

when the shorter period of treatment and the more moderate nature of the deficiency were considered.

During both the preweaning and postweaning periods, it was observed that the deficits in all the bone parameters were less in vitamin A deficiency than in food energy and protein deficiency. However, the vitamin A deficient animals differed from the protein and calorie restricted animals in having altered bone shape. These observations suggest that protein and calorie are of primary importance in the formation of the basic structure of bone while vitamin A has a specific role in regulating bone metabolism.

Skeletal retardation thus seems to be directly related to the severity of nutritional stress. The level of dietary protein during growth and the relative rate of growth of the body and its parts are important factors in calcium absorption and thus in calcification. A poor absorption of calcium is associated with undernutrition as the synthesis of CaBP is found to be affected with nutritional stress (Kalk and Pimstone, 1974). These effects in turn could be due to poor hormonal regulation of the synthesis of the metabolites of vitamin D_3 or due to the resistance of the gut to the biological effect of the vitamin (Adams et al., 1974). A number of hormones such as thyroxine (Shrader et al., 1977), growth hormone (Atinmo et al., 1976) and corticosteroids (Dearder and Espionosa, 1974) are also affected by under-nutrition.

In vitamin A deficiency calcium mobilisation from bone and its level in serum are normal suggesting no impairment of hormonal regulation (Zile et al., 1973). It may be that vitamin A critically influences the degree of calcification by affecting sulphated mucopolysaccharide metabolism in the osteoblasts. Vitamin A deficiency might affect the membrane permeability and release of enzymes thereby altering the sequence of biochemical processes that characterize calcification. Thus by influencing the activity of osteoblasts and osteoclasts, the formation and renewal of bone may be affected leading to variation in moulding the shape of bone.

The present studies on the effects of nutritional stress on bones indicate differential responses of the various bones studied and this seems to be related to their ontogenetic pattern. In this connection other investigators (Nakamoto and Miller, 1977) have found that responses of parameters (Protein:DNA) indicative of cell size and function in mandible and long bones suggest differences in their sensitivity to malnutrition. Hence it will be of interest to further study histologically and histochemically the pattern of changes in the different bones at defined sites.

Further studies on the developmental pattern of enzymes involved in bone formation and renewal and their behaviour under different nutritional stress may be useful for a better understanding of the chemical changes in bones.