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GENERAL DISCUSSION AND RECAPITULATION

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Thus these studies show that skeletal development is affected not only by the calcium content of the diet but also by protein content and quality as well as by under-nutrition.

The chief points that emerge are that whereas protein deficiency during the postweaning period is associated with an increase in moisture and fat contents, undernutrition is associated with an increase only in fat content.

The data also suggest that a poor protein quality of the diet produces effects similar to those of a low protein diet. This observation is consistent with expectation and has been made with regard to growth, brain enzymes (Rajalakshmi, Pillai and Ramakrishnan, 1969) and carbohydrate metabolism (Bahl and Venkitasubramanian, 1974).

The differential effects of protein deficiency and undernutrition are consistent with similar differential effects on other parameters such as serum proteins (Barnes, Cunnold, Zimmermann, Simmons, MacLeod and Krook, 1966), brain enzymes (Rajalakshmi and Ramakrishnan, 1969a) and psychological performance (Barnes, Moore, Reid and Pond, 1968).

These studies also suggest that the effects of calcium and protein deficiencies on the composition of the femur are

similar (e.g. on the basis of experiments III and VII). This might be because in both cases the maturation of the bone is affected.

The normal maturation of the bone is associated with a decrease in moisture content and an increase in ash, and consequently in A:R ratio (Table 37). This is also brought about by a comparison of the values obtained for the control groups in different experiments in the present studies (Table 38). It would appear then that the increase in moisture and decrease in ash associated with a low protein diet may be due to an interference with this maturation. The femur of the low protein animals resembles that of a younger animal with regard to moisture and ash contents. This is evident when we compare the values for the low protein animals with those for younger animals.

If a low protein diet affects maturation we should expect the effects to be less evident when the older animal is subjected to protein deficiency. We find this indeed to be the case from a comparison of experiments III and IV. (Table 39). The animals in the latter seemed to be less affected than those in the former. However, even in the older animal some changes might occur because of the constant renewal of the bone. It would be worthwhile to study the effects of protein deficiency at different ages.

Table 37 : Composition of femur at different ages*.

	age (weeks)			
	4	12	24	36
fat-free dry weight (mg)	251	616	854	973
ash weight (mg)	122	377	551	642
calcium content of femur (mg)	43.7	140.6	206.7	240.5
A:R ratio	0.95	1.58	1.82	1.94
<u>g per 100 g fresh bone</u>				
moisture	55.1	37.3	31.1	29.6
<u>g per 100 g fat-free dry bone</u>				
ash	48.6	61.2	64.5	66.0
calcium	17.4	22.8	24.2	24.7

* Values taken from Henry and Kon (1953).

Table 38 : The size and composition of the femur at different ages.

experiment	I	*	III	II	IV
age in weeks	3	8	14	20	22
terminal body weight (g)	33	111	203	245	275
<u>Composition of femur</u>					
length (cm)	1.6	3.08	3.1	3.3	3.3
wet weight (mg)	118.5	338	499	557	556
dry weight (mg)	46.8	185	337	389	379
fat-free dry weight (mg)	44.5	173	325	375	365
ash weight (mg)	17.0	89.2	196	228	225
calcium content of femur (mg)	6.2	34.4	73.1	87	88
calcium (mg) per 100 g of body weight	18.3	30.9	36.1	35.6	32.1
A:R ratio	0.62	1.06	1.53	1.54	1.62
<u>g per 100 g fresh bone</u>					
moisture	60.5	45.2	32.5	30.2	31.8
fat	1.9	3.5	2.3	2.4	2.5
ash	14.3	26.4	39.3	40.8	40.5
calcium	5.2	10.2	14.7	15.6	15.9

* The values for 8 weeks are taken from Ramachandran, (1968).
The remaining values are from the present investigations.

Table 39 : Comparative effects of protein deficiency at different ages on the composition of femur.

% dietary protein	5	20	5	20	5% values as per cent of 20% values	
experiment	III		IV			
age at start(weeks)	4		12			
period of treatment (weeks)	10		10		III	IV

terminal body weight (g)	69	203	173	275	34	63
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Composition of femur

length (cm)	2.3	2.1	3.0	3.3	74	91
wet weight (mg)	231	499	466	556	46	84
dry weight (mg)	142	337	300	379	44	79
fat-free dry weight (mg)	128	325	278	365	39	76
ash weight (mg)	67	196	163	225	34	72
calcium content of femur (mg)	26.9	73.1	63	88	37	72
calcium (mg) per 100 g of body weight	39.1	36.1	36.5	32.1	108	114
A:R ratio	1.09	1.53	1.40	1.62	71	86

g per 100 g fresh bone

moisture	38.6	32.5	35.4	31.8
fat	6.0	2.3	4.7	2.5
ash	29.0	39.3	34.9	40.5
calcium	11.6	14.7	13.6	15.9

The decrease in the moisture content of femur with age is consistent with the general decrease in the water content of tissues with age. In other experiments in this laboratory protein deficiency is found to be associated with slight increases in the moisture content of other tissues such as the brain and liver.

In undernutrition, this decrease in moisture content of the femur seems to proceed normally. More extensive investigations are needed on the moisture content of different tissues during undernutrition and protein deficiency. It would appear that the greater cohesion of bone mineral achieved during maturation by dehydration is not seriously affected in undernutrition.

No consistent differences were found in the fat content of the femur with age. The increase in fat content in both protein deficiency and undernutrition therefore needs explanation. It is well known that in the liver fatty infiltration is a common phenomenon in protein deficiency. It is possible that fat tends to fill in for the deficits in other constituents or that the utilization of the fat formed is affected.

Studies are also needed on changes in carbohydrate content of bone with protein deficiency. Total N and ash are found to be decreased with protein deficiency and fat

and moisture increased. In the experiments on protein deficiency, (e.g. the data on Table 39) the values obtained for carbohydrate + protein by deducting from 100 the values for moisture, fat and ash is greater for the protein deficient animal. In other experiments, protein content of bone is found to be reduced with deficiency. This suggests that the carbohydrate content of bone may be elevated in protein deficiency. It is interesting to note in this connection that carbohydrate metabolism in the liver is altered in protein deficiency (Platt, Heard and Stewart, 1964).

In this connection, kwashiorkor children are found to show gross retardation of skeletal development as judged by bone age, bone density and the cortical thickness of the metacarpals (Garn, 1966; Table 40). Similar observations have been made in studies in this laboratory (Table 40). It is generally assumed that this may be because the diet of the kwashiorkor child is poor not only in protein but also in calcium. The present studies suggest that a major factor in the etiology of skeletal retardation in such children may be a deficiency of protein as well.

The results thus underline the importance of adequate protein nutrition and energy supplies in early life for proper skeletal development. They also suggest that the administration of calcium supplements to protein deficient children may have no value unless the protein status is also improved.

Table 40 : Skeletal development of children suffering from kwashiorkor and marasmus.

Baroda studies*						Garn(1966)
age (months)	no. of subjects	cortical thickness (mm)	range	skeletal age (months)	range	cortical** thickness (mm)
18	1	0.5 (1.1)		6 (14)		1.3 (1.5)
30	1	0.8 (1.4)		24 (22)		1.5 (1.6)
36	2	0.85 (1.5)	(0.7 -1.0)	18 (28)	(15 -21)	2.1 (1.7)
42	2	0.65 (1.55)	(0.5- 0.8)	20 (33)	(12 -27)	1.8 (1.8)
48	4	1.1 (1.7)	(0.5 -1.6)	27 (34)	(21 -33)	1.0 (1.9)
60	1	1.1 (1.9)		32 (40)		1.6 (2.1)
72	2	1.1 (2.1)	(0.8 -1.4)	26 (48)	(18 -33)	1.9 (2.3)

* Values taken from Rajalakshmi (1973); values for age matched controls from rural Baroda are given in parentheses.

** Abstracted values for kwashiorkor and control cases for corresponding ages.