C H A P T E R III

RESULTS AND DISCUSSION

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As mentioned earlier, several studies on children and experimental animals suggest that skeletal maturation is affected by deficiencies not only of the mineral elements which constitute bone but also of nutrients such as food energy, protein and vitamin A. As the rate of skeletal growth varies at different ages, the effects of nutritional deficiencies on the skeleton may be expected to depend on the age at which they occur. They may also be expected to vary with the type and severity of deficiency. The present studies were undertaken on rats to investigate the effects of undernutrition and protein deficiency on the composition of femur in relation to the age of the animal as well as the severity of deficiency. Such studies seemed necessary as comprehensive studies comparing different types and degrees of deficiency have not been reported.

As skeletal maturation is associated with increase in size, decrease in moisture content and increase in ash content, the parameters studied were length, weight, moisture, ash and calcium contents. Fat content was also investigated as some studies suggest changes in the same with deficiency.

Experiment I : Effects of neonatal undernutrition

As pointed out in the introduction, a few studies on rats, pigs and cockerels suggest that skeletal maturation may be affected by nutritional deprivation in early life. But comprehensive data on the effects of such undernutrition on the composition of the bone with regard to moisture, fat, ash and calcium are not available. The present experiment was undertaken in this context.

Undernutrition was induced during the neonatal period by increasing litter size. The results are presented in Table 19. The undernutrition induced resulted in a body weight deficit of 54%. In the case of femur, length was less affected than the other parameters studied. This is consistent with the dictum that bone grows at the expense of bone. Similar observations have been made by other investigators (Widdowson and McCance, 1960). The deficits in length and weight were of the order of 19 and 41% and those in ash and calcium contents greater (51% and 48%). However, the amount of calcium in femur in relation to body weight was not affected.

The values are presented in Table 19a as percentages of wet weight, fat-free weight, dry weight and fat-free dry weight as different kinds of comparisons are made by different authors (Widdowson and McCance, 1960; Braham, Tejada, Guzman and Bressani, 1961; Dickerson and McCance, 1961).

It can be seen from Table 19a that undernutrition was associated with an increase in moisture and fat content and a decrease in ash and calcium contents. The ash and calcium

Table 19 : Effects of neonatal undernutrition on the composition of femur.

	litter size		UN values as % of control values
	16 (UN)[1]	8 (C)[1]	
	mean ± s.e.		
terminal body weight (g) at 21 days	15.5±0.19***	33.4±0.17	46
Composition of femur			
length (cm)	1.3±0.02***	1.6±0.02	81
wet weight (mg)	70.1±2.02***	118.5±3.01	59
dry weight (mg)	25.7±0.93***	46.8±1.34	55
fat-free dry weight (mg)	23.8±0.78***	44.5±1.16	53
ash weight (mg)	8.4±0.37***	17.0±0.50	49
calcium content of femur (mg)	3.2±0.16***	6.2±0.20	52
calcium(mg) per 100 g of body weight	20.1±1.05	18.3±0.35	111
A:R ratio[2]	0.55±0.20**	0.62±0.011	89

Eight animals used in each group. Values marked with asterisk significantly different from control values; p less than 0.01 for ** and 0.001 for ***.

1. UN - undernourished; C - controls.

2. Ash weight/Residual mass.

Table 19a : Effects of neonatal undernutrition on the composition of femur.

	litter size	
	16 (UN)	8 (C)
	g per 100 g fresh bone	
moisture	63.4	60.5
fat	2.7	1.9
ash	11.9	14,3
calcium	4.5	5.2
	g per 100 g fat-free bone	
moisture	65.1	61.7
ash	12.3	17.0
calcium	4.6	5.3
	g per 100 g dry bone	
fat	7.4	5.0
ash	32.8	36.2
calcium	12.4	13.1
	g per 100 g fat-free dry bone	
ash	35.4	38.1
calcium	13.4	13.8
	g per 100 g ash	
calcium	37.7	36.2

contents were reduced in absolute terms as well as in relation to fat-free bone. This is associated with a reduction in A:R ratio as might be expected. However, the calcium content of ash was not affected. It has been generally observed that in several deficiencies including those of energy, protein, and calcium, while ash contents and the proportion of ash to other components are affected, the calcium content of ash itself is not affected (Estremera and Armstrong, 1948).

No comparative data seem to be available on the effects of neonatal undernutrition except for the data reported for rat pups aged 2 weeks by Widdowson and McCance (1960) who found a marked reduction in calcium content of femur in pups whose body weight deficit was 62%. Their data for animals subjected to undernutrition for a similar period are shown below :

	UN	C
body weight (g)	12	32
	g per 100 g fat-free bone	
moisture	69.6	64.4
total N	2.72	2.56
calcium	2.82	4.38
Ca/N	1.04	1.71

The greater differences found by them might have been due to the greater degree of undernutrition achieved as judged by body weight. It is interesting to note the difference reported by them in Ca/N ratio. Although nitrogen was not determined in the present study, we can assume that protein is the major component other than ash, moisture and fat-free bone as the carbohydrate content of bone (0.24%) is negligible (Eastoe and Eastoe, 1954). The bone of the undernourished and control animals can therefore be expected to have had a protein content of 22.4 and 21.1%. The corresponding values for nitrogen content would be 3.58 and 3.37%. The calculated values for Ca/N in the two groups would be 0.89 and 1.83. These values compare with those of Widdowson and McCance (1960). However, the possibility that the carbohydrate content of bone is altered to some extent in deficiency cannot be ruled out.

In conclusion, neonatal undernutrition was associated with a reduction in femur length, an increase in moisture and fat contents and a decrease in ash and calcium contents. The A:R ratio was decreased, suggesting an altered ratio of minerals to matrix. Computations from the data suggest a similar reduction in Ca/N ratio. As the maturation of the bone during the neonatal period is associated with a decrease in moisture content and an increase in bone mineral, the results suggest a delayed maturation of the bone in the undernourished animals.

Experiment II : Effects of undernutrition during the postweaning period.

Next, studies were carried out on the effects of undernutrition during the postweaning period. Animals were given food either *ad lib.* or in amounts representing 50% of the food intake of those fed *ad lib.*. At the end of 5 weeks, the undernourished group was divided into two groups and one of them continued on the restricted diet for a further period of 11 weeks and the other fed *ad lib.*.

The food intake and body weights of these animals are shown in Table 20. As may be expected, undernutrition resulted in an appreciable deficit in body weight and this deficit was not fully restored by rehabilitation for 11 weeks. The undernourished and rehabilitated animals had a greater food intake than controls in relation to body weight. This has also been observed by other investigators (Chow, Blackwell and Shewin, 1968).

As with neonatal undernutrition, femur length was affected to a lesser extent than other parameters (Table 21). Femur weight (both fresh and dry) showed a deficit of 30%.

The values are presented in Table 21a as percentages of wet weight, fat-free weight, dry weight and fat-free dry weight. Moisture content was not affected but fat content

Table 20 : Effects of postweaning undernutrition on food intake and weight gain in rats.

	control	rehabilitated*	under-nourished
		mean ± s.e.	
food intake (g/day)			
0 - 5 weeks	7.2 ± 0.35	3.8 ± 0.13	3.9 ± 0.18
6 - 16 weeks	11.4 ± 0.11	11.1 ± 0.14	5.5 ± 0.11
body weight (g)			
initial	45 ± 1.3	45 ± 1.6	45 ± 1.2
at 5 weeks	134 ± 5.1	83 ± 2.2	82 ± 1.9
at 16 weeks	245 ± 10.6	209 ± 6.7	133 ± 4.1

Period of treatment, 15 weeks; 8 animals used in each group.

* Undernourished for the first five weeks and rehabilitated thereafter.

Table 21 : Effects of postweaning undernutrition on the composition of femur.

	control	rehabilitated@	undernourished
		mean ± s.e.	
terminal body weight (g)	245 ± 10.6	209 ± 6.7 (85)	133 ± 4.1*** (54)
composition of femur			
length (cm)	3.3 ± 0.05	3.2 ± 0.03 (97)	2.9 ± 0.02*** (88)
wet weight (mg)	557 ± 14.4	523 ± 10.7 (94)	388 ± 8.4*** (70)
dry weight (mg)	389 ± 12.5	361 ± 6.6 (93)	271 ± 6.4*** (70)
fat-free dry weight (mg)	375 ± 12.7	345 ± 7.3 (92)	235 ± 5.6*** (63)
ash weight (mg)	228 ± 9.4	209 ± 4.9 (92)	135 ± 3.8*** (59)
calcium content of femur (mg)	87 ± 3.7	80 ± 1.9 (92)	53 ± 1.1*** (61)
calcium (mg) per 100 g of body weight	35.6 ± 0.49	38.2 ± 1.60 (107)	39.6 ± 0.90** (111)
A : R ratio	1.54 ± 0.031	1.54 ± 0.027 (100)	1.35 ± 0.032*** (88)

Period of treatment, 16 weeks; 8 animals used in each group. Values given in parentheses are percentages of controls. Values marked with asterisk significantly different from control values; p less than 0.01 for ** and 0.001 for ***.

@ Undernourished for the first five weeks and rehabilitated thereafter.

Table 21a : Effects of postweaning undernutrition on the composition of femur.

	control	rehabilitated	under-nourished
	g per 100 g fresh bone		
moisture	30.2	30.9	30.0
fat	2.4	3.0	9.4
ash	40.8	40.0	34.7
calcium	15.6	15.1	13.5
	g per 100 g fat-free bone		
moisture	30.9	31.9	33.1
ash	41.8	41.3	38.4
calcium	16.0	15.7	14.9
	g per 100 g dry bone		
fat	3.5	4.4	13.3
ash	58.4	58.0	49.7
calcium	22.3	22.0	19.3
	g per 100 g fat-free dry bone		
ash	60.5	60.3	57.3
calcium	23.1	23.0	22.3
	g per 100 g ash		
calcium	38.2	37.9	38.9

was found to show a marked increase in the undernourished animals. In relation to fat-free weight, however, moisture content was found to be somewhat more in the undernourished animals.

Both ash and calcium contents were reduced either in absolute terms or when considered in terms of concentration.

In this and the following experiments the calcium content of bone ash was sometimes found to be slightly more than the value of 36% reported. This could be due to either slight variations in the proportions of calcium salts such as calcium phosphate, calcium carbonate and calcium oxide which go to make up bone ash or a normal experimental error margin in the estimation of ash weight and/or calcium content. Cumulatively, these factors could result in a small deviation from the expected value.

With dietary rehabilitation, the differences found were either reduced or abolished. No comparative data appear to be available except for the studies of McCance and associates on pigs (Dickerson and McCance, 1961) and cockerels (Lister, Cowen and McCance, 1966) subjected to prolonged and severe undernutrition from birth for a period 1-2 years in the former case and from fourteen days for six months in the latter case, the body weight deficits in these studies being more than 95%. With such severe undernutrition in pigs they found increase in moisture, decrease in calcium to collagen ratio and nitrogen, calcium and phosphorus contents. The ratio of thickness to length and of collagen N to total N were slightly increased as shown in Table 22.

In cockerels, the ratio of thickness to length was reduced and percentages of calcium and phosphorus increased. All these changes were reversed by rehabilitation (Dickerson and McCance, 1961). Abnormalities were found in the skull, jaws and teeth and were corrected with rehabilitation in the first two cases (McCance and Ford, 1961). Other findings in cockerels were disturbed endochondral ossification, a reduced activity of osteoblasts and a more fibrous periosteal bone.

In other studies, undernutrition has been found to result in reduced thickness of ephiphyseal plate, failure of cartilage cells to hypertrophy and relatively excessive amounts of cartilaginous matrix (Silberberg and Silberberg, 1940; Handler, Baylin and Follis, 1947; Saxton and Silberberg,1947; Bavetta, Bernick, Geiger and Bergran, 1954).

These studies ~~suggest~~ indicate that the decrease in bone mineral with undernutrition is primarily due to reduced activity of osteoblasts. A similar conclusion is to be drawn from the studies comparing the effects of protein deficiency and undernutrition (Frandsen _et al._, 1954).

Table 22 : Composition of pig humerus*.

	weight matched controls	under-nourished	controls
age (weeks)	4	46	43
body weight (kg)	6.58	6.55	189
length (cm)	7.87	9.00	20.9
thickness (cm)	1.17	1.55	3.65
moisture (g/100 g)	59.4	66.1	22.0
fat (g/100 g)	1.7	0.4	39.7
thickness/length	0.149	0.176	0.176
	g per 100 g fat-free bone		
moisture	60.4	66.4	36.2
total N	2.69	2.08	3.53
collagen	9.57	8.87	14.9
calcium	7.18	6.18	14.8
phosphorus	3.33	3.04	6.95
calcium / collagen	0.75	0.69	0.99
collagen N / total N	0.63	0.76	0.76

*Values taken from Dickerson and McCance (1961).

However, the results of these studies are not entirely comparable with those of the present studies because of differences in the species used as well as the duration and severity of the undernutrition induced.

In conclusion undernutrition caused by a 50% reduction in food intake in rats during the postweaning period resulted in an increase in fat content and a decrease in ash and calcium contents but no change in moisture content although it was increased slightly in relation to fat-free weight. The concentrations of ash and calcium in the fat-free dry bone as well as A:R ratio were also affected. These changes are consistent with reports of poor bone development in undernourished animals as a result of retarded endochondral ossification and reduced osteoblastic activity resulting in poor formation of the matrix.

It is interesting to note that whereas undernutrition during the neonatal period resulted in an increase in moisture content, no such increase was found with undernutrition during the postweaning period. In studies to be described in the next section, protein deficiency during the postweaning period was also found to be associated with an increase in moisture content. In other studies in this laboratory the effects of neonatal undernutrition on brain enzymes were found to be similar to those of postweaning protein deficiency whereas protein deficiency and calorie undernutrition during the postweaning period had differential effects.

Experiment III : Effects of protein deficiency during the postweaning period.

In the experiment described in the previous section undernutrition during the postweaning period was found to result in an increase in the fat content of the femur. The question arose as to whether protein deficiency also affects the composition of the femur and how far the effects of protein deficiency are similar to those of undernutrition.

Weanling rats were fed a diet either low or high in protein for a period of 10 weeks. The data on food intake and body weight are presented in Table 23. As might be expected, the low protein animals had a smaller food intake and decreased weight gain which was less even when considered in relation to food intake. This conforms to the general pattern found with protein deficiency.

The data on femur composition are presented in Table 24. Protein deficiency was found to affect the size and composition of the femur. The percentage deficits were 26% for length, 56% for dry weight, 61% for fat-free dry weight, 66% for ash and 63% for calcium content. Thus as in undernutrition, the deficit in femur length was less than that in body weight

Table 23 : Effects of protein deficiency on food intake and weight gain in rats.

	% dietary protein	
	5	20
	mean ± s.e.	
food intake (g/day)	4.7 ± 0.15	9.0 ± 0.24
protein intake (g/day)	0.24	1.80
body weight (g)		
initial	44 ± 1.4	44 ± 1.0
terminal	69 ± 4.8	203 ± 10.3
weight gain (g)		
(a) total	25 ± 3.5	159 ± 8.2
(b) per 100 kcal intake.	2.0	6.6

Period of treatment, 10 weeks; 8 animals used in each group.

Table 24 : Effects of protein deficiency on the composition of femur.

	% dietary protein	
	5	20
	mean ± s.e.	
terminal body weight (g)	69 ± 4.8*** (34)	203 ± 10.3
composition of femur		
length (cm)	2.3 ± 0.04*** (74)	3.1 ± 0.05
wet weight (mg)	231 ± 13.0*** (46)	499 ± 15.6
dry weight (mg)	142 ± 8.5*** (44)	337 ± 11.9
fat-free dry weight (mg)	128 ± 8.2*** (39)	325 ± 10.7
ash weight (mg)	67 ± 4.9*** (34)	196 ± 5.3
calcium content of femur (mg)	26.9 ± 1.83*** (37)	73.1 ± 2.83
calcium (mg) per 100 g of body weight	39.1 ± 1.05* (108)	36.1 ± 0.82
A : R ratio	1.09 ± 0.044*** (71)	1.53 ± 0.049

Period of treatment, 10 weeks; 8 animals used in each group. Values given in parentheses are percentages of 20% values. Values marked with asterisk significantly different from control valuës; p less than 0.05 for * and 0.001 for ***.

and bone weight. Calcium per 100 g of body weight was somewhat increased with protein deficiency and this was also found in other experiments.

The A : R ratio was significantly reduced in protein deficiency.

The values are presented in Table 24a as percentages of wet weight, fat-free weight, dry weight and fat-free dry weight. Protein deficiency was associated with an increase in moisture and fat contents and a decrease in ash and calcium contents but no change was found in the calcium content of ash. Calcium content was reduced both in absolute terms and as percentage of both wet weight and dry weight. A similar pattern was found in subsequent experiments and in fact a greater reduction was found in ash as percentage of dry weight in other experiments.

In studies now in progress in this laboratory, both ash and nitrogen content are found to be reduced but the former is reduced to a greater extent with a consequent decrease in the Ca/N ratio.

An increase in fat content and decreases in ash content and ash matrix ratio in protein deficient rats have also been found by other investigators (Gontea *et al.*, 1960). The changes in ash and ash matrix ratio have also been noted by El Maraghi *et al.* (1965). A reduction in ash content

Table 24a : Effects of protein deficiency on the composition of femur.

	% dietary protein	
	5	20
	g per 100 g fresh bone	
moisture	38.6	32.5
fat	6.0	2.3
ash	29.0	39.3
calcium	11.6	14.7
	g per 100 g fat-free bone	
moisture	40.9	33.3
ash	30.8	40.3
calcium	12.3	15.0
	g per 100 g dry bone	
fat	8.5	3.4
ash	47.3	58.3
calcium	19.0	21.7
	g per 100 g fat-free dry bone	
ash	52.5	60.4
calcium	21.0	22.5
	g per 100 g ash	
calcium	40.1	37.2

associated with thinning of the epiphyseal plate of cartilage and delayed maturation of the bone has been reported in pigs by Stewart and Platt (1961) who found that addition of calcium to a low protein diet was not effective in increasing the amount of bone mineral. Similar observations have been made in this laboratory. However, these authors did not find a decrease in A:R ratio in pigs (Platt and Stewart, 1962). This might be due to differences in the species studied as well as the degree of deficiency.

A comparison of the results of ~~this~~ the present experiment with those of the previous one on undernutrition suggest that the effects of protein deficiency are perhaps more severe than those of simple undernutrition. Also, a major difference between the two conditions appears to be that moisture content is increased in protein deficiency but not in post-weaning undernutrition although it is increased in neonatal undernutrition. These studies suggest the differential effects of protein and energy deficiencies during the postweaning period. In this connection similar observations have been made in this laboratory on the effects of these two conditions on brain glutamate dehydrogenase and decarboxylase. Whereas both neonatal undernutrition and postweaning protein deficiency were found to be associated with reduced activities of these two enzymes, postweaning undernutrition was not found to have a similar effect (Rajalakshmi and Ramakrishnan, 1969c).

Differential effects of protein and energy deficiencies on skeletal development are also to be inferred from the studies of Frandsen et al., (1954) who found skeletal growth and endochondral ossification to be affected by both protein deficiency and undernutrition in young rats, the effects being much more severe in the former case. They found decreased width of epiphyseal cartilage, diminished number and size of cartilage cells, increase in ground substance, slowing down and arrest of cartilage erosion and poor bone formation. El Maraghi and his associates (1965) consider these changes in young animals to be due to a defective formation of the matrix rather than poor bone mineralization. Such a view is consistent with the increase in fat and moisture contents found in the present studies and the reduction in nitrogen content found in other studies suggesting changes in the non-mineral components of the bone. Such changes may conceivably interfere with the normal mineralization of the bone and result in a low mineral content.

In conclusion, protein deficiency was found to result in an increase in fat and moisture contents and a decrease in ash and calcium contents as well as A:R ratio. These changes are consistent with the findings of other investigators.

Experiment IV : Comparative effects of protein deficiency and moderate and severe food restriction on the composition of femur in growing rats.

In the studies described in the previous section, protein deficiency was found to affect the size and composition of the femur in weanling rats. These studies were extended to 12 week old rats as the effects are found to vary with age (El Maraghi *et al.*, 1965) and the A:R ratio has been found to be unaltered in adult rats (Fontaine, *et al.*, 1950).

As the previous studies suggested the differential effects of calorie and protein deficiencies, studies were also made of the comparative effects of protein deficiency and moderate and severe food restriction in these animals.

Groups of animals, 12 weeks of age at start and matched for sex and body weight were fed either a low or high protein diet or the latter in restricted amounts for a period of 10 weeks. The amounts fed for the restricted groups were 66.7% and 33.3% of the *ad lib.* intakes of the high protein group.

Data on the food intake and weight gain of these animals are presented in Table 25. As expected, moderate food restriction and protein deficiency retarded growth. The more severe degree of food restriction (66.7%) resulted in some loss of weight.

Table 25 : Comparative effects of protein deficiency and undernutrition on food intake and weight gain in rats.

% dietary protein	5	20		
mode of feeding	ad lib.	ad lib.	food intake as % of ad lib. intake	
			66.7	33.3
	mean ± s.e.			
food intake (g/day)	9.0±0.35	12.0±0.29	8.0±0.23	4.0±0.10
protein intake (g/day)	0.45	2.40	1.60	0.80
body weight (g)				
initial	148±1.7	148±2.1	148±1.4	148±2.3
terminal	173±3.0	275±7.4	219±2.9	123±3.5
weight gain (g)				
(a) total	+25±1.6	+126±6.7	+71±3.3	-25±5.2
(b) per 100 kcal intake	1.1	3.9	3.3	-

Age at start, 12 weeks; period of treatment, 10 weeks; 6 animals used in each group.

As in other studies, utilization of energy for tissue gain was more efficient in the moderately undernourished animals than in the low protein animals. In spite of their smaller food intake as compared to low protein animals they showed a greater weight gain.

As in the previous experiment on younger animals both moisture and fat contents of femur were increased whereas ash and calcium contents were reduced in the protein deficient animals(Tables 26 and 26a), the percentage deficits were less, presumably because of the older age at which deficiency was introduced.

No appreciable changes were found with moderate food restriction. With more severe food restriction, the femur composition was comparable to that in protein deficiency except for the absence of increase in moisture content (Table 26a). In other words the changes found in protein deficiency with a food intake of 9 g per day and a terminal body weight deficit of 37% were found with undernutrition only when the food intake was drastically reduced to 4 g per day and the deficit in body weight was 55%. Even in this case, the increase in moisture content found with protein deficiency was not found with undernutrition. These observations have been confirmed in recent studies in this laboratory in younger animals (Dave, unpublished).

Table 26 : Comparative effects of protein deficiency and undernutrition on the composition of femur.

% dietary protein	5	20		
mode of feeding	ad lib.	ad lib.	food intake as % of ad lib. intake	
			66.7	33.3
		mean ± s.e.		
terminal body weight (g)	173±3.0*** (63)	275±7.4	219±2.9*** (80)	123±3.5*** (45)
composition of femur				
length (cm)	3.0±0.05** (91)	3.3±0.06	3.2±0.05 (97)	3.0±0.05** (91)
wet weight (mg)	464±11.8** (84)	556±21.7	538±18.7 (97)	454±13.2** (82)
dry weight (mg)	300±13.0*** (79)	379±11.2	365±14.6 (96)	309±12.0** (82)
fat-free dry weight (mg)	278±10.7*** (76)	365±12.2	346±12.6 (95)	272±10.1*** (75)
ash weight (mg)	163±7.5*** (72)	225±7.8	207±8.1 (92)	162±7.1*** (72)
calcium content of femur (mg)	63±2.5*** (72)	88±3.0	79±3.1 (90)	63±2.8*** (72)
calcium (mg) per 100 g of body weight	36.5±1.26 (114)	32.1±0.71	36.0±1.33* (112)	51.0±1.92*** (159)
A:R ratio	1.40±0.034*** (86)	1.62±0.044	1.49±0.038* (88)	1.46±0.029 (87)

Age at start, 12 weeks; period of treatment, 10 weeks; 6 animals used in each group. Values given in parentheses are percentages of controls. Values marked with asterisk significantly different from control values; p less than 0.05 for *, 0.01 for ** and 0.001 for ***.

Table 26a : Comparative effects of protein deficiency and undernutrition on the composition of femur.

% dietary protein	5	20		
mode of feeding	ad lib.	ad lib.	food intake as % of ad lib. intake	
			66.7	33.3
		g per 100 g fresh bone		
moisture	35.4	31.8	32.2	32.0
fat	4.7	2.5	3.2	8.0
ash	34.9	40.5	38.6	35.6
calcium	13.6	15.9	14.6	13.8
		g per 100 g fat-free bone		
moisture	37.1	32.6	32.9	34.8
ash	36.9	41.6	39.8	38.7
calcium	14.3	16.3	15.1	15.0
		g per 100 g dry bone		
fat	7.3	3.7	4.7	11.7
ash	54.1	59.4	56.8	52.4
calcium	21.1	23.3	21.6	20.3
		g per 100 g fat-free dry bone		
ash	56.3	61.8	59.9	59.4
calcium	22.7	24.2	22.7	23.0
		g per 100 g ash		
calcium	39.0	39.2	38.0	38.7

It must also be pointed out that although the animals in this experiment were older than those in the previous experiment, they were still young and growing. The result might be different in adult animals.

The differential effects of protein deficiency and undernutrition were suggested as early as 1925 by Jackson (1925) who differentiated quantitative and qualitative inanition. In the studies of Shenolikar and Narasinga Rao (1968) in which the animals were undernourished so that their body weights matched those of low protein animals, the latter were found to do worse as judged by bone calcium, calcium balance and calcium accretion per day. Similar observations have been made with regard to width of cartilage, size of cartilage cells and bone formation by Frandsen et al., (1954). The differential effects of protein and calorie deficiencies are consistent with the differences between marasmus and kwashiorkor in children and particularly with the lack of edema in the former case (Viteri, Behar and Arroyave,1964).

It is interesting to note that the size and composition of the femur were not appreciably affected with a moderate degree of food restriction. A further point of interest is that even where the weight and mineral content of the femur are decreased, the same in relation to body weight are actually increased and bone growth seems to be maintained to some extent even with overall growth retardation. A similar pattern is observed in the case of the brain, the ratio of brain weight to body weight being generally elevated in undernourished animals. This may perhaps be because the maturation of the central nervous system and the skeleton take precedence, in that order, over that of other systems as the maturation of the former is completed in early life and the latter well within the period of growth.

Experiment V : Effects of different degrees of protein deficiency on the composition of femur.

In the studies described earlier, animals fed low and high protein diets were found to differ with regard to the size and composition of the femur. The diets used in these studies contained 5% and 20% protein. A question arose as to the minimum level of protein needed in the diet to prevent the effects of a low protein diet. Studies were therefore carried out on the growth and composition of femur in animals fed different levels of protein.

Weanling albino rats were fed diets containing 5, 8, 10, 15 or 20% protein for a period of 10 weeks and studies made of food intake, weight gain and femur composition.

The data on food intake and body weight are presented in Table 27.

Table 27 : Food intake and weight gain in rats fed different levels of protein.

	% dietary protein				
	5	8	10	15	20 (controls)
	mean ± s.e.				
food intake (g/day)	4.6±0.16	6.9±0.25	8.2±0.22	8.8±0.18	9.2±0.26
protein intake (g/day)	0.23	0.55	0.82	1.32	1.84
body weight (g)					
initial	46 ± 1.4	46 ± 1.4	46 ± 0.8	46 ± 1.2	46 ± 1.0
terminal	69 ± 3.9	141± 4.8	174± 3.8	213± 8.0	213±11.3
weight gain (g)					
(a) total	23 ± 2.6	93 ± 5.4	129± 3.1	166± 7.8	168±11.1
(b) per 100 kcal intake	1.7	4.5	5.2	6.4	6.2

Period of treatment, 10 weeks; 8 animals used in each group.

It can be seen from the same that 15% protein in the diet was sufficient to ensure maximum growth and efficiency in the utilization of food. Both fell when protein content was reduced to 10 or 8% but the decrease was most marked when protein content was further reduced to 5%.

The size and weight of the femur varied with body weight and dietary protein content upto 15% (Table 28). These were associated with variations in ash and calcium contents and A:R ratio. A:R ratio was not affected with 10% protein in the diet but somewhat reduced with 8% protein.

With regard to the composition of the femur, as in previous studies, a 5% protein diet was found to be associated with an increase in moisture and fat contents and a decrease in ash and calcium contents (Table 28a). With 8 and 10% protein in the diet these changes were far less evident and only small increases were found in moisture and fat contents. The decrease in ash and calcium contents were also not appreciable with these levels of protein in the diet. No change was found in the percentage of ash in fat-free dry femur or in the amount of calcium per 100g of body weight.

No change was found with 15% protein in the diet in any of the parameters studied.

Table 28 : Composition of femur in rats fed different levels of protein.

	% dietary protein				
	5	8	10	15	20 (controls)
			mean ± s.e.		
terminal body weight (g)	69±3.9*** (32)	141±4.8*** (66)	174±3.8*** (82)	213±8.0 (100)	213±11.3
composition of femur					
length (cm)	2.3±0.03*** (72)	2.8±0.05*** (88)	2.9±0.04*** (91)	3.1±0.04 (97)	3.2±0.05
wet weight (mg)	239±8.1*** (45)	375±13.1*** (71)	433±16.6*** (82)	529±18.9 (100)	530±17.1
dry weight (mg)	143±5.9*** (40)	246±10.4*** (69)	287±12.4*** (80)	357±13.3 (99)	359±12.3
fat-free dry weight (mg)	130±6.1*** (37)	234±9.9*** (67)	276±11.4*** (79)	347±12.3 (100)	348±12.2
ash weight (mg)	69±4.3*** (33)	136±6.9*** (66)	162±7.2*** (78)	202±7.4 (98)	207±6.8
calcium content of femur (mg)	27.3±1.41*** (36)	51.7±2.35*** (68)	60.2±2.32** (79)	76.1±2.69 (100)	76.1±3.18
calcium (mg) per 100 g of body weight	39.8±1.33* (111)	36.8±1.50 (103)	34.8±0.21 (97)	35.9±1.04 (100)	35.9±0.79
A:R ratio	1.08±0.042*** (73)	1.39±0.049 (95)	1.42±0.029 (97)	1.42±0.010 (97)	1.47±0.022

Period of treatment, 10 weeks; 8 animals used in each group. Values given in parentheses are percentages of controls. Values marked with asterisk significantly different from control values; p less than 0.05 for *, 0.01 for ** and 0.001 for ***.

Table 28a : Composition of femur in rats fed different levels of protein.

	% dietary protein				
	5	8	10	15	20
			g per 100 g fresh bone		
moisture	41.5	34.6	33.7	32.8	32.3
fat	5.2	3.1	2.5	1.7	2.1
ash	28.6	36.2	37.3	38.1	39.0
calcium	11.4	13.6	13.9	14.4	14.3
			g per 100 g fat-free bone		
moisture	42.3	35.7	34.6	33.1	32.9
ash	30.1	37.3	38.3	38.7	39.9
calcium	12.0	14.2	14.3	14.6	14.7
			g per 100 g dry bone		
fat	7.4	4.7	3.8	2.5	3.1
ash	47.9	55.3	56.5	56.5	57.6
calcium	19.1	21.0	21.0	21.4	21.2
			g per 100 g fat-free dry bone		
ash	52.5	58.0	58.7	58.0	59.5
calcium	20.9	22.1	21.9	21.9	21.9
			g per 100 g ash		
calcium	39.7	38.0	37.2	37.7	36.7

These studies suggest that although a low protein diet produces some characteristic changes in the composition of femur, the amount of protein in the diet needed to prevent these changes is probably much less than that needed for preventing growth deficits as the changes in animals fed 8 and 10% protein diets were only slight although the body weight deficits were considerable (34% and 18%).

In this connection, in other studies in this laboratory, deficits in brain glutamate dehydrogenase and decarboxylase found with a low protein diet are prevented by diets containing 8% protein in the form of casein (Rajalakshmi and Ramakrishnan, 1972). In this connection, in studies carried out by El Maraghi et al. (1965) in which the percentage of net dietary protein calories was varied from 4.5 to 10.2. A:R ratio was affected at the level of 5.1% NDP calories but not at the 6% level although the weight gain of 122 g at latter level was much less than that of 183 g in controls fed 10.2% NDP calories.

In conclusion, the present studies confirm the effects of protein deficiency on femur composition in weanling rats and suggest that these effects are largely prevented by diets containing 8 or 10% of good quality protein in the diet although these levels were not sufficient to ensure optimum growth.

Experiment VI : Effects of variations in protein quality on the composition of the femur.

Several studies described earlier demonstrated the effects of a low protein diet on the composition of the femur. Since ordinary diets vary not only in protein content but also in protein quality, the question arose as to whether similar effects are found with diets of poor protein quality. Studies were therefore made of the effects of improving the protein quality of kodri, a millet very deficient in lysine, by supplementation with this amino acid. As the kodri-based diet contains 7.3% protein, a diet providing this amount of protein in the form of casein was used for comparison.

The data on the food intake and body weight of the different groups are shown in Table 29. The weight gain of animals fed kodri was about 0.6 g per week. In other studies, a 4% casein diet was found to produce no weight gain whereas one containing 5% casein was found to promote a gain of 2-4 g. The kodri diet can be presumed to correspond in protein value to a casein diet containing about 4% protein. The weight gain of the lysine supplemented group was 6.2 g per week and that of the 7.3% casein diet 8.2 g per week. The former seems to correspond to a 6-7% casein diet on the basis of other experiments. The latter compares with a

Table 29 : Effects of lysine supplementation to kodri on food intake and weight gain in rats.

diet	kodri	kodri + lysine	7.3% casein diet
		mean ± s.e.	
food intake (g/day)	4.3±0.14	7.3±0.54	7.7±0.60
protein intake (g/day)	0.31	0.53	0.56
body weight (g)			
initial	50 ± 2.0	50 ± 1.4	50 ± 1.1
terminal	56 ± 2.2	112± 5.0	132 ±7.3
weight gain (g)			
(a) total	6 ± 0.7	62 ± 4.2	82 ± 7.4
(b) per 100 kcal intake	0.6	3.3	3.6

Period of treatment, 10 weeks; 8 animals used in each group.

weight gain of 9.3 g per week found for 8% casein in previous studies and 6.2 g in those fed 7% in other studies (unpublished data).

The differences in the composition of femur in animals fed kodri alone or with lysine (Table 30) were similar in pattern but smaller as compared to those between those fed low and high protein diets. This is not surprising as supplementation with lysine increased the protein value of the diet only to that of a 6-7% casein diet. The femur in the supplemented group had smaller amounts of moisture and fat and greater amounts of ash and calcium (Table 30a). However, the values failed to reach the levels found in the group fed 7.3% casein.

The results demonstrate the importance of a diet adequate in both protein content and quality for normal skeletal development.

Experiment VII : Effects of supplements of legumes and greens to maize and wheat on the composition of femur.

In the studies described earlier, improving the quality of kodri by lysine supplementation was found to be associated with increased weight gain and a more mature composition of femur. In these studies, a salt mixture providing adequate calcium and phosphorus was added to the diets. The basal and improved diets in these studies did not vary very much with regard to nitrogen and mineral contents.

Table 30 : Effects of lysine supplementation to kodri on composition of femur.

diet	kodri	kodri + lysine	7.3 % casein diet	significance of difference between means	
group no.	1	2	3	1 ∽ 2	2 ∽ 3
		mean ± s.e.		p less than	
terminal body weight (g)	56±2.2 (42)	112±5.0 (85)	132±7.3	0.001	0.05
composition of femur					
length (cm)	2.2±0.03 (81)	2.6±0.06 (96)	2.7±0.04	0.001	N.S.
wet weight (mg)	198±7.0 (60)	285±12.0 (86)	331±12.1	0.001	0.05
dry weight (mg)	130±5.4 (57)	188±9.2 (82)	230±8.8	0.001	0.01
fat-free dry weight (mg)	114±4.8 (54)	174±8.8 (82)	211±8.6	0.001	0.01
ash weight (mg)	63±2.5 (49)	106±3.1 (83)	128±6.2	0.001	0.01

contd.

Table 30 contd.

diet	kodri	kodri + lysine	7.3 % casein diet	significance of difference between means	
group no.	1	2	3	1 ∽ 2	2 ∽ 3
		mean ± s.e.		p less than	
calcium content of femur (mg)	25.9±1.11 (52)	42.2±2.01 (84)	50.2±2.42	0.001	0.05
calcium (mg) per 100 g of body weight	46.4±0.86 (121)	37.2±0.76 (97)	38.3±1.45	0.001	N.S.
A:R ratio	1.24±0.054 (83)	1.54±0.026 (103)	1.49±0.068	0.001	N.S.

Period of treatment, 10 weeks; 8 animals used in each group. Values given in parentheses are percentages of those for casein diet.

N.S. = not significant.

Table 30a : Effects of lysine supplementation to kodri on composition of femur.

diet	kodri	kodri + lysine	7.3% casein diet
		g per 100 g fresh bone	
moisture	34.4	34.0	30.3
fat	7.7	4.9	5.7
ash	32.1	37.1	38.6
calcium	13.1	14.8	15.2
		g per 100 g fat-free bone	
moisture	37.3	35.7	32.2
ash	33.3	39.1	41.0
calcium	14.2	15.6	16.2
		g per 100 g dry bone	
fat	11.8	7.7	7.6
ash	49.7	57.3	55.4
calcium	20.1	22.5	21.8
		g per 100 g fat-free dry bone	
ash	55.5	61.0	60.4
calcium	22.7	24.4	23.8
		g per 100 g ash	
calcium	40.8	39.9	39.3

In developing countries such as India, the diets consumed by the poor are based predominantly on cereals which provide about 77% of total food energy, 77% of protein and 36% of calcium (Rajalakshmi, 1972). More than 80% of the food grains produced are consumed by the producer and the means adapted in the west of enriching flour with lysine, minerals and vitamins are neither practicable nor perhaps even desirable if this involves, as it usually does, the refinement of flour in order to improve its keeping quality. In the circumstances prevailing in this country, the use of locally available foods which can improve the nutritional quality of the poor man's diet seems to be a more practicable solution.

In the Applied Nutrition Programme conducted by this Department, the approach has been to seek nutritional improvement in rural areas by such exploitation of locally available foods (Rajalakshmi and Ramakrishnan, 1967; Rajalakshmi _et al._, 1972; Rajalakshmi _et al._, 1973). The use of cereals along with pulses (legumes) and leafy vegetables has been specially advocated as the former are richer in protein as well as lysine and the latter can supply nutrients such as carotene, riboflavine, calcium and iron which are limiting nutrients in ordinary diets.

As mentioned earlier, cereals are the major sources of not only energy but also most other nutrients in the poor man's diet. However, cereals vary appreciably with regard to their content of calcium and other nutrients (Table 31). The poor diet in Tamil Nadu based on rice provides only about 150-200 mg as against 380-400 mg provided by a mixed diet in Gujarat based on wheat, bajra (pearl millet), rice and kodri. Similarly, the diet of the Bhils, a tribal people consuming only maize provides less calcium (275 mg).

In other preliminary studies in this department, school boys in rice and maize eating areas seemed to show a greater degree of skeletal retardation than those in areas where wheat or bajra (pearl millet) are the staples (Rajalakshmi and associates, unpublished).

The present studies were carried out in this context on (a) the effects of supplementing selected cereals (wheat and maize) with legumes and greens and (b) the comparative nutritive value of combinations of staple grain + legume + leafy vegetables with variation in the staple used. The staples used were wheat, maize, rice, bajra and jowar. Bengal gram and fenugreek leaves were used as the most commonly used legume and leafy vegetable. Also, previous studies on animals and children had suggested their suitability from the point of view of nutritive value as well as acceptability (Rajalakshmi _et al._, 1972). Further, in supplementary feeding programmes organized for pre-school

Table 31 : Nutrients provided by selected food grains, bengal gram and fenugreek leaves.*
(per 100 g edible portion)

	calories	protein (g)	fat (g)	calcium (mg)	phos-phorus (mg)	iron (mg)	thiamine (mg)	ribo-flavine (mg)	niacin (mg)	vita-min A (mcg)
bajra (Pennisetum typhoideum)	361	11.6	5.0	25	296	5.0	0.33	0.25	2.3	132
wheat (Triticum aestivum)	346	11.8	1.5	41	306	4.9	0.45	0.17	5.5	64
rice (Oryza sativa)	345	6.8	0.5	10	160	3.1	0.06	0.06	1.9	0
kodri (Paspalum scorbiculatum L.)	309	8.3	1.4	27	188	5.2	0.33	0.09	2.0	0
maize (Zea mays)	342	11.1	3.6	10	348	2.0	0.42	0.10	1.8	90
jowar (Sorghum vulgare)	349	10.4	1.9	25	222	5.8	0.37	0.13	3.1	47
bengal gram (Cicer arietinum)	372	20.8	5.6	56	331	9.1	0.48	0.18	2.4	129
fenugreek leaves (Trigonella foenum graecum)	49	4.4	0.9	395	51	16.5	0.04	0.31	0.8	2340

*Values based on data given by Gopalan, Ramasastri and Balasubramanian (1971) except in the case of bajra calcium which was obtained by analysis.

and school children one dish meals such as dhokla and debra based on cereal + legume + greens are found to be convenient and highly acceptable (Rajalakshmi and Ramakrishnan, 1969b).

Groups of rats were fed either wheat or maize (75 g) or a combination of staple grain (60 g) + bengal gram (15 g) + fenugreek leaves (20 g). Four g of groundnut oil and 1.5 g each of salt and spices were added to the above. An additional group was fed a 10% casein diet reinforced with salt and vitamin mixtures for comparison. One more group was fed Modern bread in order to compare the overall nutritive value of this with a mixture of cereal, legume and greens. The comparative nutritive values of these diets are shown in Table 32. The inclusion of 'Modern bread' in these studies was governed by the following considerations. Modern bread is prepared from flour enriched with lysine, calcium, iron, thiamine, riboflavine, niacin and vitamin A and was introduced in 1969 as a potential remedy for the nutritional ills of this country. However, it costs more than twice as much as a diet based on wheat, bengal gram and greens and this difference in cost is significant for the poor who are obliged to spend more than 80% of their income on food and are yet undernourished. Only the upper classes are found to consume bread regularly and that too not more than 2-3 slices twice or thrice a week. The total production of

Table 32 : Nutritive value of the diet (values expressed per 100 g dry weight)*.

	unsupplemented		supplemented***					Modern bread**	10% casein diet
	wheat	maize	wheat	maize	rice	bajra	jowar		
calories	380	380	400	400	370	370	370	327	380
protein (g)	10.8	10.2	13.1	12.6	9.5	12.9	12.1	10.4	10.0
calcium (mg)	38	9	132	110	110	121	121	80	440
iron (mg)	4.5	1.8	9.0	6.9	7.7	9.0	9.6	5.0	5.6
thiamine (mg)	0.44	0.39	0.41	0.38	0.14	0.33	0.24	1.46	0.75
riboflavine (mg)	0.16	0.09	0.23	0.18	0.15	0.28	0.20	0.32	1.25
nicotinic acid (mg)	5.0	1.7	4.5	1.9	2.0	2.2	2.8	1.35	1.5
vitamin A (mcg)	59	82	620	638	575	668	608	137	70-100

* On the basis of values given by Gopalan, Ramasastri and Balasubramanian, (1971) except in the case of maize, carotene and in the case of bajra, calcium which were obtained by analysis.

** Values given by Modern Bakeries (India) Ltd., Ahmedabad (India) except in the case of calcium and carotene which were obtained by analysis.

*** With bengal gram and fenugreek leaves.

bread in this country is less than 1 loaf per capita per year. Further, the substitution of bread made of refined flour for chapaties made of whole wheat flour hardly seems to represent progress. The superior nutritive value of whole wheat flour as compared to refined flour was pointed by McCarrison (1936) several decades ago. The approach in this department has been, as mentioned earlier, to advocate the use of cheap and locally available foods. The present studies were part of several attempts to investigate the nutritional validity of this approach.

The data on food intake and weight gain are presented in Table 33. As in several previous experiments in this laboratory, wheat was found to promote a greater weight gain than maize. Supplementation with bengal gram and leafy vegetables increased weight gain in both cases, but the percentage increase was greater in the case of maize. However, even with both supplements weight gain was less than with the 10% casein diet except in the case of wheat. Somewhat smaller weight gains were obtained with the other combinations. It must be pointed out that the weight gain with rice might have been greater had parboiled rice been used instead of milled rice. The former is generally consumed by the poor.

The diets differed in both calcium and protein contents. They also differed in protein value on the basis of differences in weight gain. The approximate protein value of the diet in

Table 33 : Comparative nutritive value to rats of supplemented and unsupplemented cereal or millet diets and Modern bread.

	unsupplemented		supplemented*					Modern bread	10% casein diet
	wheat	maize	wheat	maize	rice	bajra	jowar		
					mean ± s.e.				
food intake (g/day)	6.5 ±0.19	5.0 ±0.28	8.7 ±0.23	7.2 ±0.20	6.8 ±0.29	7.9 ±0.15	8.0 ±0.42	8.1 ±0.46	8.5 ±0.31
protein intake (g/day)	0.70	0.51	1.14	0.91	0.65	1.02	0.97	0.84	0.85
body weight (g)									
initial	43 ±1.6	43 ±1.0	43 ±0.7	45 ±1.5	46 ±1.7	46 ±1.1	46 ±0.8	44 ±0.8	45 ±1.6
terminal	94 ±3.0	66 ±3.0	136 ±4.0	110 ±4.3	111 ±1.6	121 ±4.6	113 ±2.4	96 ±3.2	143 ±8.2
weight gain (g)									
(a) total	51 ±3.3	24 ±3.0	94 ±5.4	65 ±3.9	66 ±2.9	75 ±5.2	68 ±2.4	50 ±3.1	97 ±8.7
(b) per 100 kcal intake	3.7	2.3	4.8	4.0	4.7	4.6	4.1	3.4	5.4

Period of treatment, 8 weeks; 5-8 animals used in each group.

* With bengal gram and fenugreek leaves.

terms of casein was estimated on the basis of weight gains of animals fed different levels of casein in other studies and these estimates are also show in Table 34. According to these comparisons, maize and wheat produced weight gains comparable to those produced by 5 and 7% casein diets. A combination of wheat, bengal gram and greens must be deemed to be equal to a 10% casein diet and the other combinations, to one containing 8-9%.

Table 34 : Weight gain in relation to dietary protein content*.

% dietary protein (casein)	weight gain (g) per week
4	0
5	3.0 (2.0 - 5.0)
6	3.5 (2.0 - 5.0)
7	6.0 (4.0 - 10.0)
8	8.0 (7.0 - 11.0)
10	12.0 (10.0 - 15.0)
15	16.0 (13.0 - 20.0)
20	17.0 (13.0- 22.0)

* data compiled from several experiments in this laboratory; range given in parentheses.

The data on the composition of femur are shown in Table 35. Femur weight was less in all the groups as compared to the casein fed group but the reduction was more or less proportional to that in body weight. However, the deficits in dry weight, fat-free weight, ash and calcium contents were much greater than those in body weight.

Both the supplemented and unsupplemented cereals diets were associated with smaller amounts of bone ash and calcium and greater amounts of moisture and fat. These differences were greater with the unsupplemented diets. Supplementations of bengal gram and leafy vegetable resulted in decreasing moisture and fat and increasing ash and calcium contents (Table 35a). As the supplemented and unsupplemented diets differed in both protein value and calcium content it is not possible to identify the contribution of either factor to this improvement. In other studies in this department bone changes similar to those in protein deficiency are also found in calcium deficiency. This would appear to be the case in the present studies also on a comparison of diets not differing much in protein value (e.g. rice and bajra diets).

The animals fed the wheat diets fared better than those fed other grains. This is not surprising as wheat contains 41 mg calcium per 100 g as against 10 mg in maize and rice and 25 mg in jowar and bajra (Table 31). In diets providing

Table 35 : Composition of femur in rats fed supplemented and unsupplemented cereal or millet diets and Modern bread.

	unsupplemented		supplemented*					Modern bread	10% casein diet (controls)
	wheat	maize	wheat	maize	rice	bajra	jowar		
group no.	1	2	3	4	5	6	7	8	9
					mean ± s.e.				
terminal body weight (g)	94 ± 3.0 (66)	66 ± 3.0 (46)	136 ± 4.0 (95)	110 ± 4.3 (77)	111 ± 1.6 (78)	121 ± 4.6 (85)	113 ± 2.4 (79)	96 ± 3.2 (67)	143 ± 8.2
composition of femur									
length (cm)	2.5 ± 0.04 (89)	2.3 ± 0.05 (82)	2.7 ± 0.04 (96)	2.5 ± 0.02 (89)	2.5 ±0.04 (89)	2.7 ± 0.03 (96)	2.6 ± 0.06 (93)	2.6 ± 0.04 (93)	2.8 ± 0.04
wet weight (mg)	313 ± 10.3 (80)	226 ± 8.0 (58)	349 ± 11.9 (89)	245 ± 8.7 (62)	259 ± 8.1 (66)	304 ± 11.0 (77)	265 ± 2.6 (67)	264 ± 11.2 (67)	393 ± 13.7
dry weight (mg)	158 ± 7.1 (60)	111 ± 2.9 (42)	189 ± 5.9 (72)	131 ± 5.3 (50)	135 ± 2.0 (52)	162 ± 3.8 (62)	140 ± 5.1 (53)	137 ± 3.5 (52)	262 ± 6.3
fat-free dry weight (mg)	135 ± 5.6 (54)	91 ± 3.2 (36)	176 ± 5.6 (70)	120 ± 4.7 (48)	123 ± 2.0 (49)	150 ± 4.8 (60)	129 ± 4.9 (52)	119 ± 3.6 (48)	250 ± 7.1

contd.

Table 35 contd.

	unsupplemented		supplemented*					Modern bread	10% casein diet (controls)
	wheat	maize	wheat	maize	rice	bajra	jowar		
group no.	1	2	3	4	5	6	7	8	9
					mean ± s.e.				
ash weight (mg)	54 ± 2.9 (35)	36 ± 2.6 (23)	84 ± 3.8 (55)	54 ± 3.2 (35)	50 ± 2.2 (32)	66 ± 2.9 (43)	56 ± 4.4 (36)	48 ± 1.6 (31)	154 ± 3.1
calcium content of femur (mg)	20.5 ± 0.59 (36)	13.6 ± 0.91 (24)	32.3 ± 1.33 (56)	19.5 ± 0.94 (34)	19.1 ± 0.25 (33)	24.5 ± 1.18 (43)	21.5 ± 1.23 (37)	17.4 ± 0.55 (30)	57.6 ± 1.42
calcium (mg) per 100 g of body weight	21.8 ± 0.68 (57)	20.6 ± 0.42 (54)	23.7 ± 0.95 (62)	17.8 ± 0.51 (46)	16.6 ± 0.74 (43)	20.4 ± 0.96 (53)	19.0 ± 1.32 (49)	18.2 ± 0.79 (47)	38.4 ± 1.42
A:R ratio	0.69 ± 0.058 (43)	0.68 ± 0.062 (43)	0.93 ± 0.038 (58)	0.84 ± 0.071 (53)	0.69 ± 0.036 (43)	0.79 ± 0.023 (49)	0.77 ± 0.065 (48)	0.67 ± 0.014 (42)	1.60 ± 0.045

Period of treatment, 8 weeks; 5-8 animals used in each group. Values given in parentheses are percentages of controls.

* With bengal gram and fenugreek leaves.

Table 35a : Composition of femur in rats fed supplemented and unsupplemented cereal or millet diets and Modern bread.

	unsupplemented		supplemented*					Modern bread	10% casein diet
	wheat	maize	wheat	maize	rice	bajra	jowar		
					g per 100 g fresh bone				
moisture	49.5	50.8	45.8	46.5	47.8	46.7	47.1	48.1	33.4
fat	7.3	8.8	3.7	4.4	4.6	3.9	4.1	6.8	3.0
ash	17.2	15.9	24.0	22.0	19.3	21.7	21.1	18.1	39.1
calcium	6.5	6.0	9.3	8.0	7.4	8.1	8.1	6.6	14.7
					g per 100 g fat-free bone				
moisture	53.4	55.8	47.6	48.7	50.2	48.6	49.2	51.6	34.3
ash	18.6	17.4	25.0	23.0	20.2	22.6	22.0	19.5	40.4
calcium	7.1	6.6	9.6	8.3	7.7	8.4	8.5	7.1	15.1
					g per 100 g dry bone				
fat	14.5	18.0	6.8	8.3	8.8	7.4	7.8	13.1	4.5
ash	34.1	32.4	44.4	41.2	37.0	40.7	40.0	35.0	58.7
calcium	13.0	12.3	17.1	14.9	14.1	15.1	15.4	12.7	22.0
					g per 100 g fat-free dry bone				
ash	40.0	39.5	47.7	45.0	40.6	44.0	43.4	40.3	61.6
calcium	15.2	14.9	18.4	16.3	15.5	16.3	16.7	14.6	23.0
					g per 100 g ash				
calcium	38.0	37.8	38.5	36.1	38.2	37.1	38.4	36.3	37.4

* With bengal gram and fenugreek leaves.

Table 35b : Statistical significance of the data of Table 35.

groups compared	significance of different between means								
	1 ∽ 2	1 ∽ 3	2 ∽ 4	3 ∽ 8	3 ∽ 9	3 ∽ 4	3 ∽ 5	3 ∽ 6	3 ∽ 7
					p less than				
terminal body weight (g)	0.001	0.001	0.001	0.001	N.S.	0.001	0.001	0.05	0.001
composition of femur									
length (cm)	N.S.	0.01	0.01	N.S.	N.S.	0.001	0.01	N.S.	N.S.
wet weight (mg)	0.001	0.05	N.S.	0.001	0.05	0.001	0.001	N.S.	0.001
dry weight (mg)	0.001	0.01	0.01	0.001	0.001	0.001	0.001	0.01	0.001
fat-free dry weight (mg)	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001
ash weight (mg)	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.01	0.001
calcium content of femur (mg)	0.001	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.001
calcium (mg) per 100 g of body weight	N.S.	N.S.	0.01	0.001	0.001	0.001	0.001	0.05	N.S.
A:R ratio	N.S.	0.01	N.S.	0.001	0.001	N.S.	0.001	0.01	N.S.

N.S. = not significant.

300-400g of cereals, this would make a difference in calcium content of 90-120 mg, a critical amount in diets in which the total supply is of the order of 300 mg. As mentioned earlier in preliminary studies carried out in this department, skeletal development appeared to be somewhat more delayed in children in a tribal area where maize is the staple and in Madras where rice is the staple than in Baroda and Uttar Pradesh where wheat and bajra are the staples.

In the present studies the differences between the basal and supplemented diets were less than those in the previous studies on kodri and kodri + lysine. This might have been because calcium was a limiting nutrient in the present case.

In the experiments described earlier in which protein or energy was the limiting nutrient, even when the animals were growth retarded, the amount of calcium in femur in relation to body weight was either not affected or increased. In these studies, calcium was not a limiting nutrient. It is interesting to note that in the present experiment femur calcium in relation to body weight was depressed with diets deficient in calcium.

It is interesting to note that animals fed Modern bread fared no better than those fed unsupplemented wheat. This must be presumed to be because bread is prepared with refined flour and not all the nutrients removed during refinement are restored during enrichment. Further, cereals contain different proteins such as albumin, globulin, protamine and glutelin and the former two, rich in lysine, methionine and tryptophan and present in the embryonic protein (Naik and Das, 1972) are removed during refinement. On the other hand, protamine and glutelin present in the endosperm and retained in the refined flour are deficient in these amino acids. Several decades ago McCarrison (1936) found that animals fed refined flour fared worse than those fed whole wheat flour even when vitamins and minerals were added to the former. Similar observations were made in studies in this laboratory aimed at the role of studying the effects of phytate in wheat flour (Varkey, 1967). It is interesting to note that animals fed Modern bread were not found to show a better bone status than those fed unsupplemented wheat diet containing much less calcium (38 mg per 100 g as compared to 80 mg in Modern bread) inspite of the fact that whole wheat flour contains more phytate. Similar observations were made in earlier experiments in which animals fed whole wheat flour or the same with bran added showed better growth and bone calcification than those fed refined flour although salt and vitamin mixtures were added to all the diets. The data are reproduced as given in Table 36.

The results are far from supporting the wisdom of advocating Modern bread or other bread made from refined flour for the alleviation of malnutrition. It is noteworthy

Table 36 : Weight gain and calcium content of femur in rats fed refined wheat flour or whole wheat flour with or without bran*.

diet	weight gain (g/week)	calcium content of femur (mg)
refined wheat flour	2	18.3 ± 1.5
whole wheat flour	4.5	22.5 ± 1.4
90 g whole wheat flour + 10 g bran	4.5	22.8 ± 1.2

Period of treatment, 4 weeks; 6 animals used in each group.
* Varkey (1967).

in this connection that in war-time Britain, the manufacture of bread without a certain proportion of whole wheat flour was prohibited although the nutritional status of the average Briton was much more satisfactory even during the war than that of the poor man in this country in normal times.

It is interesting to note that in the present studies, bone growth was apparently maintained in spite of grossly deficient intakes of calcium. The animals used in the present studies were four weeks old at the start of treatment. In studies described earlier, three week old animals in the stock colony were found to have an average femur length of 1.6 cm; weight of 118.5 mg and calcium content of 6.2 mg. In spite of grossly deficient intakes, some thing like these values must have increased to the values found at the end of treatment in the present experiment with cereal based diets.

As a matter of fact, in the group receiving the unsupplemented maize and wheat diets, the dietary calcium was barely sufficient to meet the needs of endogenous metabolism as a diet containing 4% protein are found to be required for maintenance of body weights in these animals. According to Mitchell (1962) the amount required for meeting the maintenance requirements for calcium is of the order of 1% of protein requirements so that the calcium concentration of the diet should be 40 mg. The unsupplemented wheat diet provided 38 mg and the maize diet, 9 mg.

Even if we assume that endogenous losses of calcium are drastically reduced with a calcium deficient diet, the question arises whether the calcium content of the diet was adequate to promote the observed increments in bone. In other studies in this laboratory, calcium is found to form 0.8% of body weight. With a body weight increment of 0.4 g per day the normal accretion of calcium per day would be about 3 mg. Thus the calcium economy of these animals seems to have been rather precarious and its utilization efficient.

Further studies are needed on calcium retention and the calcium content of the whole body to understand the calcium economy of these animals.

In summary, the nutritional improvement of food grains such as maize and wheat by the addition of legumes and greens is found to have a favourable effect on both skeletal and overall growth presumably because of increase in the calcium and protein contents of the diet together with an improvement in protein quality. Although the improved diets were grossly deficient in calcium for the rat, they may not be so for man except in the case of very young children as they would provide about 275-325 mg per 1000 calories.