CHAPTER III

RESULTS AND DISCUSSION

Experiment-I

THE PATTERN OF CHANGES IN THE COMPOSITION OF SELECTED BONES DURING GROWTH AND MATURATION IN THE RAT

As pointed out earlier, 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 and composition. Studies were made of the pattern of changes in the chemical composition of bone during development and the possible variations in this pattern in different bones. Data on bone compositions were obtained in rats at birth and at 1, 2, 3, 5, 9, 12 and 26 weeks of age. For certain ages namely, 3, 9 and 12 weeks, data obtained for control animals in experiments designed to study the effects of nutritional stress were taken and for the other ages the animals chosen were representative of their age group for body weight.

The results are presented in Tables 14-a, 15-a and 16-a. Further data derived from the values are presented in tables 17-a, b and c, in order to facilitate comparison with published values as different investigators have given the values in terms of fresh weight (Nakamoto and Miller, 1977; Rajalakshmi and Dave, 1977), dry weight (Pavlovitch et al., 1977; Le Roith and Pimstone, 1973) and fat-free dry weight (Dickerson, 1962; Wolinsky and Guggenheim, 1970).

The tabulation enables the study of the data from different angles. The values for moisture, total lipid, ash, hydroxyproline, hexosamine and uronic acid are calculated as g per 100 g of fresh bone.

Although moisture content is very difficult to measure reliably, the values obtained are presented to get an overall picture as maturation of the bone is associated with changes in moisture content. The pattern of the results obtained gives us a reasonable degree of confidence about their validity.

It may be added that whatever criticism applies to moisture applies also in some measure to lipids and ash and that variations in these components have been used for monitoring changes with maturation (Dickerson, 1962; Sakai et al., 1969) as well as nutritional stress (Widdowson and McCance, 1960; Dickerson and McCance, 1961). In previous studies in this laboratory the values for moisture as determined gravimetrically were compared with those derived by calculation as the difference between the total bone weight and the major components other than moisture, namely, lipids, proteins (taken as N x 6.25) and ash. The values were found to compare well before 3 weeks of age. Thereafter, some disparity was found (Table 12). This could be due to several factors including failure to determine moisture and lipids not extracted by the procedure used. It has been suggested that the bone should be dried

Table 12: Chemical composition of femur[@]

Age (in weeks)	g per 100 g of fresh bone: At birth	1	2	3	13	38
Moisture (a)	74.3	72.6	71.7	64.7	35.7	31.5
Total Lipid (b)	1.1	0.8	0.9	1.3	2.0	2.0
Ash (c)	7.4	7.0	9.1	12.7	38.2	43.2
Protein Determined (d) (N x 6.25)	16.9	17.5	16.9	18.1	18.1	16.9
Protein calculated $\{100 - (a+b+c)\}$	17.2	19.6	18.3	21.3	24.1	23.3
$\frac{\text{Protein Determined}}{\text{Protein Calculated}} \times 100$	98	89	92	85	75	73
Moisture calculated $\{100 - (b+c+d)\}$	75.6	74.7	73.1	68.9	41.7	37.9

@ - Data taken from Dave, (1976)

at 100°C in order to achieve complete dehydration. Even so, the difference observed between birth and 38 weeks of age (43%) is large enough to be significant. Therefore the data may be taken as valid for what may be considered a 'labile' component.

On the basis of previous studies in this laboratory (Dave, 1976), although body and bone growth continue in the rat well beyond 13 weeks of age the rates of growth as judged by percentage increments level off after 13 weeks (Table 13). The present studies were therefore, done till 26 weeks of age and the data for this age used as a standard for comparison.

The body weights in these studies conformed to the normal pattern of development expected, the rate of growth being rapid during the initial stages, but declining progressively with age (Tables 14 a and c). In the following discussion of the parameters studied, the bone weights and morphometric measurements are considered first followed by the bone chemical composition.

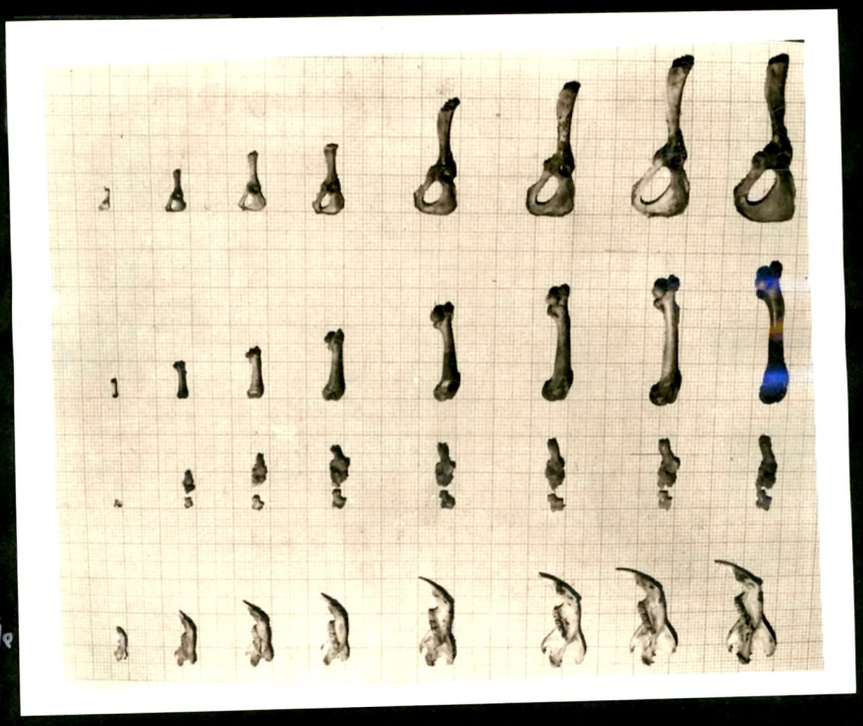
In figure 5, the four bones studied, namely, pelvis, femur, tarsus and mandible are shown in their various stages of growth from birth to 26 weeks.

The pattern of growth with regard to bone as judged by weights and lengths was essentially similar to that of

Table 13: Per cent increment per week in the body weight, bone weight and length during the development in the rat (Dave, 1976)

Age (weeks)	0-1	1-2	2-3	3-4	4-8	8-13	13-26	26-52
Body weight	141	86	54	50	33	17	2	2.0
Bone length	59	35	29	17	11	5	0.4	0.5
Bone weight	320	151	70	49	18	12	1	1.7

Figure: 5 Stages of development in selected bones



whole body growth. But the percentage of increments in the weights of the bones (Table 14-c) 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, e.g. muscle or body fat.

The bone values corresponding to 25% and 50% of the 26 week values were reached much earlier in the case of bone length (Table 14-b) and somewhat later in the case of other parameters (Table 15-b). It was also observed that these values were reached earlier in the case of tarsus and mandible than for femur and pelvis. The percentage increments were comparable in the case of pelvis and femur.

The ratio of the internal width of the pelvic bone to its length remained almost constant (Table 14-a). A similar observation was made by Harrison (1958) who suggested that pelvic growth in rat is not subjected to specialized local control, but is regulated by general growth determinants. This was also true of the ratio of mandible width at the broadest region to the length. On the other hand the ratio of the epiphyseal width to the length of femur at 26 weeks was half the value at birth, as may be expected in a 'long' bone in which elongation would be greater than widening.

Table 14-a: Bone size and Morphology

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
No. of animals	6	6	5	6	6	7	6	7
Body weight (g)	6.0 ±0.06	12.7 ±0.36	23.6 ±0.97	43 ±0.78	88 ±3.3	170 ±3.4	281 ±18.3	389 ±5.1
Fresh weight (mg):								
Mandible	12.6 ±0.54	30.5 ±0.78	51.2 ±1.29	81 ±2.0	118 ±1.8	224 ±1.5	241 ±3.0	346 ±9.7
Femur	10.5 ±0.18	29.2 ±1.07	94.0 ±4.14	164 ±1.2	295 ±16.1	539 ±10.4	652 ±40.2	1006 ±19.2
Pelvis	8.1 ±0.20	20.2 ±0.23	61.0 ±1.78	100 ±0.3	210 ±10.7	437 ±7.3	525 ±20.0	830 ±13.3
Tarsus	4.7 ±0.17	11.5 ±0.40	48.6 ±1.44	75 ±1.9	108 ±4.0	160 ±4.9	176 ±10.0	184 ±5.8

Table 14-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Bone measurements (cm):								
Length:								
Mandible	0.96 ±0.015	1.32 ±0.030	1.65 ±0.013	2.05 ±0.019	2.20 ± 0.019	2.41 ±0.034	2.60 ±0.035	2.89 ±0.034
Femur	0.68 ±0.041	0.95 ±0.030	1.37 ±0.010	1.85 ±0.030	2.29 ±0.099	2.98 ±0.045	3.26 ±0.065	3.90 ±0.040
Pelvis	0.79 ±0.023	1.05 ±0.047	1.57 ±0.018	2.10 ±0.038	2.64 ±0.080	3.47 ±0.044	3.83 ±0.077	4.60 ±0.064
Tarsus	0.32 ±0.015	0.57 ±0.010	0.75 ±0.008	1.10 ±0.015	1.17 ±0.022	1.20 ±0.023	1.23 ±0.015	1.26 ±0.02
Width:								
Mandible	0.43 ±0.014	0.57 ±0.017	0.78 ±0.007	0.94 ±0.017	1.06 ± 0.018	1.22 ±0.018	1.37 ±0.035	1.43 ±0.023
Femur	0.26 ±0.029	0.27 ±0.009	0.41 ±0.005	0.54 ±0.006	0.57 ± 0.018	0.65 ±0.013	0.69 ±0.017	0.75 ±0.014
Pelvis	0.37 ±0.012	0.46 ±0.031	0.71 ± 0.009	0.96 ±0.015	1.07 ±0.014	1.34 ±0.029	1.49 ±0.026	1.84 ±0.026

Table 14-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
Width/Length:								
Mandible	0.45	0.49	0.48	0.46	0.50	0.51	0.53	0.50
Femur	0.38	0.29	0.30	0.29	0.25	0.22	0.21	0.19
Pelvis	0.47	0.47	0.45	0.46	0.41	0.39	0.39	0.40
Length ratio:								
Mandible/Femur	1.41	1.39	1.20	1.11	0.93	0.79	0.79	0.74
Pelvis/Femur	1.12	1.11	1.16	1.13	1.15	1.18	1.17	1.18
Tarsus/Femur	0.47	0.60	0.55	0.59	0.51	0.40	0.38	0.32
Bone weight/Body weight (mg/g):								
Mandible	2.1	2.6	2.0	1.9	1.3	1.3	0.95	0.9
Femur	1.8	2.4	3.7	3.8	3.4	3.0	2.30	2.6
Pelvis	1.4	1.7	2.4	2.3	2.4	2.6	1.90	2.1
Tarsus	0.8	0.9	2.0	1.8	1.2	0.9	0.60	0.5

Table 14-b

Per cent of value at 26 weeks

Postnatal age (week)	0	1	2	3	5	9	12	26
Body weight:	1.5	3.3	6.1	10.9	22.5	43.7	72.0	100
Fresh weight:								
Mandible	3.6	8.8	14.7	23.5	34.2	64.7	69.7	100
Femur	1.0	3.0	9.3	16.2	29.9	53.5	64.6	100
Pelvis	1.0	2.4	7.3	12.0	25.3	52.6	63.3	100
Tarsus	2.6	6.3	26.7	40.7	58.7	88.0	95.6	100
Bone measurements:								
Length:								
Mandible	33.2	45.7	56.7	70.9	74.0	83.4	90.0	100
Femur	17.4	24.4	35.1	47.4	58.7	75.9	83.6	100
Pelvis	17.1	22.8	34.1	45.4	57.4	75.7	83.3	100
Tarsus	25.4	45.3	59.5	87.3	92.8	95.2	97.6	100
Width:								
Mandible	30.1	39.9	49.7	65.7	74.1	85.3	95.8	100
Femur	34.7	36.0	54.7	72.0	76.0	85.3	92.0	100
Pelvis	20.1	25.0	38.6	52.2	58.2	73.4	81.0	100

Table 14-c

per cent increment per week

Postnatal age (week)	0-1	1-2	2-3	3-5	5-9	9-12	12-26
Body weight:	112	86	82	53	24	22	2.7
Fresh weight:							
Mandible	142	69	57	23	22	3	0.3
Femur	178	221	74	40	21	7	4.0
Pelvis	149	202	64	55	27	7	4.0
Tarsus	145	328	54	20	12	4	0.6
Bone measurements:							
Length:							
Mandible	38	24	26	4	2.4	1.2	0.8
Femur	40	44	35	12	7.0	3.3	1.4
Pelvis	33	49	33	13	8.0	3.3	1.4
Tarsus	78	32	47	3	0.7	0.8	0.2
Width:							
Mandible	33	25	32	6	4	4	0.3
Femur	4	51	32	3	3	3	0.6
Pelvis	24	54	35	6	6	3	1.6

The bone maturation is associated with an increase in the ash content and consequently in the A:R ratio (ratio of ash to non-ash components in the fat-free dry bone). As might be expected this was associated with an increase in calcium and phosphorus. The maturation is also accompanied by a decrease in the moisture content.

As seen from the tables 15-a and c the increment of mineral in all the bones was high during the suckling period. This is in agreement with the observation of Kuftinec and Miller (1975) that in rat the major portion of mineralization occurs at approximately 8-19 days post partum. Also it is consistent with the finding that demands for calcium and phosphorus by suckling rats are very high and that rat milk is rich in these nutrients (Spray, 1950). The concentration of calcium in rat milk is 345 mg per dl as compared with about 30 mg per dl in human milk (Cox and Mueller, 1937) and lactation seems to be associated in the rat with considerable depletion of calcium from the maternal skeleton. Even when a diet with an adequate Ca content is given, 15-25% of femur Ca may be lost (Warnock and Duckworth, 1944). It may be mentioned here that among the different bones of the mother, the femur seems to be sensitive to this demand (Rasmussen, 1977) and that it is the ends, not the shafts of bones which provide the source of available calcium (Warnock and Duckworth, 1944).

Table 15-a: The pattern of changes in the composition of selected bones during growth and maturation in the rat.

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
No. of animals	6	6	5	6	6	7	6	7
Body weight (g)	6.0 ±0.06	12.7 ±0.36	23.6 ±0.97	43 ±0.78	88 ±3.3	170 ±3.4	281 ±18.3	389 ±5.1
Fresh weight of bones (mg):								
Mandible	12.6 ±0.54	30.5 ±0.78	51.2 ±1.29	81 ±2.0	118 ±1.8	224 ±1.5	241 ±3.0	346 ±9.7
Femur	10.5 ±0.18	29.2 ±1.07	94.0 ±4.14	164 ±1.2	295 ±16.1	539 ±10.4	652 ±40.0	1006 ±19.2
Pelvis	8.1 ±0.20	20.2 ±0.23	61.0 ±1.78	100 ±0.3	210 ±10.7	437 ±7.3	525 ±20.0	830 ±13.3
Tarsus	4.7 ±0.17	11.5 ±0.40	48.6 ±1.44	75 ±1.9	108 ±4.0	160 ±4.9	176 ±10.0	184 ±5.8

Table 15-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Dry weight (mg):								
Mandible	4.1 ±0.19	11.3 ±0.36	21.2 ±0.82	39 ±1.4	69 ±2.1	161 ±1.5	179 ±4.1	279 ±7.6
Femur	2.6 ±0.08	7.7 ±0.34	30.0 ±0.91	57 ±1.3	132 ±6.2	305 ±5.9	414 ±28.0	691 ±15.7
Pelvis	2.1 ±0.06	5.9 ±0.14	21.5 ±0.63	39 ±0.2	100 ±5.4	243 ±2.1	326 ±14.0	560 ±13.5
Tarsus	0.8 ±0.09	2.8 ±0.18	13.3 ±0.41	32 ±0.5	63 ±2.6	109 ±3.7	123 ±5.0	143 ±4.7
Fat-free dry weight (mg)*:								
Femur	2.4 ±0.08	6.4 ±0.22	27.4 ±0.76	54 ±1.2	126 ±6.0	297 ±3.8	402 ±28.0	683 ±15.3
Pelvis	1.9 ±0.06	5.3 ±0.14	20.0 ±0.59	37 ±0.4	95 ±5.4	232 ±2.0	316 ±14.0	552 ±13.3
Tarsus	0.7 ±0.04	1.9 ±0.06	11.3 ±0.37	27 ±0.5	54 ±2.2	99 ±3.6	117 ±5.6	138 ±4.4

Table 15-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Ash: (mg)								
Mandible	2.0 ±0.13	5.7 ±0.12	10.9 ±0.41	21.0 ±0.73	39 ±1.0	101 ±1.0	113 ±2.6	194 ±4.9
Femur	0.65 ±0.040	2.2 ±0.11	10.0 ±0.30	20.4 ±0.47	62 ±3.8	168 ±3.1	237 ±17.9	448 ±14.7
Pelvis	0.57 ±0.023	1.9 ±0.08	7.7 ±0.20	15.3 ±0.37	45 ±3.0	131 ±2.5	176 ±9.9	341 ±7.4
Tarsus	negligible	0.37 ±0.010	2.9 ±0.16	10.2 ±0.30	26 ±1.7	55 ±2.4	66 ±3.0	82 ±3.1
Collagen (Hydroxyproline) (mg):								
Mandible	0.101 ±0.0098	0.218 ±0.0139	0.49 ±0.031	0.88 ±0.011	1.68 ±0.068	3.29 ±0.240	4.06 ±0.169	5.52 ±0.449
Femur	0.058 ±0.0053	0.183 ±0.0170	0.68 ±0.039	1.53 ±0.052	3.06 ±0.216	6.40 ±0.160	7.81 ±0.480	11.55 ±0.234
Pelvis	0.049 ±0.0051	0.143 ±0.0051	0.51 ±0.020	1.03 ±0.053	2.48 ±0.095	5.20 ±0.186	6.88 ±0.300	10.23 ±0.473
Tarsus	0.023 ±0.0011	0.091 ±0.0058	0.47 ±0.008	1.07 ±0.052	1.86 ±0.057	2.93 ±0.174	3.59 ±0.099	4.59 ±0.298

Table 15-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Total MPS (Hexosamine) (mg):								
Mandible	0.044 ±0.0030	0.063 ±0.0026	0.10 ±0.010	0.21 ±0.011	0.27 ±0.030	0.41 ±0.046	0.48 ±0.035	0.50 ±0.020
Femur	0.092 ±0.0040	0.267 ± 0.0183	0.44 ±0.016	0.65 ±0.029	0.74 ±0.092	0.93 ±0.033	1.03 ±0.042	1.21 ±0.050
Pelvis	0.054 ±0.0016	0.165 ± 0.0120	0.19 ±0.011	0.38 ±0.014	0.53 ±0.035	0.74 ±0.046	0.81 ±0.027	1.00 ±0.031
Tarsus	0.062 ±0.0020	0.129 ± 0.0100	0.28 ±0.021	0.33 ±0.013	0.34 ± 0.014	0.38 ±0.025	0.39 ±0.034	0.48 ±0.037

* - the amount of fat was not measurable with realiability in mandible

Table 15-b: Per cent of value at 26 weeks

Postnatal age (week)	0	1	2	3	5	9	12	26
Body weight	1.5	3.3	6.5	10.9	23	44	72	100
Fresh weight:								
Mandible	3.6	8.8	14.7	23.5	34	65	70	100
Femur	1.0	3.0	9.3	16.2	30	54	65	100
Pelvis	1.0	2.4	7.3	12.0	25	53	63	100
Tarsus	2.6	6.3	26.7	40.7	59	88	96	100
Dry weight:								
Mandible	1.5	4.0	7.5	13.9	25	58	64	100
Femur	0.4	1.1	4.3	8.2	19	44	60	100
Pelvis	0.4	1.0	3.8	6.9	18	43	58	100
Tarsus	0.6	2.0	9.6	23.4	44	76	86	100
Fat-free dry weight:								
Femur	0.4	0.9	4.0	7.9	18	43	59	100
Pelvis	0.3	0.9	3.6	6.7	17	42	57	100
Tarsus	0.5	1.4	8.1	19.4	39	72	85	100

Table 15-b continued

Postnatal age (week)	0	1	2	3	5	9	12	26
Ash:								
Mandible	1.0	2.9	5.6	10.8	20	53	58	100
Femur	0.2	0.5	2.2	4.6	14	38	53	100
Pelvis	0.2	0.5	2.5	4.5	13	38	52	100
Tarsus	N.D.	0.5	4.1	12.4	31	67	80	100
Collagen (Hydroxyproline):								
Mandible	1.8	3.9	8.9	16.1	30	60	74	100
Femur	0.5	1.6	5.9	13.2	27	56	67	100
Pelvis	0.5	1.4	5.0	10.0	24	51	67	100
Tarsus	0.5	1.9	10.2	23.4	41	64	78	100
Mucopolysaccharide (Hexosamine):								
Mandible	8.8	12.6	20.2	42.0	54	82	96	100
Femur	7.6	22.1	36.4	54.0	61	77	85	100
Pelvis	5.4	16.5	19.0	37.6	53	74	81	100
Tarsus	12.9	26.9	58.3	68.0	71	79	81	100

N.D. - Not determined

Table 15-c: per cent increment per week

Postnatal age (week)	0-1	1-2	2-3	3-5	5-9	9-12	12-26
Body weight:	112	86	82	53	24	22	2.7
Fresh weight:							
Mandible	142	69	57	23	22	3	0.3
Femur	178	221	74	40	21	7	4.0
Pelvis	149	202	64	55	27	7	4.0
Tarsus	145	328	54	20	12	4	0.6
Dry weight:							
Mandible	173	88	83	37	33	4	4.0
Femur	196	290	90	62	32	12	5.0
Pelvis	181	264	86	80	38	12	5.4
Tarsus	211	392	132	49	18	5	1.1
Fat-free dry weight:							
Femur	185	327	92	65	33	12	5.0
Pelvis	191	292	85	80	35	12	5.3
Tarsus	184	500	137	50	20	7	1.2

Table 15-c continued

Postnatal age (week)	0-1	1-2	2-3	3-5	5-9	9-12	12-26
Ash:							
Mandible	183	93	94	43	40	4	5.1
Femur	238	354	104	97	45	13	6.0
Pelvis	233	307	99	94	49	11	6.7
Tarsus	-	684	252	78	28	7	1.7
Hydroxyproline:							
Mandible	110	133	80	44	24	8	2.5
Femur	216	272	125	50	30	7	3.4
Pelvis	200	248	101	73	27	11	3.5
Tarsus	278	479	112	49	10	8	2.0
Hexosamine:							
Mandible	16	100	104	16	13	5	0.5
Femur	102	130	49	9	6	4	1.2
Pelvis	72	108	76	20	4	6	1.0
Tarsus	108	117	17	8	3	1	1.6

In the early stages of development, the Ca:P ratio (Table 16-b) is low and 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; Posner, 1971). In the rat the average apatite crystallinity is reported to increase through the first 47 days with little change thereafter (Menczel et al., 1965). Theoretically the Ca:P value for hydroxyapatite is 2.1, that in the whole body being 1.8. The Ca:P ratio in the rat epiphysis was reported to be 1.0 at birth and increased to 1.9 at 21 days attaining a value of 2.2 at 158 days (Dickerson, 1962). The Ca:P ratio is known to vary markedly under different nutritional conditions.

The A:R ratio increases steadily during the course of the development of bones (Table 16-b). Since mandible is highly mineralised it has the highest A:R ratio at any age. A similar increase is found in the calcium:hydroxyproline ratio and this is due to progressively increasing mineralization of the matrix.

The calcium:hydroxyproline ratio (Table 16-b) is low in the case of tarsal bone, due to the presence of excess non-bone collagen. The non-bone collagen includes the tendon, part of which lies buried in the tarsal bone and the fibres which link together the members of the tarsal bone. These fibres are not easily removable at the time of dissection.

Table 16-a: The pattern of changes in the composition of selected bones during growth and maturation in the rat.

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Ash (mg):								
Mandible	2.0 ±0.13	5.7 ±0.120	10.9 ±0.41	21.0 ±0.73	39 ±1.0	101 ±1.0	113 ±2.6	194 ±4.9
Femur	0.65 ±0.040	2.2 ±0.11	10.0 ±0.30	20.4 ±0.47	62 ±3.8	168 ±3.1	237 ±17.9	448 ±14.7
Pelvis	0.57 ±0.023	1.9 ±0.08	7.7 ±0.20	15.3 ±0.37	45 ±3.0	131 ±2.5	176 ±9.9	341 ±7.4
Tarsus	negligible	0.37 ±0.010	2.9 ±0.16	10.2 ±0.30	26 ±1.7	55 ±2.4	66 ±3.0	82 ±3.1
Calcium (mg):								
Mandible	0.75 ±0.032 (36.3)	2.09 ±0.040 (37.0)	4.1 ±0.17 (37.7)	7.8 ±0.16 (37.2)	14.7 ±0.35 (37.8)	38 ±0.5 (38.0)	43 ±1.2 (37.9)	75 ±1.9 (39.3)
Femur	0.21 ±0.012 (33.1)	0.78 ±0.030 (35.2)	3.5 ±0.14 (35.0)	7.6 ±0.15 (35.8)	22.7 ±1.38 (36.8)	65 ±1.2 (36.5)	87 ±5.9 (36.8)	168 ±5.9 (37.3)

Table 16-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Calcium (mg) contd.								
Pelvis	0.17	0.66	2.7	5.4	16.0	48	65	124
	±0.004	±0.022	±0.07	±0.16	±1.04	±1.6	±3.8	±3.0
	(30.3)	(35.1)	(34.9)	(35.7)	(36.1)	(36.4)	(36.6)	(36.2)
Tarsus	N.D.	N.D.	1.0	3.6	9.7	21	25	33
			±0.06	±0.12	±0.45	±0.6	±1.1	±1.3
			(35.3)	(35.0)	(37.0)	(38.2)	(38.2)	(39.1)
Phosphorus (mg):								
Mandible	0.42	1.12	2.2	4.1	7.4	19	20	33
	±0.026	±0.040	±0.10	±0.11	±0.19	±0.4	±0.5	±1.1
	(20.4)	(19.9)	(20.1)	(19.5)	(19.0)	(18.6)	(18.1)	(17.4)
Femur	0.13	0.44	2.0	4.1	11.5	32	42	72
	±0.007	±0.022	±0.05	±0.11	±0.67	±0.7	±2.5	±3.0
	(19.2)	(19.7)	(20.0)	(19.9)	(18.7)	(18.2)	(17.8)	(16.0)
Pelvis	0.11	0.38	1.6	3.0	8.3	23	32	57
	±0.004	±0.014	±0.06	±0.07	±0.56	±0.6	±1.8	±1.4
	(19.8)	(19.5)	(20.0)	(20.0)	(18.8)	(17.9)	(17.9)	(16.6)

Table 16-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Phosphorus (mg) contd.								
Tarsus	N.D.	N.D.	0.58 ±0.029 (20.0)	1.9 ±0.07 (18.9)	4.9 ±0.28 (18.9)	10 ±0.4 (18.1)	12 ±0.7 (17.9)	15 ±0.5 (17.6)
Chondroitin Sulphate (Uronic acid) (mg):								
Mandible	0.027 ±0.0018	0.041 ±0.0049	0.06 ±0.008	0.08 ±0.005	0.12 ±0.005	0.13 ±0.016	0.13 ±0.014	0.031 ±0.0057
Femur	0.073 ±0.0029	0.220 ±0.0230	0.39 ±0.030	0.56 ±0.012	0.53 ±0.018	0.22 ±0.020	0.24 ±0.011	0.142 ±0.0092
Pelvis	0.043 ±0.0023	0.128 ±0.0055	0.16 ±0.013	0.32 ±0.025	0.31 ±0.013	0.18 ±0.030	0.17 ±0.014	0.075 ±0.0060
Tarsus	0.045 ±0.0024	0.106 ±0.0060	0.25 ±0.024	0.29 ±0.007	0.18 ±0.020	0.17 ±0.017	0.09 ±0.016	0.087 ±0.0069

Values in parentheses are as per cent of ash.

N.D. - Not determined

Table 16-b: Ratios of inter-relations among chemical components in bone during development.

Postnatal age (week)	0	1	2	3	5	9	12	26
				mean ± s.e.				
Ca:P ratio:								
Mandible	1.78 ±0.040	1.86 ±0.050	1.87 ±0.026	1.90 ±0.036	1.97 ±0.016	2.06 ±0.052	2.10 ±0.018	2.26 ±0.018
Femur	1.72 ±0.050	1.76 ±0.040	1.75 ±0.028	1.86 ±0.026	1.98 ±0.012	2.00 ±0.012	2.08 ±0.020	2.29 ±0.011
Pelvis	1.51 ±0.023	1.75 ±0.020	1.72 ±0.031	1.76 ±0.031	1.95 ±0.012	2.03 ±0.029	2.04 ±0.013	2.26 ±0.014
Tarsus	N.D.	N.D.	1.73 ±0.016	1.86 ±0.043	1.97 ±0.014	2.12 ±0.061	2.14 ±0.018	2.26 ±0.018
A/R ratio:*								
Mandible	1.00 ±0.052	1.00 ±0.036	1.05 ±0.025	1.17 ±0.033	1.30 ±0.040	1.70 ±0.061	1.74 ±0.044	2.23 ±0.152
Femur	0.39 ±0.025	0.54 ±0.021	0.58 ±0.015	0.60 ±0.002	0.88 ±0.046	1.32 ±0.036	1.42 ±0.060	1.79 ±0.034
Pelvis	0.48 ±0.032	0.57 ±0.011	0.65 ±0.008	0.66 ±0.016	0.84 ±0.046	1.13 ±0.042	1.26 ±0.039	1.65 ±0.022
Tarsus	N.D.	0.29 ±0.041	0.43 ±0.025	0.62 ±0.025	0.90 ±0.028	1.27 ±0.015	1.30 ±0.040	1.50 ±0.057

Table 16-b continued

Postnatal age (week)	0	1	2	3	5	9	12	26
Uronic acid / Hexosamine ratio:								
Mandible	0.61	0.65	0.60	0.38	0.44	0.32	0.27	0.06
Femur	0.77	0.82	0.88	0.86	0.72	0.24	0.23	0.11
Pelvis	0.80	0.78	0.77	0.84	0.58	0.24	0.21	0.08
Tarsus	0.73	0.82	0.89	0.85	0.53	0.45	0.23	0.18
Hexosamine / Hydroxyproline ratio:								
Mandible	0.44	0.29	0.20	0.24	0.16	0.12	0.12	0.09
Femur	1.59	1.46	0.65	0.42	0.24	0.15	0.13	0.10
Pelvis	1.10	1.29	0.37	0.37	0.21	0.14	0.13	0.10
Tarsus	2.70	1.22	0.60	0.31	0.18	0.13	0.11	0.10
Calcium / Hydroxyproline ratio								
Mandible	7.4	9.6	8.4	8.6	8.8	11.5	10.6	13.6
Femur	3.6	4.3	5.1	5.0	7.4	10.1	11.1	14.5
Pelvis	3.5	4.5	5.2	5.2	6.5	9.2	9.4	12.2
Tarsus	-	-	2.1	3.4	5.2	7.2	7.0	7.2

* Ratio of ash to non-ash components in the fat-free dry bone in femur, pelvis and tarsus and in the dry bone in mandible.

N.D. - Not determined.

In an earlier study on rat femur in this department (Dave, 1976), the total nitrogen, as per cent of fresh weight of the bone, has been shown to remain unchanged with age. In the present study it is observed that the hydroxyproline measured as per cent of fresh weight increases with age. This may be due to the presence of appreciable amounts of noncollagenous proteins in the early stages, which are removed with maturation of bone, thus leading to a reduction in their amount in later stages. The values for femur collagen in this study are compared with those for femur N in the previous study on similar ages, body weights, femur weights and femur composition (Table 17). Similar observations have been made by Dickerson (1962) on the development of cortex of rat femur.

In this connection it may be noted that Pugliarello et al. (1970) in a study of the bone mineralisation has reported a sharp decline in the noncollagenous protein in the intervel between the formation of the osteoid and calcification of the matrix. It has been suggested that the insoluble noncollagenous protein is essential for the localisation of the initial mineral deposits (Van de Putte and Urist, 1965; Bang and Urist, 1967) and that the efflux of the soluble noncollagenous protein prepares the stage for the influx of calcium, phosphate and other ions in the formation first of amorphous and later crystalline calcium phosphate (Pugliarello et al., 1970).

Table 17: Comparison of femur collagen in the present study with femur-N of previous study (Dave, 1976).

	0 day		3 week		12 week	
	Previous study	Present study	Previous study	Present study	Previous study	Present study
Body weight (g)	5.8	6.0	44	43	284	281
Femur fresh weight (mg)	9.1	10.5	163	164	666	652
	Composition of femur. g/100 g of Fresh weight					
Moisture	74.3	74.7	64.7	64.4	35.7	36.6
Lipid	1.1	2.9	1.3	2.2	2.0	1.5
Ash	7.4	6.3	12.7	12.5	38.2	36.2
MPS	3.99	3.3	1.67	1.6	0.7	0.7
Total Protein (Total-N x 6.25)	16.9		18.1		18.1	
Collagen (Hydroxyproline x 7.5)		4.0		6.8		9.0
$\frac{\text{Collagen}}{\text{Total Protein}} \times 100$		24%		38%		50%

Both total mucopolysaccharides, measured as hexosamine, and chondroitin sulphate, measured as uronic acid (Table 17-a) decline with age, the rate of decline being rapid in chondroitin sulphate in the later stages. At any age studied, the amount of these two components as per gram of tissue was the highest in the tarsus and the lowest in the mandible.

The exact mechanism of action of mucopolysaccharides (proteoglycans) in biological mineralisation is yet to be elucidated. However, they are found to have an inhibitory effect on bone mineral deposition and degradation of proteoglycan seems to be essential for biological mineralisation to proceed (Blumenthal et al., 1979). The major glycosaminoglycan in bone is chondroitin-4-sulphate (Vejlens, 1971). Chondroitin-6-sulphate is specifically mobilized during mineralisation of osteoid (Dorey and Bick, 1977).

In cartilage keratan sulphate increases with the advance in age (Kaplan and Meyer, 1959) and aged human cartilage is composed mainly of keratan sulphate and chondroitin-6-sulphate with a trace of chondroitin-4-sulphate (Mathews and Glagov, 1966).

Svejcar (1974) observed that in human fetal development, the presence of chondroitin sulphate in the embryo corresponds with the chondrification (formation of cartilage) of the skeleton. Chondroitin sulphate is

Table 17-a: The pattern of changes in the percentage composition of selected bones during growth and maturation in the rat.

Postnatal age (week)	0	1	2	3	5	9	12	26
	g per 100 g of fresh bone mean ± s.e.							
Moisture:								
Mandible	67.3 ±0.32	63.2 ±0.49	60.0 ±0.58	51.6 ±0.23	41.5 ±1.01	28.1 ±0.45	25.5 ±0.70	19.3 ±0.35
Femur	74.7 ±0.51	73.2 ±0.34	67.9 ±0.30	64.4 ±0.22	55.3 ±0.50	43.4 ±0.72	36.6 ±0.62	31.4 ±0.53
Pelvis	73.7 ±1.46	70.5 ±0.54	64.7 ±0.48	61.2 ±0.30	52.2 ±0.85	45.3 ±0.42	37.9 ±0.45	32.4 ±0.68
Tarsus	82.0 ±2.29	76.8 ±1.00	72.7 ±0.52	58.4 ±0.62	40.7 ±0.72	33.3 ±0.35	28.5 ±1.22	22.6 ±1.12
Total lipid:								
Femur	2.9 ±0.22	2.9 ±0.23	2.7 ±0.13	2.2 ±0.06	2.0 ±0.13	2.0 ±0.14	1.8 ±0.12	1.0 ±0.05
Pelvis	3.0 ±0.09	2.9 ±0.08	2.5 ±0.08	2.2 ±0.12	2.3 ±0.14	2.4 ±0.12	2.0 ±0.20	1.0 ±0.10
Tarsus	3.6 ±0.96	4.8 ±0.48	4.8 ±0.41	7.0 ±0.20	7.9 ±0.70	6.4 ±0.38	4.8 ±0.28	2.8 ±0.36

Table 17-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
	g per 100 g of fresh bone mean ± s.e.							
Ash:								
Mandible	15.7 ±0.68	18.6 ± 0.42	21.9 ±0.64	25.7 ±0.64	33.0 ±0.62	45.0 ±0.65	47.0 ±0.25	54.8 ±1.00
Femur	6.3 ±0.33	7.6 ±0.19	10.8 ±0.33	12.5 ±0.22	20.1 ±0.68	31.3 ±0.31	36.3 ±0.82	44.7 ±0.76
Pelvis	7.4 ±0.35	9.8 ±0.39	13.1 ±0.41	15.2 ±0.33	21.3 ±0.64	29.9 ±0.40	34.1 ±0.80	42.2 ±0.67
Tarsus	N.D.	3.6 ±0.35	5.9 ±0.17	13.5 ±0.43	24.3 ±1.15	33.6 ±0.61	38.1 ±1.05	45.2 ±1.00
Collagen (Hydroxyproline):								
Mandible	0.67 ±0.048	0.70 ±0.017	0.94 ±0.038	1.16 ±0.038	1.47 ±0.062	1.50 ±0.070	1.53 ±0.034	1.57 ±0.105
Femur	0.53 ±0.036	0.59 ±0.050	0.85 ±0.055	0.93 ±0.029	1.11 ±0.062	1.18 ±0.027	1.19 ±0.003	1.14 ±0.041
Pelvis	0.60 ±0.058	0.64 ±0.024	0.97 ±0.035	0.99 ±0.012	1.22 ±0.036	1.22 ±0.020	1.32 ±0.025	1.20 ±0.052
Tarsus	0.46 ±0.021	0.53 ±0.022	0.96 ±0.024	1.28 ±0.066	1.70 ±0.081	1.84 ±0.048	2.16 ±0.106	2.29 ±0.145

Table 17-a continued

Postnatal age (week)	0	1	2	3	5	9	12	26
	g per 100 g of fresh bone mean ± s.e.							
Mucopolysaccharide (Hexosamine):								
Mandible	0·31 ±0·010	0·28 ±0·018	0·23 ±0·018	0·26 ±0·018	0·22 ±0·010	0·19 ±0·018	0·19 ±0·009	0·17 ±0·008
Femur	0·88 ±0·040	0·72 ± 0·060	0·68 ±0·028	0·42 ±0·029	0·25 ±0·023	0·18 ±0·006	0·18 ± 0·009	0·12 ±0·004
Pelvis	0·69 ±0·015	0·61 ±0·026	0·43 ±0·008	0·39 ±0·014	0·23 ±0·014	0·18 ±0·008	0·16 ±0·005	0·12 ±0·004
Tarsus	1·44 ±0·085	0·97 ±0·075	0·80 ±0·062	0·43 ±0·021	0·34 ±0·022	0·26 ±0·010	0·25 ±0·018	0·24 ±0·015
Chondroitin sulphate (Uronic acid):								
Mandible	0·19 ±0·012	0·17 ±0·020	0·12 ±0·013	0·11 ±0·009	0·10 ±0·004	0·089 ±0·0029	0·073 ±0·0110	0·011 ±0·0020
Femur	0·76 ±0·030	0·59 ±0·047	0·50 ±0·040	0·37 ±0·006	0·17 ±0·006	0·051 ±0·0040	0·042 ±0·0098	0·015 ±0·0050
Pelvis	0·61 ±0·020	0·58 ±0·069	0·33 ±0·029	0·31 ±0·022	0·15 ±0·003	0·051 ±0·0060	0·038 ±0·0030	0·010 ±0·0007
Tarsus	1·00 ±0·030	0·71 ±0·041	0·55 ±0·056	0·37 ±0·009	0·19 ±0·016	0·12 ±0·016	0·060 ±0·0090	0·048 ±0·0031

Table 17-b

g per 100 g dry bone

Postnatal age (week)	0	1	2	3	5	9	12	26
Total lipid:								
Femur	11.3	11.5	8.5	5.9	4.4	3.3	2.8	1.0
Pelvis	10.6	10.5	8.2	6.0	4.6	4.3	3.1	1.8
Tarsus	20.0	18.5	16.9	16.8	13.1	9.4	6.4	3.1
Ash:								
Mandible	48.8	50.3	52.0	53.7	56.5	63.1	63.6	69.5
Femur	23.2	28.9	33.5	35.0	44.8	55.3	57.0	61.0
Pelvis	26.7	30.0	36.7	39.2	43.8	54.0	54.0	59.1
Tarsus	-	14.5	25.1	31.9	39.5	50.4	53.2	57.5
Collagen (Hydroxyproline):								
Mandible	1.72	1.94	2.62	2.43	2.44	2.06	2.06	1.93
Femur	2.19	2.12	2.61	2.58	2.16	2.17	1.85	1.64
Pelvis	2.09	2.20	2.80	2.60	2.32	2.24	2.02	1.75
Tarsus	2.51	2.53	3.48	2.87	2.83	2.73	2.97	3.08
Mucopolysaccharide (Hexosamine):								
Mandible	0.97	0.66	0.55	0.52	0.36	0.26	0.27	0.19
Femur	3.54	2.14	1.67	1.10	0.53	0.36	0.27	0.17
Pelvis	2.82	1.82	1.22	0.95	0.48	0.35	0.26	0.17
Tarsus	7.56	4.05	2.29	1.09	0.51	0.38	0.36	0.32

Table 17-c: g per 100 g fat-free dry bone

Postnatal age (week)	0	1	2	3	5	9	12	26
Ash:								
Femur	27.6	34.4	36.5	37.6	46.7	56.8	58.7	64.1
Pelvis	30.2	35.9	38.6	41.3	45.9	56.1	56.2	61.8
Tarsus	-	18.9	25.7	38.2	47.3	55.2	57.1	60.2
Collagen (Hydroxyproline):								
Femur	2.39	2.11	3.19	2.74	2.31	2.27	1.90	1.65
Pelvis	2.24	2.35	3.40	2.71	2.41	2.51	2.07	1.79
Tarsus	3.06	3.00	4.45	3.39	3.40	3.03	3.27	3.15
Mucopolysaccharide (Hexosamine):								
Femur	3.96	2.38	1.85	1.23	0.55	0.34	0.30	0.17
Pelvis	2.66	2.01	1.22	1.04	0.50	0.36	0.27	0.18
Tarsus	9.05	5.08	2.66	1.27	0.56	0.42	0.39	0.33

considered to be specific to cartilage. In bone, though it is present only in small amounts, it seems to have a definite physiological function in bone formation and resorption.

The presence of a high amount of mucopolysaccharide and chondroitin sulphate at birth in the tarsus is a biochemical indication that it is still in a cartilagenous state.

In the case of the pelvis and femur, the growing ends have epiphyseal cartilage and growth does not completely cease in these bones. This may explain the presence of mucopolysaccharide at 26 weeks.

Although the mandible undergoes membranous bone formation not involving cartilage, secondary cartilage is formed at points of contact in the bone and this may account for part of the relatively small amounts measured.

The proportion of water in all the bones decreased during the course of development (Table 17-a). It fell from 67% at birth to 19% at 26 weeks in the mandible, from 75% to 31% in the femur, from 74% to 32% in the pelvis and from 82% to 23% in the tarsus. The decline was gradual in the pelvic bone and femur, whereas it was faster in the tarsus and mandible. During the preweaning period this decline was rapid in the bones as this was associated with the increased rate of deposition of mineral.

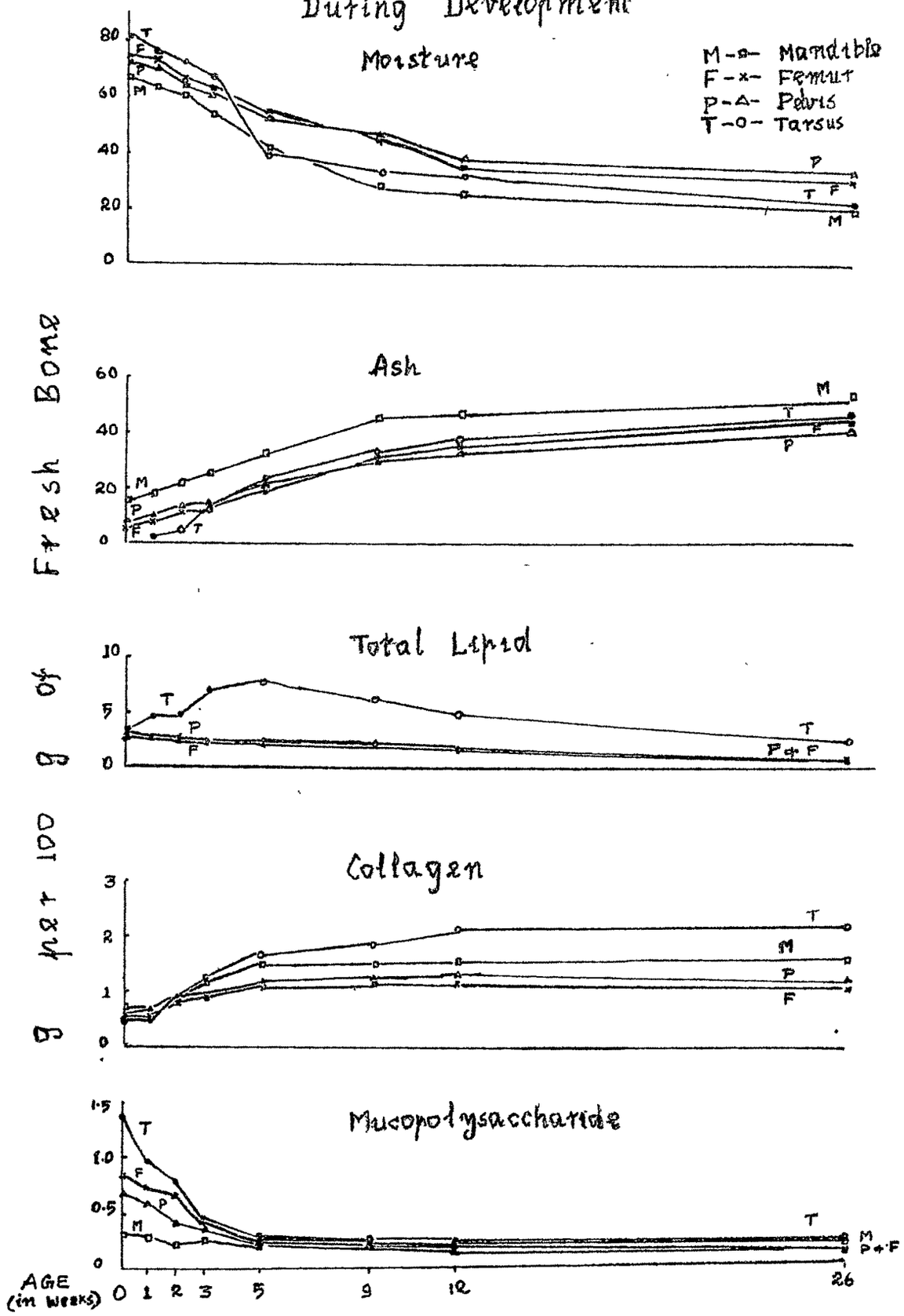
Among the four bones, a similarity in the developmental pattern is observed in pelvis and femur, whereas the tarsus and mandible differ from each other and also from the former two bones with reference to chemical composition at birth and changes in the postnatal period (Figure 6).

Based on moisture and ash content, it is evident that among the bones studied mandible is the most highly calcified bone at birth while tarsus is the least calcified whereas pelvis and femur fall between mandible and tarsus.

It may be mentioned in this context that growth curves and patterns of ossification at birth show large variations among mammals (Harrison, 1933) and some of the biochemical studies (Zika and Klein, 1975) also confirm these variations. According to Zika and Klein (1975) animals which walk at birth, such as the guinea pig, present an advanced degree of ossification which is similar to that of young adults. In this connection, the maturation of the CNS is also more advanced in the guinea pigs than in species such as the rat (Dobbing, 1968). Rodents such as rats and mice are born with immature skeletons and are equivalent to 4-month old human fetus. Newborn dogs and cats are intermediate in skeletal maturity and are comparable to the 7-month old fetus.

The mandible is one of the few bones to ossify in late gestation in the rat and it undergoes membranous ossification. Since it is an organ attached to the head which develops

Figure: 6 Chemical Composition of Bone During Development



prenatally, the greater biochemical maturity of the mandible at birth as compared to other bones is understandable. But after birth, the growth of the mandible slows down compared to the long bones which are calcified less at birth and grow faster immediately after birth. Though the mandible grows at a slow rate after weaning, it increases in mineralization and has the highest A:R value at 26 weeks.

A comparison of pelvic and femur bones with regard to concentration of moisture and ash and A:R ratio shows that the pelvic bone is slightly more advanced in calcification than the femur prior to weaning, but is surpassed in this regard by the femur after weaning.

The tarsus is only a tissue of cartilage at birth with almost no calcification. In the human tarsus, though some of the members are calcified towards the final stages of fetal life, cuboid gets calcified only after birth. In the rat the appearance of ossification centres in the tarsus is postnatal. The mineralization of tarsus is very high between the first and second postnatal week. This period also coincides with the time when the pups begin to stand on their feet and move about actively, requiring a strong tarsal bone to bear the weight of the body. By about 12 weeks of age the tarsus has reached its maximum mineralization after which a conspicuous slowing down of growth is seen.

In conclusion, 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 and collagen, calcium and phosphorus and a decrease in moisture and mucopolysaccharides. The concentrations of total lipids remain more or less the same.

Fig 61
Table -

Experiment-II

THE EFFECTS OF DIFFERENT DEGREES OF UNDERNUTRITION DURING THE SUCKLING PERIOD ON THE COMPOSITION OF SELECTED BONES.

The postnatal period is a critical period for the growth of the animal as the accelerated growth and maturation of tissues which begin in the fetal period continue during this period, and in the case of tissues such as the brain are almost completed during this period. It is not surprising that attempts have been made to study the impact of nutritional stress during this period on the growth and maturation of different tissues.

As mentioned earlier, an appreciable prevalence of skeletal retardation is found in infants and young children. Previous studies in this laboratory (Dave, 1976) showed that, in the rat, undernutrition during the suckling period is associated with a number of changes such as lighter and thinner bones, increased concentrations of moisture and the organic constituents and decreased concentrations of inorganic constituents. Similar observations concerning some of these parameters have been made by a number of investigators (Dickerson and Widdowson, 1960; Di Orio *et al.*, 1973; Nakamoto and Miller, 1977).

Most of these studies were confined to the femur or in some cases both femur and tibia which show similar patterns of development and response to stress. Comparative studies have also been made of mandible and long bones for

calcium, protein and DNA (Nakamoto and Miller, 1977), of the mandible and tibia for their weights and the growth of incisors and molars (Di Orio et al., 1973) and of the long bones and parietal bones for bone weights (Dickerson and Widdowson, 1960). No other studies appear to have been performed in a comprehensive way on the comparative effects of nutritional stress on chemical composition of different bones which differ appreciably in chemical composition and patterns of maturation. Also very seldom has a systematic effort been made to interpret the changes observed in terms of a maturational framework. The present studies were designed in this context to study the impact of nutritional status on selected bones, namely, mandible, femur, pelvis and tarsus, which were also studied from the development point of view.

As mentioned earlier, different degrees of under-nutrition were induced either 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 the normal diet (LL). In this connection it should be mentioned that there are quite a few reports claiming that feeding the mothers a low protein diet during lactation results in diminished milk production but does not affect the composition of milk (Perisse and Salmon-Leganeur et al., 1960; Chow and Lee, 1964; Mueller and Cox, 1946; Luckey et al., 1954) so that the net effect of a low protein diet is believed to be

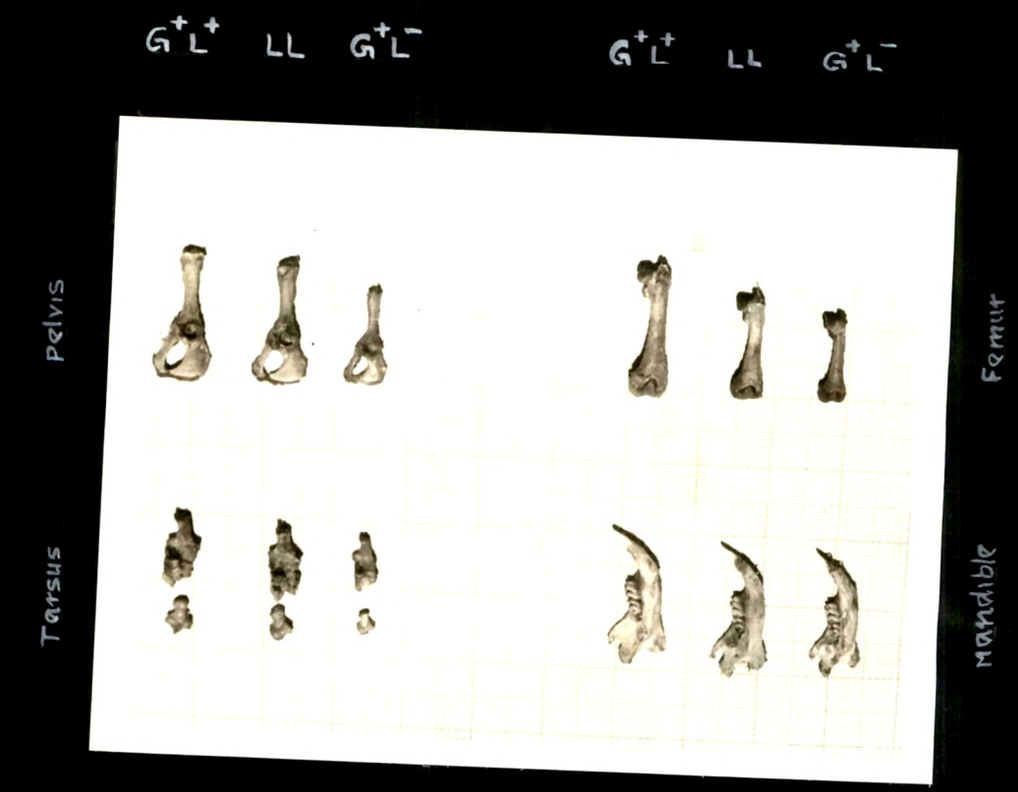
undernutrition for the progeny.

The results of these studies are presented in Tables 18-a to 22-b. The bones studied are seen in Figure 7. As expected, the deficits in body weight, bone weight and length were found in both experimental groups, with greater deficits in $G^{+}L^{-}$ group (Table 18-a and Figure 8). They were found to be the least in the mandible and increased progressively in the order mandible, tarsus, femur and pelvis.

The percentage deficits in body weight with undernutrition were of the order of 44% in LL and 69% in $G^{+}L^{-}$ (Table 18-a). The deficits in the weights of mandible and tarsus were less and those of femur and pelvis more than those in body weight. However, the deficits in linear measurements in all the bones were much less than those in either body weight or bone weight, and were of a similar order in all the bones in spite of the variations in weight deficits suggesting that bone growth is maintained even in the face of nutritional adversity.

When expressed as mg/g of body weight (Table 18-a), the weights of mandible and tarsus, in relation to body weight, from the malnourished group were higher than those from controls. This observation suggests that the growth of mandible and tarsus is protected to some extent in spite of overall growth retardation. On the other hand, the weights

Figure: 7 Effects of undernutrition during the suckling period



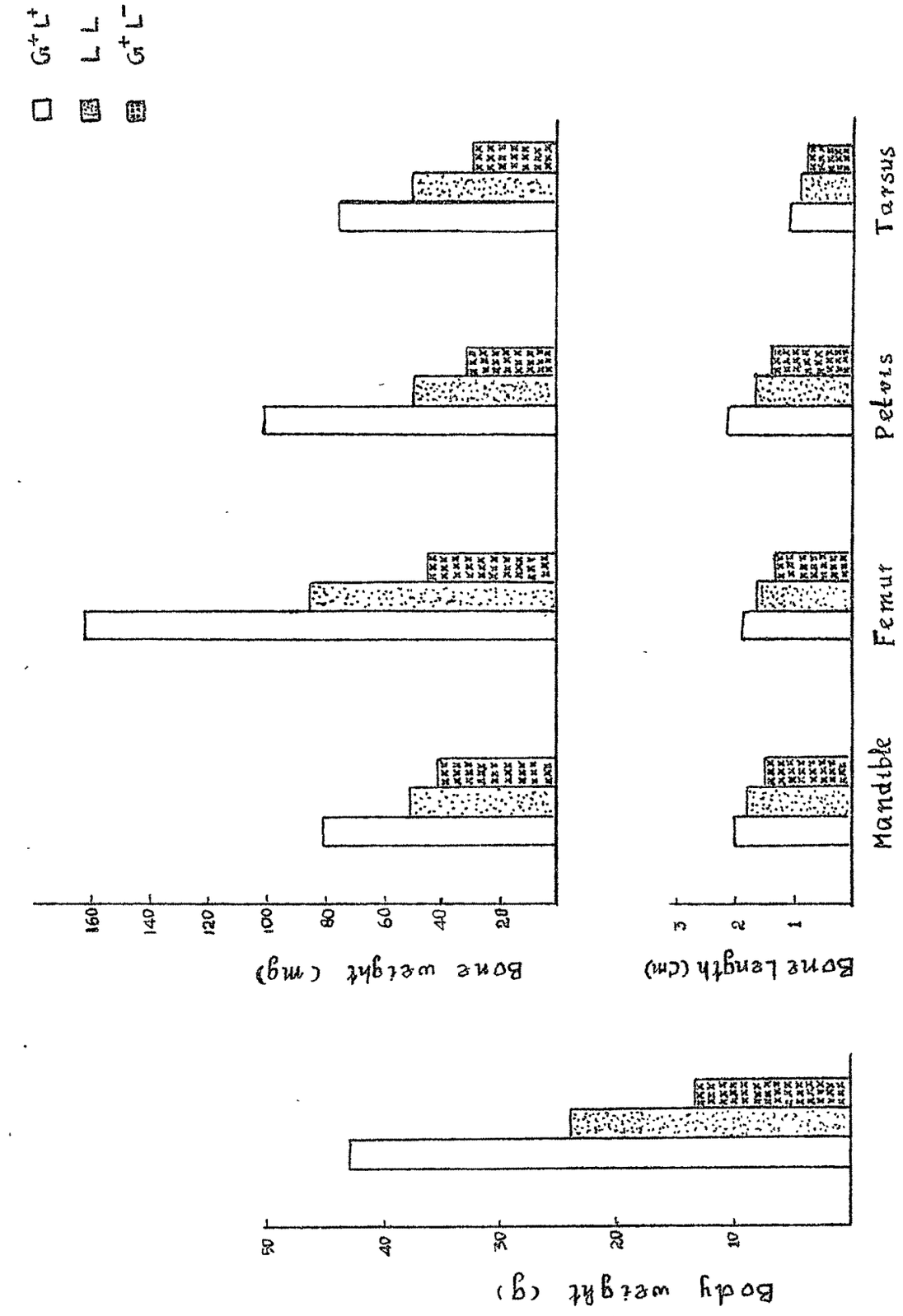


Figure: 8. The effects of different degrees of undernutrition during the suckling period on body weight and bone size

Table 18-a: The effects of different degrees of undernutrition during the suckling period on bone size and morphology.

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
No. of animals	6	6	9		
Body weight (g)	42.7 ± 0.78	24.0 ± 1.22	13.3 ± 0.25	56	31
Fresh weight (mg):					
Mandible	81.0 ± 2.02 (1.9)**	52.3 ± 0.92 (2.2)	41.2 ± 0.65 (3.1)	65	51
Femur	163.7 ± 1.25 (3.9)	84.6 ± 1.96 (3.5)	46.3 ± 1.71 (3.4)	52	28
Pelvis	100.5 ± 0.24 (2.4)	49.5 ± 1.19 (2.1)	27.2 ± 0.76 (2.0)	50	27
Tarsus	75.3 ± 1.93 (1.8)	51.2 ± 2.00 (2.1)	30.7 ± 1.91 (2.3)	68	41

Table 18-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Bone measurements (cm):					
No. of animals	6	6	7		
Length:					
Mandible	2.05 ± 0.019	1.78 ± 0.033	1.51 ± 0.042	87	74
Femur	1.85 ± 0.030	1.57 ± 0.039	1.29 ± 0.008	85	70
Pelvis	2.10 ± 0.038	1.70 ± 0.063	1.39 ± 0.031	81	66
Tarsus	1.09 ± 0.015	0.93 ± 0.028	0.82 ± 0.017	85	75
Width:					
Mandible	0.94 ± 0.017	0.82 ± 0.017	0.76 ± 0.009	87	81
Femur	0.54 ± 0.006	0.48 ± 0.020	0.39 ± 0.008	89	72
Pelvis	0.96 ± 0.015	0.76 ± 0.027	0.60 ± 0.018	79	63

Table 18-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
Width/Length:					
Mandible	0.46	0.46	0.50	100	109
Femur	0.29	0.31	0.30	107	103
Pelvis	0.46	0.45	0.43	98	93
Comparison of bone lengths:					
Mandible/Femur	1.11	1.13	1.17	102	105
Pelvis/Femur	1.14	1.08	1.08	95	95
Tarsus/Femur	0.59	0.59	0.64	100	108

* (1) G^+L^+ - Mothers fed on 20% protein diet ad libitum and standard litter size (8) maintained during lactation.

(2) LL - Mothers fed on 20% protein diet ad libitum; but the litter size was increased to 16 during lactation.

(3) G^+L^- - Mothers fed on 5% protein diet ad libitum during lactation and standard litter size (8) maintained.

** Values in parentheses are bone weight expressed as mg per g of body weight.

For statistical significance of differences see Table 18-b.

Table 18-b: Statistical significance of differences between groups in table 18-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
Fresh weight:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Bone measurements:			
Length:			
Mandible	0.001	0.001	0.010
Femur	0.010	0.001	0.001
Pelvis	0.001	0.001	0.010
Tarsus	0.010	0.001	0.050
Width:			
Mandible	0.050	0.001	0.050
Femur	0.050	0.001	0.010
Pelvis	0.001	0.001	0.001

Table 18-c: Per cent increment over values at birth.

	Group			Values as % of control values	
	G^+L^+	LL	G^+L^-	LL	G^+L^-
Body weight:	611	300	121	49	20
Fresh weight:					
Mandible	545	315	223	58	41
Femur	1462	706	332	48	23
Pelvis	1141	520	227	46	20
Tarsus	545	315	223	58	41
Bone measurements:					
Length:					
Mandible	125	93	64	74	51
Femur	249	196	143	79	57
Pelvis	256	188	136	73	53
Tarsus	336	272	228	81	68
Width:					
Mandible	154	122	105	79	68
Femur	260	220	160	85	62
Pelvis	194	138	88	71	45

of the femur and pelvis in relation to body weight were reduced suggesting that the long bones are more susceptible to the effects of the overall growth retardation. Di Orio et al. (1973) and Nakamoto and Miller (1977) also found that the developmental deficits in the mandible and long bones differed in malnourished animals. Similar observations were made of other bones in rats reared in large litters. The skull was found to be less affected than the spine and the brain case less than the face (Williams and Hughes, 1978).

The increment in body weight over the value at birth is about 50% in LL group, whereas it is less than 25% in the $G^{+}L^{-}$ group (Table 18-c).

The percentage increments in the lengths (ranging from 51 to 68%) of the bones are higher than those with regard to bone weight (ranging from 20 to 41) an observation consistent with the smaller impact of malnutrition on linear measurements.

The increments in bone weight seem to be higher in femur and pelvic bones than in the other two in all the three groups. But in terms of dry weight, the increment was the highest in the tarsus of the three groups an observation consistent with the fact that the appearance of ossification centres and the rapid phase of development are postnatal in these bones (Table 19-c).

Table 19-a: The effects of different degrees of undernutrition during the suckling period on the chemical composition of bones.

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
No. of animals	6	6	9		
Body weight (g)	42.7 ± 0.78	24.0 ± 1.22	13.3 ± 0.25	56	31
Fresh weight (mg):					
Mandible	81.0 ± 2.02	52.3 ± 0.92	41.2 ± 0.65	65	51
Femur	163.7 ± 1.25	84.0 ± 1.96	46.3 ± 1.71	52	28
Pelvis	100.5 ± 0.24	49.5 ± 1.19	27.2 ± 0.76	50	27
Tarsus	75.3 ± 1.93	51.2 ± 2.00	30.7 ± 1.91	68	41
Dry weight (mg):					
Mandible	39.2 ± 1.37	25.4 ± 0.89	19.2 ± 0.36	65	49
Femur	57.3 ± 1.35	29.2 ± 0.82	15.7 ± 0.57	52	27
Pelvis	39.1 ± 0.22	18.3 ± 0.39	9.7 ± 0.30	47	25
Tarsus	31.8 ± 0.52	19.7 ± 0.48	11.2 ± 0.66	62	35

Table 19-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Fat free dry weight (mg):					
Mandible**					
Femur	54.3 ± 1.28	26.7 ± 0.91	13.9 ± 0.62	49	26
Pelvis	37.0 ± 0.42	16.6 ± 0.36	8.4 ± 0.26	45	23
Tarsus	26.6 ± 0.49	15.5 ± 0.35	8.3 ± 0.52	58	31
Chemical composition:					
Ash content (mg):					
No. of animals	6	6	9		
Mandible	21.0 ± 0.73	12.1 ± 0.14	9.2 ± 0.20	58	45
Femur	20.4 ± 0.50	9.9 ± 0.46	4.5 ± 0.20	49	22
Pelvis	15.3 ± 0.31	6.1 ± 0.09	3.1 ± 0.12	40	20
Tarsus	10.2 ± 0.30	5.1 ± 0.09	2.6 ± 0.12	50	26

Table 19-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Collagen (Hydroxyproline)(mg):					
No. of animals	6	5	6		
Mandible	0.88 ± 0.011	0.59 ± 0.035	0.45 ± 0.015	67	51
Femur	1.53 ± 0.052	0.92 ± 0.065	0.49 ± 0.019	60	32
Pelvis	1.03 ± 0.052	0.61 ± 0.015	0.28 ± 0.013	59	27
Tarsus	1.07 ± 0.052	0.72 ± 0.057	0.42 ± 0.025	67	39
Total Mucopolysaccharide (Hexosamine)(mg):					
No. of animals	6	6	6		
Mandible	0.21 ± 0.011	0.15 ± 0.006	0.11 ± 0.007	71	52
Femur	0.65 ± 0.029	0.52 ± 0.008	0.26 ± 0.012	80	40
Pelvis	0.38 ± 0.015	0.30 ± 0.021	0.14 ± 0.008	79	37
Tarsus	0.33 ± 0.013	0.26 ± 0.028	0.19 ± 0.014	79	58

* (1) G^+L^+ - Mothers fed on 20% protein diet ad libitum and standard litter size (8) maintained during lactation.

(2) LL - Mothers fed on 20% protein diet ad libitum; but the litter size was increased to 16 during lactation.

(3) G^+L^- - Mothers fed on 5% protein diet ad libitum during lactation and standard litter size (8) maintained.

** Practically the same as dry weight.

For statistical significance of differences see Table 19-b.

Table 19-b: Statistical significance of differences between groups in Table 19-a.

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Body weight:	0.001	0.001	0.001
Fresh weight:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Dry weight:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Fat-free dry weight:			
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Ash:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001

Table 19-b continued

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	p less than		
Hydroxyproline:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.010
Hexosamine:			
Mandible	0.001	0.001	0.010
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.050

Table 19-c: Per cent increment over values at birth

	Group			Values as % of control values	
	G^+L^+	LL	G^+L^+	LL	G^+L^-
Body weight	611	300	121	49	20
Fresh weight of bones:					
Mandible	545	315	223	58	41
Femur	1462	706	332	48	23
Pelvis	1141	520	227	46	20
Tarsus	545	315	223	58	41
Dry weight:					
Mandible	873	520	358	60	41
Femur	2108	1025	481	49	23
Pelvis	1719	765	347	45	20
Tarsus	3791	2310	1261	61	33

Table 19-c continued

	Group			Values as % of control values	
	G^+L^+	LL	G^+L^-	LL	G^+L^-
Fat-free dry weight:					
Femur	2245	1062	465	47	21
Pelvis	1868	820	331	44	18
Tarsus	3900	2258	1138	58	29
Ash:					
Mandible	950	505	370	53	39
Femur	3038	1426	592	47	19
Pelvis	2584	1005	444	39	17
Tarsus	10.2 mg*	5.1 mg	2.6 mg	50	25
Collagen (Hydroxyproline):					
Mandible	781	504	336	65	43
Femur	2452	1486	728	61	30
Pelvis	1960	1340	500	68	26
Tarsus	4552	2455	1856	54	41

Table 19-c continued

	Group			Values as % of control values	
	G^+L^+	LL	G^+L^-	LL	G^+L^-
Total MPS (Hexosamine):					
Mandible	377	241	150	64	40
Femur	606	465	183	77	30
Pelvis	607	307	159	51	26
Tarsus	170	113	48	66	28

* absolute values; Ash in tarsus at birth is negligible.

In the case of the mandible, the increment during the postnatal period is less than in other bones, presumably because its critical development period is prenatal. Even at the time of birth the development of mandible is well advanced over that of other bones as judged by the A:R ratio.

Among the constituents of bone, ash, both in terms of content (Table 19-a) and concentration (Table 21-a) was significantly reduced. The reduction in ash was least in mandible and highest in tarsus, especially in the G^+L^- group. However, the composition of ash was not changed. The phosphorus content tended to be lower in the undernourished groups, and this raised the Ca:P ratio (Table 20-a). A similar increase in Ca:P ratio has been reported in pups born of mothers deficient in B_2 and B_{12}, suggesting an impaired utilization of phosphorus (Grainger et al., 1954). Supporting this suggestion is the observation that bone alkaline phosphatase activity and the incorporation of labelled phosphorus were reduced in these animals (Grainger et al., 1954). In this connection intestinal alkaline phosphatase activity has been found to be decreased with neonatal undernutrition (Subramaniam, 1978). The increase in the Ca:P ratio in the present case could be due to a similar phenomenon.

The decrease in A:R ratio (Table 20-a) in the bones of the undernourished animals reflects the decrease in ash

Table 20-a: The effects of different degree of undernutrition during the suckling period on the chemical composition of bones.

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Ash content (mg):					
No. of animals	6	6	9		
Mandible	21.0 ± 0.73	12.1± 0.14	9.2 ± 0.20	58	44
Femur	20.4 ± 0.50	9.9 ± 0.46	4.5 ± 0.20	49	22
Pelvis	15.3 ± 0.31	6.1 ± 0.09	3.1 ± 0.12	40	20
Tarsus	10.2 ± 0.30	5.1 ± 0.09	2.6 ± 0.12	50	25
Calcium (mg):					
Mandible	7.8 ± 0.16 ($\bar{37}.2$)**	4.5 ± 0.08 ($\bar{37}.4$)	3.4 ± 0.11 ($\bar{37}.8$)	58	44
Femur	7.6 ± 0.20 ($\bar{35}.8$)	3.4 ± 0.11 ($\bar{36}.1$)	1.6 ± 0.10 ($\bar{37}.0$)	45	21
Pelvis	5.4 ± 0.14 ($\bar{35}.7$)	2.2 ± 0.09 ($\bar{36}.5$)	1.1 ± 0.05 ($\bar{37}.4$)	41	20
Tarsus	3.6 ± 0.12 ($\bar{35}.0$)	1.9 ± 0.05 ($\bar{36}.9$)	0.88± 0.034 ($\bar{37}.9$)	53	24

Table 20-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Phosphorus (mg):					
Mandible	4.1 ± 0.10 (19.5)	2.3 ± 0.09 (18.9)	1.7 ± 0.05 (18.7)	56	41
Femur	4.1 ± 0.11 (19.9)	1.7 ± 0.06 (18.5)	0.78 ± 0.052 (17.9)	41	19
Pelvis	3.0 ± 0.06 (20.0)	1.2 ± 0.02 (18.6)	0.54 ± 0.010 (17.9)	40	18
Tarsus	1.9 ± 0.10 (18.9)	0.9 ± 0.04 (18.3)	0.40 ± 0.018 (17.5)	47	21
Chondroitin sulphate (Uronic acid)(mg):					
No. of animals	6	6	5		
Mandible	0.08 ± 0.005	0.07 ± 0.004	0.048± 0.0022	88	60
Femur	0.56 ± 0.012	0.40 ± 0.034	0.24 ± 0.012	71	43
Pelvis	0.32 ± 0.025	0.19 ± 0.025	0.12 ± 0.009	59	37
Tarsus	0.29 ± 0.003	0.23 ± 0.023	0.14 ± 0.012	79	48

Table 20-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	mean ± s.e.				
Ca:P ratio:					
Mandible	1·90 ± 0·036	1·99 ± 0·028	2·00 ± 0·050	105	105
Femur	1·86 ± 0·026	1·97 ± 0·040	2·07 ± 0·041	106	111
Pelvis	1·76 ± 0·031	1·95 ± 0·039	2·06 ± 0·043	111	117
Tarsus	1·86 ± 0·043	2·03 ± 0·050	2·14 ± 0·040	109	115
A/R ratio:***					
Mandible	1·17 ± 0·034	0·98 ± 0·010	0·92 ± 0·020	84	79
Femur	0·60 ± 0·002	0·53 ± 0·013	0·51 ± 0·015	88	85
Pelvis	0·66 ± 0·016	0·57 ± 0·007	0·56 ± 0·010	86	85
Tarsus	0·62 ± 0·025	0·48 ± 0·010	0·43 ± 0·011	77	69

Table 20-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
Uronic acid / Hexosamine ratio:					
Mandible	0·38	0·47	0·44	124	116
Femur	0·86	0·77	0·92	90	107
Pelvis	0·84	0·63	0·86	75	102
Tarsus	0·88	0·88	0·74	100	84
Hexosamine / Hydroxyproline ratio:					
Mandible	0·24	0·25	0·24	104	100
Femur	0·42	0·57	0·54	136	129
Pelvis	0·37	0·49	0·50	132	135
Tarsus	0·31	0·36	0·45	116	145

Table 20-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
Calcium / Hydroxyproline ratio:					
Mandible	8.86	7.63	7.56	86	85
Femur	4.97	3.70	3.33	74	67
Pelvis	5.24	3.61	3.92	69	75
Tarsus	3.36	2.64	2.10	79	63

* (1) G^+L^+ - Mothers fed on 20% protein diet ad libitum and standard litter size (8) maintained during lactation.

(2) LL - Mothers fed on 20% protein diet ad libitum; but the litter size was increased to 16 during lactation.

(3) G^+L^- - Mothers fed on 5% protein diet ad libitum during lactation and standard litter size (8) maintained.

** Values in parentheses are as per cent of ash.

*** Ratio of ash to non - ash components in the fat-free dry bone in femur, pelvis and tarsus; Ratio of ash to non-ash components in the dry bone in mandible.

For statistical significance of differences see Table 20-b.

Table 20-b: Statistical significance of differences between groups in Table 20-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	p less than		
Uronic Acid:			
Mandible	N.S.	0.001	0.001
Femur	0.010	0.001	0.001
Pelvis	0.010	0.001	0.001
Tarsus	0.050	0.001	0.001
Calcium:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Phosphorus:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Ca:P ratio:			
Mandible	N.S.	N.S.	N.S.
Femur	0.050	0.010	N.S.
Pelvis	0.010	0.001	N.S.
Tarsus	0.050	0.001	N.S.
A/R ratio:			
Mandible	0.001	0.001	0.050
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	0.010

Table 21-a: The effects of different degrees of undernutrition during the suckling period on the percentage composition of bones.

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	g per 100 g fresh bone mean ± s.e.				
Moisture:					
Mandible	51.6 ± 0.40	52.9 ± 0.30	53.5 ± 0.27	103	104
Femur	64.4 ± 0.40	65.5 ± 0.26	66.6 ± 0.26	102	103
Pelvis	61.2 ± 0.25	63.0 ± 0.55	65.5 ± 0.61	103	107
Tarsus	57.9 ± 1.18	62.0 ± 1.01	64.0 ± 0.50	107	111
Total lipid:					
Femur	2.1 ± 0.09	3.0 ± 0.27	4.4 ± 0.64	143	210
Pelvis	2.1 ± 0.21	3.6 ± 0.23	4.2 ± 0.20	171	200
Tarsus	7.0 ± 0.38	8.2 ± 0.27	9.1 ± 0.70	117	130

Table 21-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	g per 100 g fresh bone mean ± s.e.				
Ash:					
Mandible	25·7 ± 0·64	23·5 ± 0·25	22·6 ± 0·47	91	88
Femur	12·5 ± 0·22	11·2 ± 0·10	10·1 ± 0·26	90	81
Pelvis	15·2 ± 0·33	12·4 ± 0·19	11·3 ± 0·27	82	76
Tarsus	13·5 ± 0·43	10·0 ± 0·45	8·2 ± 0·22	74	61
Collagen (Hydroxyproline):					
Mandible	1·16 ± 0·035	1·09 ± 0·053	1·02 ± 0·009	94	88
Femur	0·91 ± 0·035	0·84 ± 0·005	0·82 ± 0·019	92	90
Pelvis	0·99 ± 0·012	0·90 ± 0·020	0·87 ± 0·035	91	88
Tarsus	1·28 ± 0·066	1·16 ± 0·029	1·11 ± 0·031	91	87
Total MPS (Hexosamine):					
Mandible	0·26 ± 0·019	0·27 ± 0·012	0·27 ± 0·013	104	104
Femur	0·42 ± 0·029	0·60 ± 0·012	0·61 ± 0·027	143	145

Table 21-a continued

	Group*			Values as % of control values	
	G^+L^+ (1)	LL (2)	G^+L^- (3)	LL	G^+L^-
	g per 100 g fresh bone mean ± s.e.				
Total MPS (Hexosamine) contd.					
Pelvis	0·39 ± 0·014	0·59 ± 0·028	0·61 ± 0·004	151	156
Tarsus	0·43 ± 0·021	0·54 ± 0·050	0·78 ± 0·060	126	181
Chondroitin sulphate (Uronic acid):					
Mandible	0·11 ± 0·009	0·13 ± 0·008	0·13 ± 0·006	118	118
Femur	0·35 ± 0·010	0·44 ± 0·032	0·59 ± 0·031	129	169
Pelvis	0·31 ± 0·022	0·35 ± 0·037	0·50 ± 0·023	113	161
Tarsus	0·37 ± 0·009	0·42 ± 0·025	0·62 ± 0·019	114	168

* (1) G^+L^+ - Mothers fed on 20% protein diet ad libitum and standard litter size (8) maintained during lactation.

(2) LL - Mothers fed on 20% protein diet ad libitum; but the litter size was increased to 16 during lactation.

(3) G^+L^- - Mothers fed on 5% protein diet ad libitum during lactation and standard litter size (8) maintained.

For statistical significance of differences see Table 21-b.

Table 21-b: Statistical significance of differences between groups in Table 21-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	G per 100 g of fresh bone		
	p less than		
Moisture:			
Mandible	0.050	0.010	N.S.
Femur	0.050	0.001	0.010
Pelvis	0.050	0.001	0.001
Tarsus	0.050	0.001	N.S.
Total lipid:			
Femur	0.010	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.050	0.050	N.S.
Ash:			
Mandible	0.010	0.001	N.S.
Femur	0.001	0.001	0.010
Pelvis	0.001	0.001	0.050
Tarsus	0.001	0.001	0.001
Hydroxyproline:			
Mandible	N.S.	0.001	N.S.
Femur	N.S.	0.050	N.S.
Pelvis	0.010	0.001	N.S.
Tarsus	N.S.	N.S.	N.S.

Table 21-b continued

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g of fresh bone p less than		
Hexosamine:			
Mandible	N.S.	N.S.	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	N.S.	0.001	0.050
Uronic acid:			
Mandible	N.S.	N.S.	N.S.
Femur	0.050	0.001	0.050
Pelvis	N.S.	0.001	0.010
Tarsus	N.S.	0.001	0.001

Table 22-a: The effects of different degrees of under-nutrition during the suckling period on the percentage composition of bones.

	Group*		
	G^+L^+ (1)	LL (2)	G^+L^- (3)
	g per 100 g of dry bone		
Total lipid:			
Femur	5·7 ± 0·40	9·6 ± 0·21	13·0 ± 1·18
Pelvis	5·2 ± 0·84	9·5 ± 0·61	12·1 ± 0·60
Tarsus	16·5 ± 0·55	21·1 ± 0·58	24·9 ± 2·34
Ash:			
Mandible	53·7 ± 0·67	49·8 ± 0·52	48·1 ± 0·59
Femur	35·7 ± 0·19	31·5 ± 0·47	29·2 ± 0·71
Pelvis	39·2 ± 0·85	33·2 ± 0·37	31·5 ± 0·66
Tarsus	31·9 ± 0·85	25·9 ± 0·54	22·6 ± 0·68
Collagen (Hydroxyproline):			
Mandible	2·37 ± 0·076	2·12 ± 0·087	2·08 ± 0·050
Femur	2·60 ± 0·105	2·46 ± 0·016	2·45 ± 0·049
Pelvis	2·60 ± 0·048	2·45 ± 0·026	2·38 ± 0·109
Tarsus	2·99 ± 0·158	2·72 ± 0·026	2·64 ± 0·053
Total MPS (Hexosamine):			
Mandible	0·52 ± 0·037	0·58 ± 0·020	0·57 ± 0·034
Femur	1·18 ± 0·074	1·68 ± 0·037	1·81 ± 0·088
Pelvis	0·97 ± 0·038	1·67 ± 0·114	1·89 ± 0·130
Tarsus	1·04 ± 0·087	1·35 ± 0·150	2·21 ± 0·150

Table 22-a continued

	Group*		
	G^+L^+ (1)	LL (2)	G^+L^- (3)
	g per 100 g of fat-free dry bone		
Ash:			
Femur	37·6 ± 0·13	35·2 ± 0·41	33·2 ± 0·91
Pelvis	41·3 ± 1·14	36·9 ± 0·29	35·5 ± 0·60
Tarsus	38·2 ± 0·97	32·8 ± 0·57	30·2 ± 1·05
Collagen (Hydroxyproline):			
Femur	2·81 ± 0·117	2·68 ± 0·028	2·77 ± 0·062
Pelvis	2·71 ± 0·059	2·65 ± 0·021	2·77 ± 0·107
Tarsus	3·49 ± 0·178	3·36 ± 0·031	3·41 ± 0·107
Total MPS (Hexosamine):			
Femur	1·23 ± 0·074	1·85 ± 0·030	2·09 ± 0·118
Pelvis	1·04 ± 0·050	1·84 ± 0·135	2·19 ± 0·152
Tarsus	1·27 ± 0·094	1·56 ± 0·140	2·91 ± 0·228

* (1) G^+L^+ - Mothers fed on 20% protein diet ad libitum and standard litter size (8) maintained during lactation.

(2) LL - Mothers fed on 20% protein diet ad libitum; but the litter size was increased to 16 during lactation.

(3) G^+L^- - Mothers fed on 5% protein diet ad libitum during lactation and standard litter size (8) maintained.

For statistical significance of differences see Table 22-b.

Table 22-b: Statistical significance of differences between groups in Table 22-a.

	Significance of difference between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g dry bone		
	p less than		
Total lipid:			
Femur	0.001	0.001	0.050
Pelvis	0.001	0.001	0.050
Tarsus	0.001	0.001	N.S.
Ash:			
Mandible	0.001	0.001	0.050
Femur	0.001	0.001	0.050
Pelvis	0.001	0.001	0.050
Tarsus	0.001	0.001	0.010
Collagen:			
Mandible	N.S.	0.010	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	0.050	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS:			
Mandible	N.S.	N.S.	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	N.S.	0.001	0.001

Table 22-b continued

	Significance of difference between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g fat-free dry bone p less than		
Ash:			
Femur	0.001	0.010	N.S.
Pelvis	0.010	0.010	N.S.
Tarsus	0.001	0.001	0.050
Collagen:			
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS:			
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	N.S.	0.001	0.001

content. This could conceivably be due to deficiencies in the matrix which prevent proper mineralization and poor absorption of calcium as the synthesis of calcium binding protein (CaBP) is found to be impaired with nutritional stress (Kalk and Pimstone, 1974). These effects in turn could be due to poor hormonal regulation of the synthesis of $25(OH)D_3$, $1,25,(OH)D_3$ as a number of hormones such as thyroxine (Shrader et al., 1977; Roberts and Zeman, 1978), growth hormone (Atinmo et al., 1976) and cortieosteroids (Dearder and Espinosa, 1974) are affected by undernutrition. It is also relevant that calcitonin involved in the regulation of osteoblastic activity is produced by the thyroid.

Collagen (Hydroxyproline) too was reduced both in content (Tables 19-a and b) and concentration (Tables 21-a and b). However, the decrease fell short of significance in the LL group except in the case of pelvis, but was significant in the G^+L^- except in the tarsus. Similar observations have been made by Singh (1978) in the case of bones of rat pups whose mothers were fed a 3% protein diet during the suckling period.

On the other hand as reported in an earlier study in this laboratory (Dave, 1976), the total-N concentration of femur was greater in the undernourished rats. From this it may be inferred that in undernutrition the bone formed has an increased proportion of under-hydroxylated collagen

and/or an increased amount of non-collagenous protein as in immature bone.

In this connection, an increase of proline:hydroxyproline ratio has been reported (Singh, 1978) in the bones of progeny born of protein deficient rats suggesting underhydroxylation. But the mechanism by which the hydroxylation is reduced in the undernourished animal is not known.

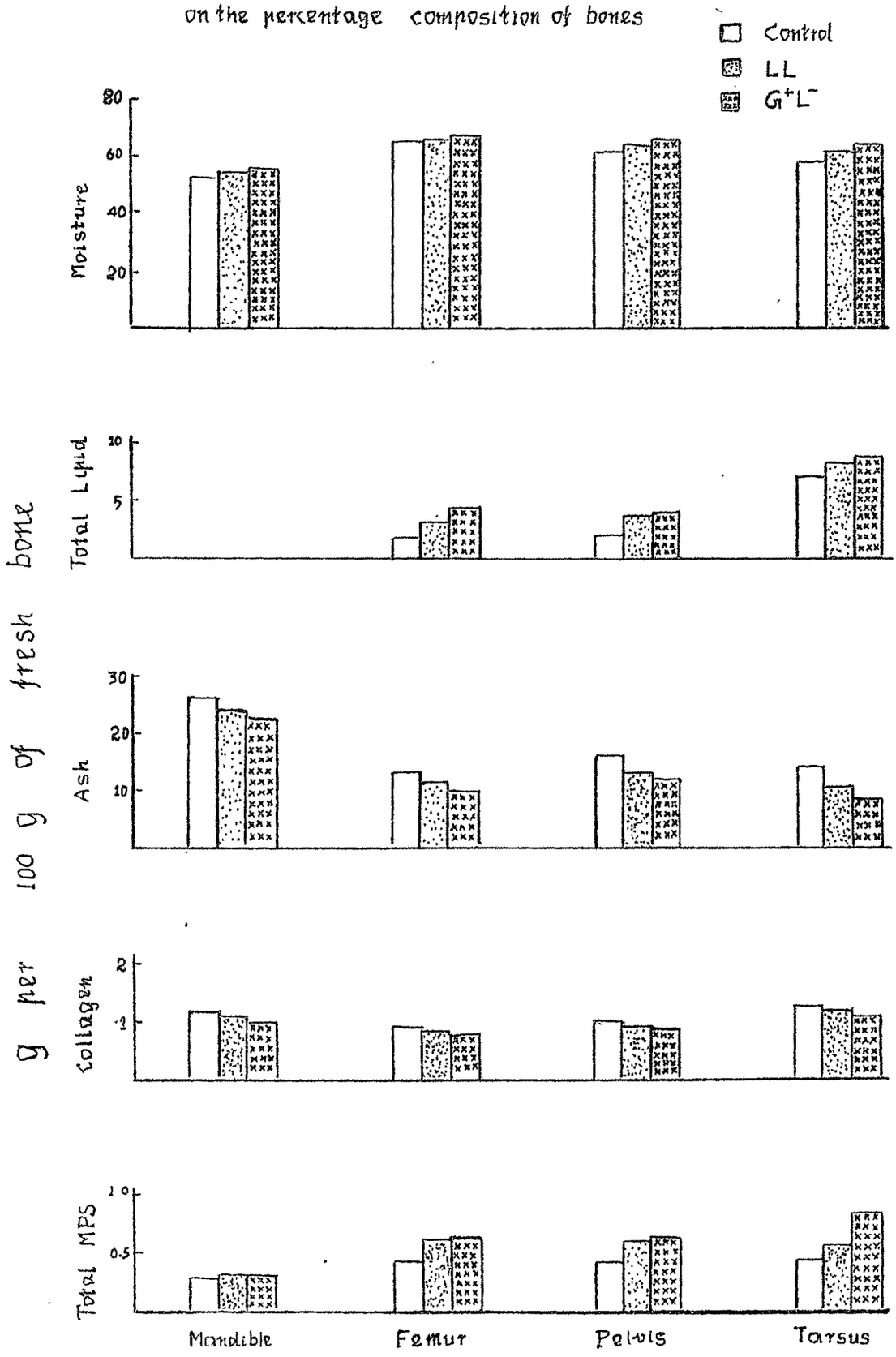
A higher concentration of mucopolysaccharide (Table 21-a) with undernutrition was found generally although the difference fell short of significance in the mandible of the two undernourished groups and the tarsus of LL group.

Among the constituents of the bone (Table 21-a), moisture content was greater in all the bones in the undernourished groups, the lowest moisture content being found in the mandibles, indicating its advanced stage of calcification compared to the other bones. The size of the difference varied with the severity of undernutrition, being generally more in $G^{+}L^{-}$ group than in the LL group.

The lipid content of the mandible was not measured as stated earlier. The lipid contents of the other three bones were greater in the undernourished groups and again the difference was less in the LL group. Since the lipid content was determined on the whole bone the increase in lipid concentration may be due to the presence of more marrow spaces in the bones of the undernourished animals.

Undernourished animals were found to differ from control with reference to bone size and composition as judged by weight, linear measurements and concentrations of ash and collagen which were found to be less and concentrations of moisture, total lipid, mucopolysaccharide and chondroitin sulphate which were found to be greater (Figure 9). This pattern of changes suggests a delayed maturation, as the overall composition of the undernourished animals resemble that of a younger animal. 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 in the face of appreciable differences between the two in chemical composition and pattern of maturation is of interest. It is interesting to consider that 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 the tarsus soon after birth.

Figure: 9 The effects of different degrees of undernutrition on the percentage composition of bones



Experiment-III

THE EFFECTS OF POSTWEANING DEFICIENCIES OF PROTEIN AND ENERGY ON THE CHEMICAL COMPOSITION OF SELECTED BONES

The observations on the effects of undernutrition on bones during the suckling period prompted further studies on the effects of nutritional stress after weaning. In human protein calorie malnutrition which occurs mainly after weaning overall growth retardation is believed to be associated with retarded skeletal growth (Garn et al., 1964) marked by thinning and immaturity of the epiphysis (Jones and Deans, 1956; Higginson, 1954) and trabecular bone loss (Wayburne, 1968).

In experimental animals postweaning deficiency of protein is found to be associated with several bone changes such as thinning of the epiphyses, rarefaction of bone, appearance of transverse trabeculae, altered shape and anatomical relationship of jaws in pigs (Platt and Stewart, 1962; Tonge and McCance, 1965), delayed ossification, increase in the diameter of the Haversian canal and reduction in the lamellae in the sheep (Floresey, 1974), reduced appositional growth and remodelling of bone (Jha et al., 1968) and changes in skeletal proportions, pattern and shape (Fleagle et al., 1975).

Growth retardation in rats subjected to undernutrition has been found to be associated with reduced density

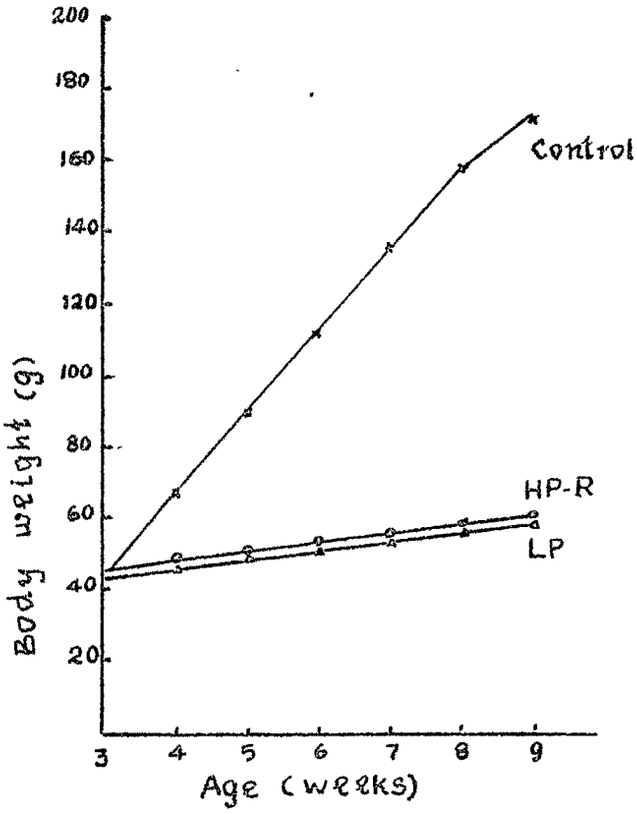
(Reddy, 1972) and osteoporosis (El Maraghi et al., 1965). Narrow epiphyseal plates and reduced calcium accretion were also common features (Le Roith and Pimstone, 1973).

In previous studies in this laboratory undernutrition during the postweaning period in the rat was found to be associated with a decrease in ash and an increase in the concentration of total lipids and moisture in the femur (Rajalakshmi and Dave, 1977). In these studies the effects of protein deficiency were found to differ in some respects from those of undernutrition. As pointed out earlier, since the microenvironment (Papapolcychroniou et al., 1979) as well as the developmental history of each particular bone (as evident from the developmental studies in the present series of investigations) is different, the sensitivity of different bones to nutritional stress may be expected to vary. The present studies were an extension of previous studies in this laboratory on the femur to other selected bones.

For this purpose weanling rats of standard weight (40-45 g) were fed for 6 weeks a diet containing either a 5% or 20% protein diet ad libitum or the latter in amounts so restricted as to get a group matched for weight with low protein rats.

The body weights (Figure 10) and the daily food intake of these rats in the different groups are shown in

Figure:10 The effects of postweaning deficiencies of food energy and protein on growth in rats



Control : Weanling rats fed on 20% protein diet ad libitum
HP-R : Weanling rats fed on 20% protein diet in restricted amounts
LP : Weanling rats fed on 5% protein diet ad libitum

Table 23. As expected, the animals fed a low protein diet had a reduced food intake throughout. As in previous studies in this laboratory (Rajalakshmi et al., 1974) and elsewhere (Miller and Payne, 1962; Samonds and Fleagle, 1973), the high protein animals needed much less food than the low protein animals for comparable weight gains.

Figure 11 shows the four selected bones at 9 weeks of age in the control and in experimental rats subjected to postweaning nutritional stress.

The results of these studies are given in Tables 24-a to 28-b. As expected, the body weights of the calorie restricted as well as the protein deficient groups were significantly reduced, the deficits being 65% in both. This growth retardation was associated with a reduction in the weights and size of all the bones in both the experimental groups. As in the preweaning experiment, the deficits in bone weight were less than those in body weight but more than in bone length and width (Table 24-a and Figure 12).

Although the bone weights were found to be affected with undernutrition in relation to body weight they were more than in the controls (Table 24-a).

During the postweaning period the pelvis has the highest rate of growth and the tarsus, the least on the basis of bone weights. Deficits varied in different bones

Table 23: The effects of postweaning deficiencies of food energy and protein on the chemical composition of selected bones.

	Group*		
	Control	HP-R	LP
No. of observations	7	6	6
Food intake (g/day)	10.5-11.5	2.5-3.0	5.5-6.0
Body weight:			
Initial	44	44	44
Final**	171	59	59
Weight as % of control value		35	35
Weight gain (g/week)	21.0	2.5	2.5
Weight gain (g) per g of food intake	0.27	0.13	0.06

* Control - Weanling rats fed on 20% protein diet ad libitum.
HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
LP - Weanling rats fed on 5% protein diet ad libitum.

** Period of treatment, 6 weeks.

Figure: 11 Effects of postweaning nutritional stress

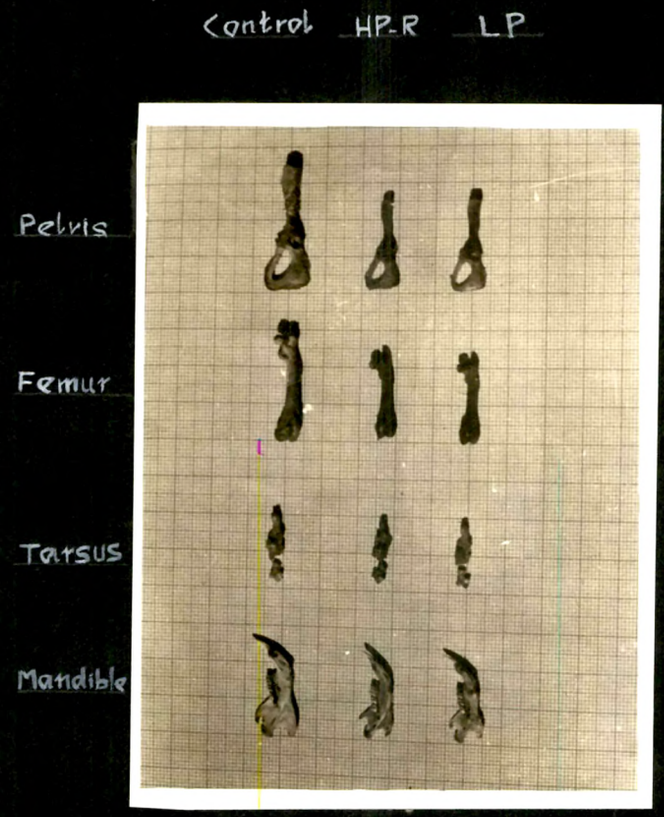


Table 24-a: The effects of postweaning deficiencies of food energy and protein on bone size and morphology.

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
No. of animals	7	6	6		
Body weight (g)	171 ± 3.4	59 ± 0.9	59 ± 4.3	35	35
Fresh weight of bones (mg):					
Mandible	224 ± 1.5 (1.3)**	106 ± 5.7 (1.8)	111 ± 5.8 (1.8)	47	50
Femur	539 ± 10.4 (3.2)	230 ± 9.9 (4.0)	224 ± 4.3 (3.8)	43	42
Pelvis	437 ± 7.3 (2.6)	156 ± 8.4 (2.6)	163 ± 4.9 (2.7)	36	37
Tarsus	160 ± 4.9 (1.3)	107 ± 5.1 (1.8)	112 ± 4.0 (1.9)	67	70

Table 24-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
Bone measurements (cm):					
Length:					
Mandible	2·41 ± 0·040	2·16 ± 0·024	2·11 ± 0·020	90	88
Femur	2·96 ± 0·040	2·24 ± 0·041	2·20 ± 0·021	76	74
Pelvis	3·48 ± 0·030	2·50 ± 0·053	2·50 ± 0·016	72	72
Tarsus	1·20 ± 0·020	1·14 ± 0·049	1·10 ± 0·020	97	92
Width:					
Mandible	1·22 ± 0·020	1·03 ± 0·026	0·97 ± 0·009	84	80
Femur	0·64 ± 0·010	0·57 ± 0·019	0·56 ± 0·015	89	88
Pelvis	1·35 ± 0·020	1·00 ± 0·032	0·96 ± 0·031	74	70

Table 24-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
Width/Length:					
Mandible	0·51	0·47	0·45	88	88
Femur	0·22	0·25	0·25	114	114
Pelvis	0·39	0·40	0·38	103	97
Comparison of bone lengths:					
Mandible/Femur	0·81	0·96	0·92	118	114
Pelvis/Femur	1·18	1·12	1·14	95	97
Tarsus/Femur	0·41	0·51	0·50	124	120

* (1) Control - Weanling rats fed on 20% protein diet ad libitum.
(2) HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
(3) LP - Weanling rats fed on 5% protein diet ad libitum.

** Values in parentheses are bone weight expressed as mg per g of body weight.

For statistical significance of differences see Table 24-b.

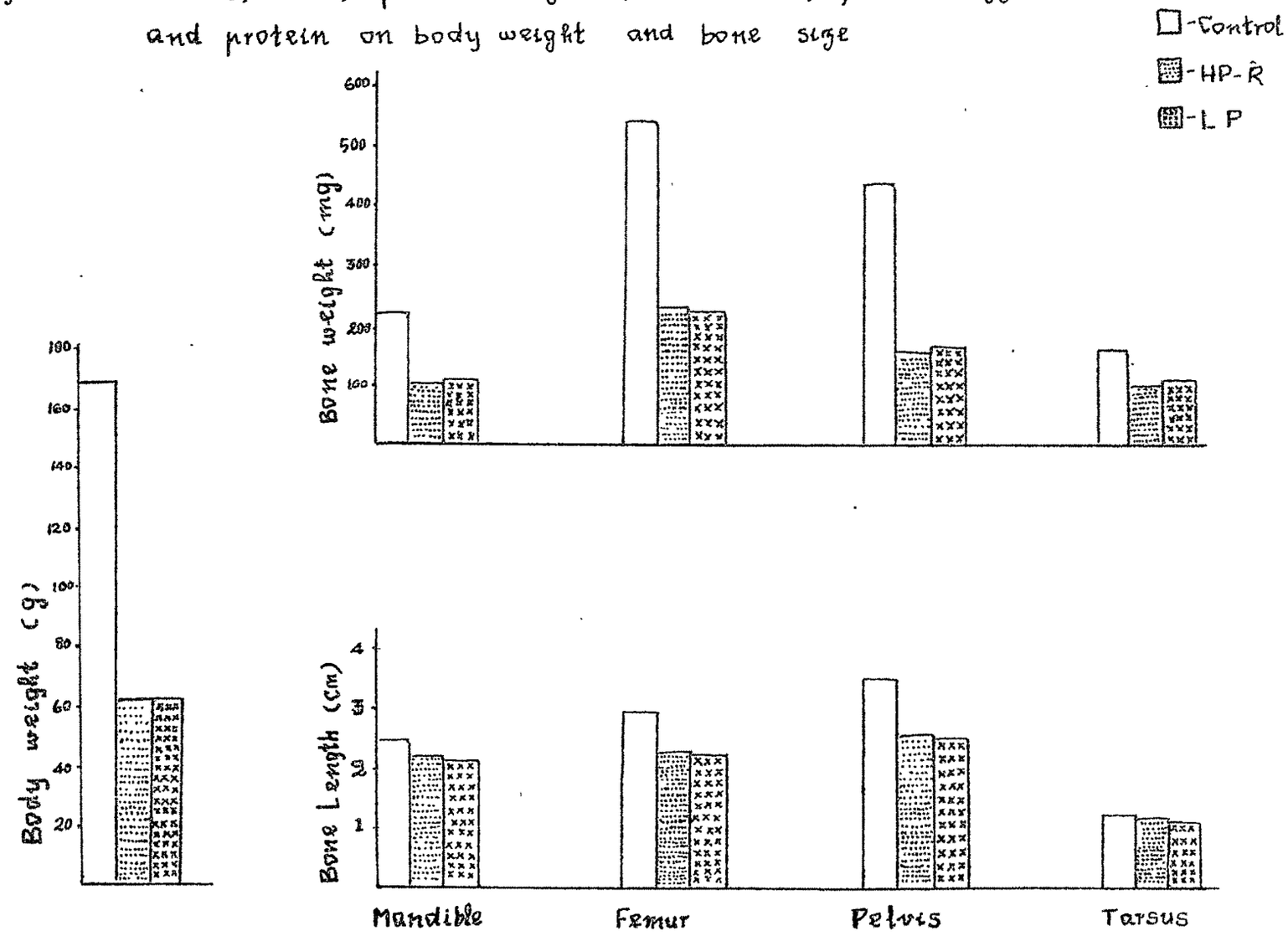
Table 24-b: Statistical significance of differences between groups in Table 24-a.

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Body weight:	0.001	0.001	N.S.
Fresh weight:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Bone measurements:			
Length:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	N.S.	0.010	N.S.
Width:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.

Table 24-c: Per cent increment over values for weanling rats.

	Group			Values as % of control values	
	Control	HP-R	LP	HP-R	LP
Body weight	300	38	38	13	13
Fresh weight:					
Mandible	176	31	37	18	21
Femur	232	41	37	18	16
Pelvis	335	55	61	16	18
Tarsus	112	42	49	38	44
Bone measurements:					
Length:					
Mandible	8	5	3	28	17
Femur	60	22	19	37	32
Pelvis	66	19	19	29	29
Tarsus	10	5	1	50	10
Width:					
Mandible	30	7	3	23	10
Femur	19	6	4	32	21
Pelvis	44	6	1	14	2

Figure: 12 The effects of post weaning deficiencies of food energy and protein on body weight and bone size



but were found to be related to the rate of growth during the period under study as seen in the percentage increments in the control between 3 and 9 weeks of age (Tables 24-c and 25-c). The same pattern applies to bone length. Bone width was less affected than length in the case of femur as judged by epiphyseal width and the reverse being true of the mandible (Table 24-a).

Although increments in bone parameters were less affected than increments in body weight, the tarsus was the least affected with regard to growth in the postweaning period and the pelvis the most affected with the femur and mandible coming next in that order.

The deficits in ash in the undernourished animals were found to be greater in the femur and pelvis than in the mandible and tarsus (Table 25-a). The ash contents at weaning of the mandible and tarsus were 10.8% and 12.4% respectively of adult values whereas those of the pelvis and femur were 4.5% and 4.6% respectively (Table 15-b). Thus mandible and tarsus were more advanced with reference to ontogenetic development than pelvis and femur. It is not surprising that the latter bones were more affected than the former with undernutrition during the postweaning period when their chemical maturation was more rapid as judged by increments in ash content.

The A:R ratio (the ratio of ash to non-ash components in the fat-free bone) (Table 26-a) was less in the

Table 25-a: The effects of postweaning deficiency of food energy and protein on the chemical composition of selected bones.

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
No. of animals	7	6	6		
Body weight (g)	171 ± 3.4	59 ± 0.9	59 ± 4.3	35	35
Fresh weight (mg):					
Mandible	224 ± 1.5	106 ± 5.7	111 ± 5.8	47	50
Femur	539 ± 10.4	230 ± 9.9	224 ± 4.3	43	42
Pelvis	437 ± 7.3	156 ± 8.4	163 ± 4.9	36	37
Tarsus	160 ± 4.9	107 ± 5.1	112 ± 4.0	67	70
Dry weight (mg):					
Mandible	161 ± 1.5	74 ± 4.0	75 ± 4.5	46	47
Femur	305 ± 5.9	121 ± 6.0	110 ± 2.0	40	36
Pelvis	243 ± 2.1	82 ± 4.5	84 ± 3.0	34	35
Tarsus	109 ± 3.7	69 ± 4.2	71 ± 2.9	63	65

Table 25-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
Fat-free dry weight (mg):					
Mandible**					
Femur	297 ± 3.8	99 ± 3.6	103 ± 2.4	34	35
Pelvis	232 ± 2.0	69 ± 3.2	78 ± 2.5	30	34
Tarsus	99 ± 2.6	54 ± 3.1	56 ± 3.1	56	58
Chemical composition:					
Ash (mg):					
No. of animals	7	5	6		
Mandible	101 ± 1.0	42 ± 2.6	45 ± 2.8	42	45
Femur	168 ± 3.0	45 ± 2.3	52 ± 1.9	27	31
Pelvis	131 ± 2.5	32 ± 1.6	39 ± 1.3	24	30
Tarsus	55 ± 2.4	25 ± 1.9	29 ± 1.1	46	53

Table 25-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
		mean ± s.e.			
Collagen (Hydroxyproline)(mg):					
No. of animals	6	4	6		
Mandible	3.30 ± 0.230	1.55 ± 0.161	1.59 ± 0.150	47	48
Femur	6.41 ± 0.160	2.13 ± 0.160	2.34 ± 0.098	33	37
Pelvis	5.20 ± 0.180	1.58 ± 0.087	1.82 ± 0.089	30	35
Tarsus	2.93 ± 0.170	1.50 ± 0.063	1.69 ± 0.080	51	57
Total Mucopolysaccharide (Hexosamine)(mg):					
No. of animals	6	6	6		
Mandible	0.41 ± 0.046	0.26 ± 0.021	0.26 ± 0.029	63	63
Femur	0.93 ± 0.033	0.65 ± 0.061	0.65 ± 0.064	70	70
Pelvis	0.73 ± 0.046	0.44 ± 0.025	0.44 ± 0.041	60	60
Tarsus	0.38 ± 0.025	0.37 ± 0.016	0.35 ± 0.037	97	92

* (1) Control - Weanling rats fed on 20% protein diet ad libitum.
(2) HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
(3) LP - Weanling rats fed on 5% protein diet ad libitum.

** Practically the same as dry weight.

For statistical significance of differences see Table 25-b.

Table 25-b: Statistical significance of differences between groups in Table 25-a.

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Body weight:	0.001	0.001	N.S.
Fresh weight:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Dry weight:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Fat-free dry weight:			
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Ash:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	0.050
Pelvis	0.001	0.001	0.050
Tarsus	0.001	0.001	N.S.

Table 25-b continued

	significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Collagen (Hydroxyproline):			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Total MPS (Hexosamine):			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.050	0.050	N.S.

Table 25-c: Per cent increment over values for weanling rats.

	Group			Values as per cent of control	
	Control	HP-R	LP	HP-R	LP
Body weight	300	38	38	13	13
Fresh weight:					
Mandible	176	31	37	18	21
Femur	232	41	37	18	16
Pelvis	335	55	61	16	18
Tarsus	112	42	49	38	44
Dry weight:					
Mandible	311	89	91	29	29
Femur	427	111	92	26	22
Pelvis	519	111	115	21	22
Tarsus	236	117	123	50	52
Fat-free dry weight:					
Femur	439	82	90	19	21
Pelvis	524	89	111	17	21
Tarsus	265	99	111	37	42
Ash:					
Mandible	381	100	114	26	30
Femur	724	121	155	17	21
Pelvis	756	109	155	14	21
Tarsus	439	145	184	33	42

Table-25-c continued

	Group			Values as per cent of control	
	Control	HP-R	LP	HP-R	LP
Collagen (Hydroxyproline):					
Mandible	275	76	81	28	29
Femur	319	39	53	12	17
Pelvis	405	53	77	13	19
Tarsus	174	40	58	23	33
Total MPS (Hexosamine):					
Mandible	95	24	24	25	25
Femur	43	0	0	0	0
Pelvis	92	16	16	17	17
Tarsus	15	12	6	80	40

Table 26-a: The effects of postweaning deficiency of food energy and protein on the chemical composition of selected bones.

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
Ash (mg):					
No. of animals	7	5	6		
Mandible	101 ± 1.0	42 ± 2.6	45 ± 2.8	42	45
Femur	168 ± 3.0	45 ± 2.3	52 ± 1.9	27	31
Pelvis	131 ± 2.5	32 ± 1.6	39 ± 1.3	24	30
Tarsus	55 ± 2.4	25 ± 1.9	29 ± 1.1	46	53
Calcium (mg):					
Mandible	38.4 ± 0.47 (38)**	16.4 ± 0.98 (40)	17.0 ± 0.86 (40)	43	44
Femur	65.4 ± 1.15 (37)	18.0 ± 0.57 (40)	20.2 ± 1.00 (39)	28	31

Table 26-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
Calcium (mg) contd.					
Pelvis	47.7 ± 1.60 (37)	13.2 ± 0.57 (40)	15.0 ± 0.63 (38)	28	31
Tarsus	21.0 ± 0.64 (38)	10.0 ± 0.84 (40)	11.2 ± 0.54 (38)	48	53
Phosphorus (mg):					
Mandible	18.8 ± 0.42 (19)	8.2 ± 0.57 (20)	8.4 ± 0.40 (20)	44	45
Femur	32.1 ± 0.67 (18)	9.1 ± 0.28 (20)	10.3 ± 0.56 (20)	28	32
Pelvis	23.4 ± 0.56 (18)	6.5 ± 0.25 (20)	7.4 ± 0.34 (19)	28	32
Tarsus	9.9 ± 0.37 (18)	5.0 ± 0.42 (20)	5.6 ± 0.21 (19)	51	57

Table 26-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	mean ± s.e.				
Ca:P ratio:					
Mandible	2·06 ± 0·052	2·00 ± 0·024	1·99 ± 0·025	98	97
Femur	2·00 ± 0·012	1·99 ± 0·030	1·97 ± 0·040	100	99
Pelvis	2·03 ± 0·029	2·02 ± 0·032	2·00 ± 0·030	100	99
Tarsus	2·12 ± 0·061	2·02 ± 0·080	2·00 ± 0·040	95	94
A:R ratio:***					
Mandible	1·70 ± 0·061	1·29 ± 0·036	1·56 ± 0·040	76	92
Femur	1·32 ± 0·036	0·91 ± 0·030	1·01 ± 0·029	69	77
Pelvis	1·13 ± 0·042	0·81 ± 0·040	0·99 ± 0·030	72	88
Tarsus	1·27 ± 0·015	0·87 ± 0·050	1·12 ± 0·070	69	88

Table 26-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
Calcium / Hydroxyproline ratio:					
Mandible	11.6	10.6	10.7	91	92
Femur	10.2	8.5	8.6	83	84
Pelvis	9.2	8.4	8.2	91	89
Tarsus	7.2	6.7	6.6	93	92
Hexosamine / Hydroxyproline ratio:					
Mandible	0.12	0.17	0.16	142	133
Femur	0.15	0.31	0.28	207	187
Pelvis	0.14	0.28	0.24	200	171
Tarsus	0.13	0.25	0.21	192	162

* (1) Control - Weanling rats fed on 20% protein diet ad libitum.
(2) HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
(3) LP - Weanling rats fed on 5% protein diet ad libitum.

** Values in parentheses are the per cent of ash.

*** Ratio of ash to non-ash components in the fat-free dry bone (for femur, pelvis and tarsus) and in the dry bone for mandible.

For statistical significance of differences see Table 26-b.

Table 26-b: Statistical significance of differences between groups in Table 26-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
Ash:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	0.050
Pelvis	0.001	0.001	0.050
Tarsus	0.001	0.001	N.S.
Calcium:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Phosphorus:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Ca:P ratio:			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
A:R ratio:			
Mandible	0.001	N.S.	0.001
Femur	0.001	0.001	0.050
Pelvis	0.001	0.050	0.010
Tarsus	0.001	N.S.	0.050

undernourished animals as might be expected from the foregoing observations, suggesting the presence of an increased amount of uncalcified matrix in the bone of the deficient animals. However, the decrease was not significant in the mandible and tarsus. The A:R ratio was more affected in the calorie restricted group than in the protein deficient group.

The calcium and phosphorus contents of bones in the deficient animals followed the same pattern as ash. The Ca:P ratio was not affected (Table 26-a).

Among the bones studied the concentration of ash in the mandible was the highest, the other three bones having almost the same concentration (Table 27-a). The percentage of bone ash was less in the deficient animals showing a poor calcification or mineralization of bone (Table 27-a). This may be due to improper calcium utilization because of reduced intestinal CaBP activity resulting from a reduced synthesis of CaBP found in protein deficiency (Kalk and Pimstone, 1974). Or it may be due to excessive faecal loss of calcium of endogenous origin (Shenolikar and Rao, 1968).

The contents of collagen (Hydroxyproline) and total mucopolysaccharide (hexosamine) were significantly less in the experimental groups (Table 25-a and b). However, the difference in collagen content followed the same pattern as

Table 27-a: The effects of postweaning deficiencies of food energy and protein on the percentage composition of bones.

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	g per 100 g of fresh tissue mean ± s.e.				
Moisture:					
Mandible	28.1 ± 0.45	30.4 ± 0.30	32.3 ± 1.13	108	115
Femur	43.4 ± 0.72	47.9 ± 0.62	50.5 ± 0.33	110	116
Pelvis	45.3 ± 0.42	46.8 ± 0.43	49.1 ± 0.38	103	108
Tarsus	33.3 ± 0.35	35.1 ± 0.67	36.8 ± 0.50	105	111
Total lipid:					
Femur	2.0 ± 0.12	9.2 ± 0.94	3.2 ± 0.42	460	160
Pelvis	2.4 ± 0.12	9.3 ± 0.81	3.5 ± 0.48	388	146
Tarsus	6.4 ± 0.38	14.7 ± 1.35	13.9 ± 0.94	230	217

Table 27-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	g per 100 g of fresh tissue mean ± s.e.				
Ash:					
Mandible	45.0 ± 0.69	38.5 ± 0.82	40.9 ± 0.81	86	91
Femur	31.3 ± 0.31	19.1 ± 0.48	23.0 ± 0.71	61	73
Pelvis	29.9 ± 0.40	20.0 ± 0.15	24.3 ± 0.48	67	81
Tarsus	33.6 ± 0.61	23.0 ± 1.00	25.8 ± 0.78	68	77
Collagen (Hydroxyproline):					
Mandible	1.50± 0.070	1.42± 0.117	1.43± 0.068	94	95
Femur	1.18± 0.028	1.06± 0.055	1.12± 0.080	90	95
Pelvis	1.22± 0.020	1.19± 0.062	1.20± 0.054	97	98
Tarsus	1.84±0. 050	1.61± 0.098	1.68± 0.079	88	91

Table 27-a continued

	Group*			Values as % of control values	
	Control (1)	HP-R (2)	LP (3)	HP-R	LP
	g per 100 g of fresh tissue mean ± s.e.				
Total Mucopolysaccharide (Hexosamine):					
Mandible	0·19 ± 0·017	0·26 ± 0·021	0·25 ± 0·019	136	132
Femur	0·18 ± 0·006	0·29 ± 0·021	0·29 ± 0·027	145	145
Pelvis	0·18 ± 0·008	0·33 ± 0·024	0·28 ± 0·024	173	147
Tarsus	0·26 ± 0·010	0·36 ± 0·013	0·35 ± 0·033	138	135

* (1) Control - Weanling rats fed on 20% protein diet ad libitum.
(2) HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
(3) LP - Weanling rats fed on 5% protein diet ad libitum.

For statistical significance of differences see Table 27-b.

Table 27-b: Statistical significance of differences between groups in Table 27-a.

	Significance of differences between means		
	1 & 2	1 & 3	2 & 3
	g per 100 g of fresh bone		
	p less than		
Moisture:			
Mandible	0.001	0.010	N.S.
Femur	0.001	0.001	0.010
Pelvis	0.050	0.001	0.050
Tarsus	0.050	0.001	N.S.
Total lipid:			
Femur	0.001	0.050	0.001
Pelvis	0.001	0.050	0.001
Tarsus	0.001	0.001	N.S.
Ash:			
Mandible	0.001	0.010	N.S.
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.050
Collagen (Hydroxyproline):			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS: (Hexosamine):			
Mandible	0.050	0.050	N.S.
Femur	0.050	0.050	N.S.
Pelvis	0.010	0.050	N.S.
Tarsus	0.001	0.050	N.S.

Table 28-a: The effects of postweaning deficiencies of food energy and protein on the percentage composition of bones.

	Group*		
	Control (1)	HP-R (2)	LP (3)
	g per 100 g of dry tissue		
Total lipid:			
Femur	3·3 ± 0·37	18·9 ± 3·70	6·9 ± 1·06
Pelvis	4·3 ± 0·39	18·1 ± 1·02	6·5 ± 0·87
Tarsus	8·8 ± 0·78	24·5 ± 3·77	21·7 ± 1·82
Ash:			
Mandible	61·5 ± 0·86	56·1 ± 0·90	60·9 ± 0·54
Femur	55·3 ± 0·67	36·4 ± 1·19	46·8 ± 1·10
Pelvis	54·0 ± 0·89	37·6 ± 0·85	46·7 ± 0·45
Tarsus	50·4 ± 0·70	35·1 ± 1·56	40·9 ± 1·11
Collagen (Hydroxyproline):			
Mandible	2·06 ± 0·110	2·05 ± 0·200	2·10 ± 0·100
Femur	2·17 ± 0·070	2·00 ± 0·123	2·24 ± 0·150
Pelvis	2·24 ± 0·030	2·23 ± 0·096	2·40 ± 0·122
Tarsus	2·73 ± 0·060	2·50 ± 0·256	2·72 ± 0·091
Total MPS (Hexosamine):			
Mandible	0·26 ± 0·028	0·37 ± 0·027	0·38 ± 0·030
Femur	0·33 ± 0·010	0·55 ± 0·040	0·60 ± 0·055
Pelvis	0·34 ± 0·014	0·62 ± 0·042	0·54 ± 0·063
Tarsus	0·38 ± 0·018	0·58 ± 0·036	0·55 ± 0·052

Table 28-a continued

	Group*		
	Control (1)	HP-R (2)	LP (3)
	g per 100 g of Fat-free dry tissue		
Ash:			
Femur	56·8 ± 0·90	45·2 ± 1·12	50·6 ± 0·84
Pelvis	56·1 ± 0·76	45·4 ± 0·76	50·2 ± 0·82
Tarsus	55·2 ± 0·75	46·0 ± 0·80	52·5 ± 1·66
Collagen (Hydroxyproline):			
Femur	2·27 ± 0·089	2·32 ± 0·187	2·38 ± 0·152
Pelvis	2·51 ± 0·171	2·52 ± 0·166	2·55 ± 0·124
Tarsus	3·08 ± 0·088	3·13 ± 0·230	3·45 ± 0·090
Total MPS (Hexosamine):			
Femur	0·34 ± 0·009	0·65 ± 0·037	0·63 ± 0·055
Pelvis	0·36 ± 0·016	0·69 ± 0·041	0·61 ± 0·056
Tarsus	0·42 ± 0·016	0·70 ± 0·057	0·72 ± 0·068

* (1) Control - Weanling rats fed on 20% protein diet ad libitum.
(2) HP-R - Weanling rats fed on 20% protein diet in restricted amounts.
(3) LP - Weanling rats fed on 5% protein diet ad libitum.

For statistical significance of difference see Table 28-b.

Table 28-b: Statistical significance of differences between groups in Table 28-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g dry bone p less than		
Total lipid:			
Femur	0.010	0.010	0.010
Pelvis	0.001	0.050	0.001
Tarsus	0.001	0.001	N.S.
Ash:			
Mandible	0.001	N.S.	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.050
Collagen:			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS:			
Mandible	0.050	0.010	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.050	N.S.
Tarsus	0.001	0.050	N.S.

Table 28-b continued

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	g per 100 g fat-free dry bone p less than		
Ash:			
Femur	0.001	0.001	0.010
Pelvis	0.001	0.001	0.010
Tarsus	0.001	N.S.	0.010
Collagen:			
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	0.050	N.S.
Total MPS:			
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.010	N.S.
Tarsus	0.001	0.010	N.S.

bone weight and concentration was not affected. On the other hand total MPS was significantly increased (Tables 27-a and b).

In the rat, protein and calorie restriction affect collagen metabolism as evident from the reduced urinary hydroxyproline excretion which is attributed to decreased turnover of body collagen (Anasuya and Rao, 1970). Angeleli et al. (1978) found that the amount of collagen nitrogen in carcass, liver and muscle was maintained whereas the amount in skin diminished as periods of protein deprivation increased in young adult rats. Also in protein deficient rats the cross linking and maturation of skin collagen seem to be impaired (Prasad and Bose, 1974) and the incorporation of ^{14}C-proline into hydroxyproline decreased (LeRoith and Pimstone, 1973).

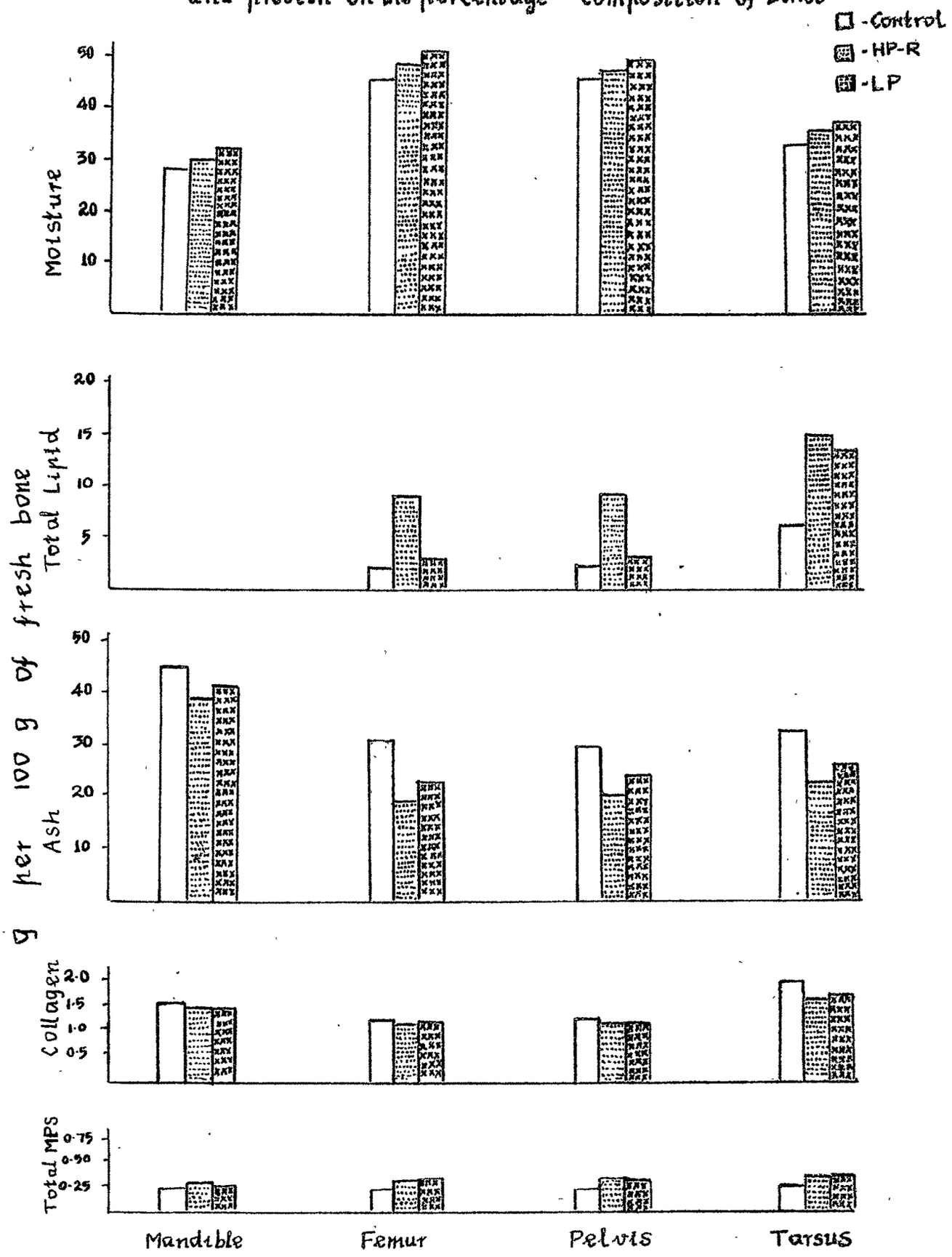
In the femur of adult mouse the amount of collagen was found to be unchanged after 20 days on a protein-free diet (Harkness et al., 1958). In the present study although the amount of collagen was significantly decreased, the concentration remained unchanged when compared to the age-matched controls. This may be because of differences in the age of the experimental animal and the period of treatment.

The concentrations of moisture, total lipid and total mucopolysaccharide were greater in the experimental

groups whereas that of ash was less (Tables 27-a and b; Figure 13). No significant differences were found with regard to collagen. 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. Similar differences between protein-deficient and calorie-deficient animals with regard to moisture and lipid have been found in this laboratory (Upadyay, 1974; Dave, 1976) and elsewhere (Gontea et al., 1974). In these studies similar differences were not found with regard to ash, but this might be because the undernourished groups used in these studies have been generally those pairfed with the protein group whereas in the present study food intake was restricted so as to achieve a weight matched group. However, this observation concerning ash is in accordance with the finding of some other investigators (Shenolikar and Rao, 1968).

Differences with regard to moisture content were more or less comparable in all the four bones studied. In the calorie deficient group the femur, pelvis and tarsus had higher lipid concentrations when compared to control in that order. In the protein deficient group, however, the order was tarsus, femur and pelvis. With regard to concentration of ash the mandible was the least affected and the femur the most affected. In this connection the ash content of femur has been generally used as a sensitive

Figure:13 The effects of postweaning deficiencies of food energy and protein on the percentage composition of bones



index of bone mineralization specially in nutritional stress. This approach seems justified on the basis of this.

In conclusion, deficiencies of food energy and protein in the bones are associated with a reduction in ash and an increase in the moisture and total lipid. The concentration of collagen is not affected whereas the concentration of the total mucopolysaccharide is greater than in controls. As maturation is associated with a decrease in moisture and mucopolysaccharide and an increase in ash, the observations with regard to these constituents are consistent with a pattern of delayed maturation. The disturbance in intestinal calcium transport and the depressed collagen synthesis seem to affect growth of bones leading to formation of bones which are shorter and lighter than in age-matched controls. Of the bones selected for the study the mandible and tarsus which seem to have an ontogenetic priority are relatively spared when compared with the pelvic and femur bones which are severely affected.

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 (Table 29). 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.

Table 29: Comparative deficits for the preweaning and postweaning undernutrition.

	Undernutrition as % of control values			
	Preweaning		Postweaning	
	LL	G^+L^-	HP-R	LP
Body weight:	44	69	65	65
Fresh weight:				
Mandible	35	49	53	50
Femur	48	72	58	59
Pelvis	50	73	64	63
Tarsus	32	59	33	30
Dry weight:				
Mandible	35	51	54	53
Femur	48	73	60	64
Pelvis	53	75	66	65
Tarsus	38	65	35	34
Fat-free dry weight:				
Femur	51	74	66	65
Pelvis	55	77	70	66
Tarsus	42	69	44	42
Bone measurements:				
Length:				
Mandible	13	26	10	12
Femur	15	30	24	26
Pelvis	19	34	28	28
Tarsus	15	25	3	8
Width:				
Mandible	13	19	16	20
Femur	11	28	11	12
Pelvis	21	37	26	30

Table 29 continued

	Undernutrition as % of control values			
	Preweaning		Postweaning	
	LL	G^+L^-	HP-R	LP
Chemical composition:				
Ash:				
Mandible	42	55	58	55
Femur	51	78	73	69
Pelvis	60	80	76	70
Tarsus	50	74	54	47
Collagen (Hydroxyproline):				
Mandible	33	49	53	52
Femur	40	69	67	63
Pelvis	41	73	70	65
Tarsus	33	61	49	43
Total MPS (Hexosamine):				
Mandible	29	48	37	37
Femur	20	60	30	30
Pelvis	21	63	40	40
Tarsus	21	42	3	8
A:R ratio:				
Mandible	16	21	24	8
Femur	12	15	31	23
Pelvis	14	15	28	12
Tarsus	23	31	31	12

Experiment-IV

EFFECTS OF MATERNAL VITAMIN A DEFICIENCY DURING GESTATION AND LACTATION ON THE BONES OF THE OFFSPRING

As mentioned earlier, vitamin A deficiency has an appreciable prevalence among the poor in developing countries, particularly in the interior rice-eating areas, as rice is practically devoid of carotene. Vitamin A plays a role in the formation and renewal of cartilage and bone and skeletal changes are seen in both hypo- and hypervitaminosis A (Irving, 1949; Howell and Thompson, 1967; Lucy et al., 1961; Grey et al., 1965; Wolke et al., 1969).

A number of studies have shown that various bone components are affected when vitamin A deficiency is imposed on growing animals (Dziewiatkowski, 1954; Havivi and Wolf, 1967; Havivi and Tal, 1974; Harris and Navia, 1978). Owing to variations in their experimental designs and experimental animals the results were not in agreement. The effects of vitamin A deficiency on different tissues are shown in Table 30.

The present experiment was concerned with the effects on bone of vitamin A deficiency during the suckling period in rats. To induce vitamin A deficiency during the neonatal period mothers were fed a standard diet with vitamin A content reduced to 500 I.U. per kg or 10% of the amount fed to control,

Table 30: Effects of vitamin A deficiency on different tissues.

Type			Vitamin-A Deficiency
Epithelial tissues (DeLuca, 1977)			
Rat:	Small Intestine	Columnar, mucus secreting	Decrease in goblet cell
	Testis	Columnar, germinal epithelium	Degeneration
	Pancreatic Islets	Columnar mucus secreting	Squamous metaplasia, keratinization
	Urinary tract	Columnar	Squamous metaplasia, keratinization
	Tracheal Epithelium	Columnar, mucus secreting	Squamous metaplasia keratinization
	Vagina	Columnar, mucus secreting	Squamous metaplasia keratinization
Mammalian:			
	Epidermis	Keratinized with mucous sebaceous glands	Squamous metaplasia of glands
	Taste bud	Columnar, mucus secreting	Squamous metaplasia keratinization
	Glandular Tissues	Mucus secreting	Squamous metaplasia keratinization
Rat: Eye (Dowling, 1961)		Rods and Cones	atrophied
Bone:			
Guinea pigs:			
	Bone implant (Harris et al.,1978)	Osteoblasts	More osteoblasts and more coarsely woven bone
Rat: Bone (Mellanby, 1950)		Osteoblasts and Osteoclasts	Excessive osteoblastic activity and periosteal bone formation and reduced osteoclastic resorptions.
Brain: (Ridgon, 1962)		Optic thalamus, optic femoral and sciatic nerves	Degeneration
(Bhat and Rama Rao, 1978)		Optic thalamus, Optic femoral and Sciatic nerves	Impairment of myelination

from a month before mating until partus after which a diet devoid of vitamin A was fed. As mentioned earlier, a diet containing at least a small amount of vitamin A was found to be needed to prevent resorption of the fetus and to get viable pups while achieving vitamin A deficiency in the pups. The controls were fed the standard diet containing 5000 I.U. of vitamin A acetate per kg diet throughout. The pups were killed at 21 days of age. The liver vitamin A in the experimental mothers was found to be reduced to 10% of control values (Table 31-a).

The data obtained were compared with those obtained previously for animals reared in large litters (LL) with a comparable degree of growth retardation in order to ensure that any effects observed in the deficient animals were not due to growth retardation produced by undernutrition.

The results of these studies are given in the Tables 31-a to 35-b and the bones studied are shown in Figure 14. The body weights of the weanling rats reared by the vitamin A deficient mother and those in large litters were respectively 71% and 56% of the control values (Table 31-a).

As in the case of the undernourished rats bone weight and length, ash, calcium, phosphorus, collagen (hydroxy-proline), total MPS (hexosamine) and chondroitin sulphate (uronic acid) content were reduced in the deficient group.

Figure : 14 Effects of maternal vitamin A deficiency

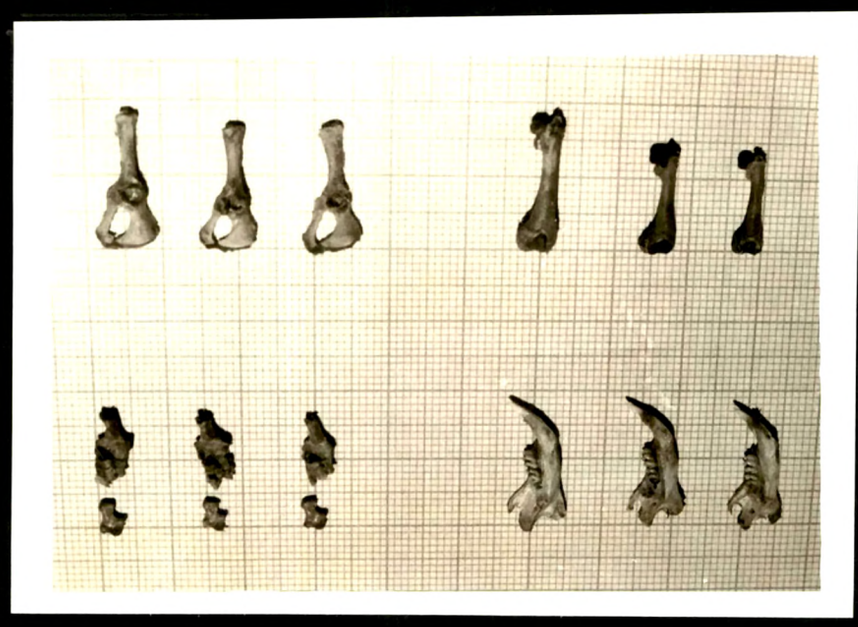


Table 31-a: The effects of maternal vitamin A deficiency during gestation and lactation on bone size and morphology in the progeny.

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit,A⁻	LL
	mean ± s.e.				
Body weight (g):					
At birth	6.6 ± 0.12	6.5 ± 0.11	6.6 ± 0.12		
1 week	12.6 ± 0.75	9.4 ± 0.57			
2 week	23.9 ± 1.20	18.6 ± 1.31			
At weaning	42.7 ± 0.92	30.3 ± 0.73	24.0 ± 0.78	71	56
No. of animals	6	6	6		
Vitamin A level in the liver of the mother (µg/g liver)†	145	15	N.D.	10	-
Fresh weight (mg):					
Mandible	76.8 ± 2.52 (1.8)**	66.2 ± 1.86 (2.2)	52.3 ± 0.92 (2.2)	86	68
Femur	164.0 ± 3.20 (3.8)	111.0 ± 3.20 (3.7)	84.0 ± 1.96 (3.5)	68	51
Pelvis	100.5 ± 1.35 (2.4)	72.8 ± 1.82 (2.3)	49.5 ± 1.19 (2.1)	72	49

Table 31-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Fresh weight (mg) contd.					
Tarsus	80.2 ± 0.52 (1.9)	59.0 ± 1.90 (2.0)	51.2 ± 2.00 (2.1)	74	64
Bone measurements (cm):					
Length:					
Mandible	2.08 ± 0.038	1.85 ± 0.023	1.78 ± 0.033	89	86
Femur	1.84 ± 0.017	1.54 ± 0.020	1.57 ± 0.039	84	85
Pelvis	2.06 ± 0.020	1.74 ± 0.030	1.70 ± 0.063	84	82
Tarsus	1.11 ± 0.020	0.97 ± 0.023	0.93 ± 0.028	87	84
Width:					
Mandible	0.92 ± 0.012	0.82 ± 0.018	0.82 ± 0.017	89	89
Femur	0.54 ± 0.007	0.49 ± 0.007	0.48 ± 0.020	91	89
Pelvis	0.94 ± 0.014	0.80 ± 0.010	0.76 ± 0.027	85	81

Table 31-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A$^-$ (2)	LL (3)	Vit.A$^-$	LL
Width/Length:					
Mandible	0.44	0.44	0.46	100	105
Femur	0.29	0.32	0.31	110	107
Pelvis	0.46	0.46	0.45	100	98
Comparison of bone lengths:					
Mandible/Femur	1.13	1.20	1.13	106	100
Pelvis/Femur	1.12	1.13	1.08	101	96
Tarsus/Femur	0.60	0.63	0.59	105	98

* (1) Control - Mothers fed on a 20% protein diet (Vit.A$^+$) ad libitum.
(2) Vit.A$^-$ - Mothers fed on a 20% protein, vitamin A deficient diet.
(3) LL - Mothers fed on a 20% protein diet (Vit.A$^+$), but the litter size increased to 16.

** Values in parentheses are bone weight expressed as mg per g of body weight.

† Average for 2 rats.

N.D. - Not determined.

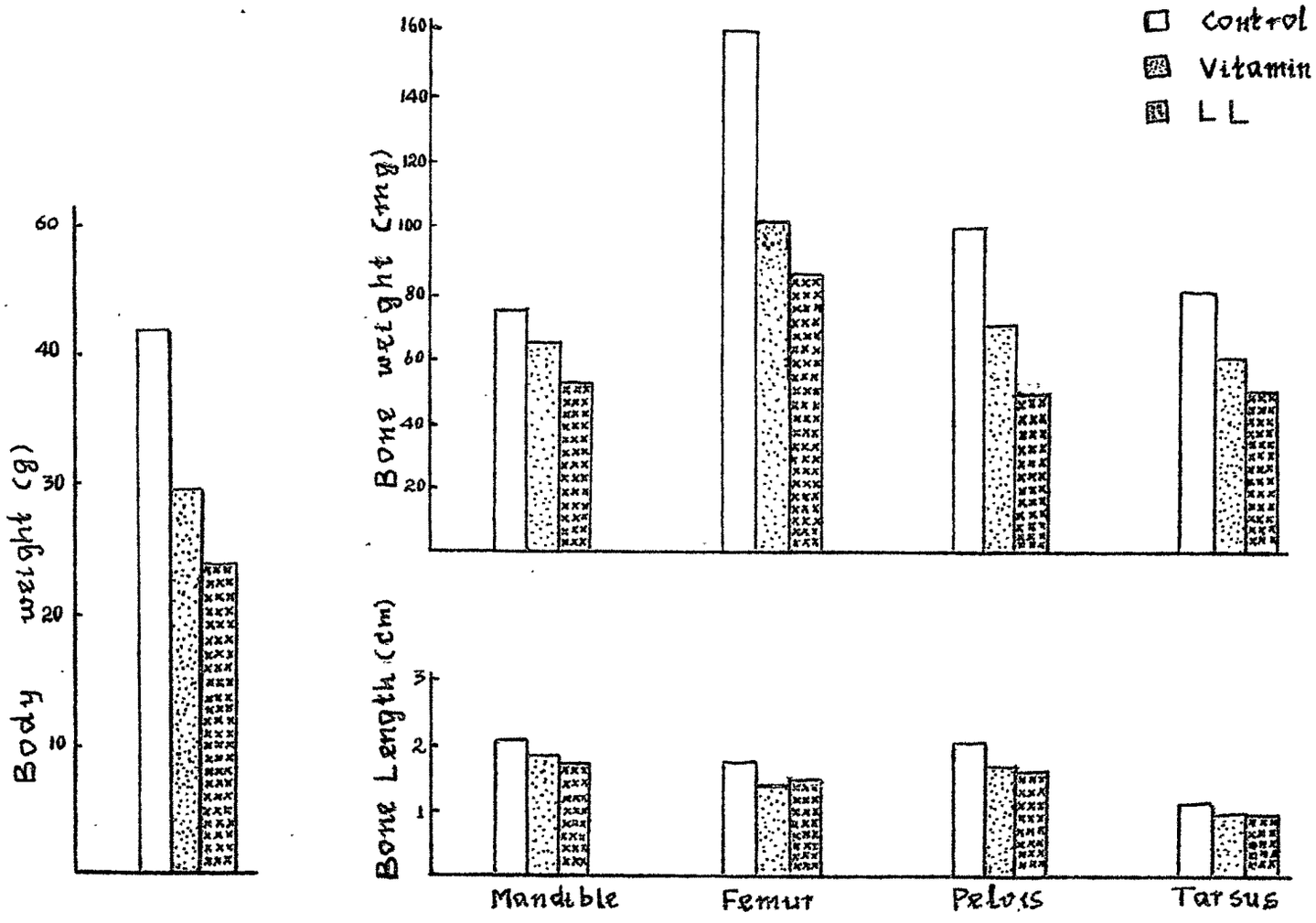
For statistical significance of differences see Table 31-b.

Table 31-b: Statistical significance of differences between the groups in Table 31-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
Fresh weight:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Bone measurements:			
Length:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Width:			
Mandible	0.001	0.010	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.

N.S. Not Significant

Figure: 15. The effects of maternal Vitamin A deficiency on Body weight and Bone size in the progeny



As in undernutrition, the length of the bones was less affected than bone weight and body weight (Figure 15). The length of the pelvis and femur were found to be affected relatively more than the tarsus and mandible. The same pattern was observed when the bone weights are expressed as mg per g of the total body weight (Table 31-a).

The mean values for the fresh weight of different bones ranged from 68% to 86% of the control values in the vitamin A deficient rats whereas they ranged from 49% to 68% in the LL rats, the deficits being less in the mandible and tarsus in both groups (Table 31-a). The deficits in fresh weight, dry weight and fat-free dry weight of the bones 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 (Table 32-a).

In the bones of vitamin A deficient rats, the amounts of ash, collagen, total mucopolysaccharide and chondroitin sulphate were less than in the controls (Table 32-a). A similar decrease was found in the content of calcium and phosphorus (Table 33-a).

Although decrease was observed in the content of calcium and hydroxyproline in both the groups, the decrease in the calcium:hydroxyproline ratio, was greater in the undernourished rats, than in the vitamin A deficient animals. A:R ratio was significantly decreased in the pelvis and femur,

Table 32-a: The effects of maternal vitamin A deficiency during gestation and lactation on the chemical composition of the bones in the progeny.

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
No. of animals	6	6	6		
Fresh weight (mg):					
Mandible	76.8 ± 2.52	66.2 ± 1.86	52.3 ± 0.92	86	68
Femur	164.0 ± 3.20	111.0 ± 3.20	84.0 ± 1.96	68	51
Pelvis	100.5 ± 1.35	72.8 ± 1.82	49.5 ± 1.19	72	49
Tarsus	80.2 ± 0.52	59.0 ± 2.00	51.2 ± 2.00	74	64
Dry weight (mg):					
Mandible	37.8 ± 1.57	32.3 ± 0.83	25.4 ± 0.89	85	67
Femur	62.5 ± 1.59	42.4 ± 0.80	29.2 ± 0.82	68	47
Pelvis	42.0 ± 0.30	29.2 ± 0.71	18.3 ± 0.39	70	44
Tarsus	37.7 ± 0.83	25.1 ± 1.09	19.7 ± 0.48	67	52

Table 32-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Fat-free dry weight (mg):					
Mandible**					
Femur	57.6 ± 1.16	38.8 ± 0.88	26.7 ± 0.91	67	47
Pelvis	39.2 ± 0.34	26.8 ± 0.71	16.6 ± 0.36	68	42
Tarsus	31.0 ± 0.57	19.8 ± 0.83	15.5 ± 0.35	64	50
Ash (mg):					
No. of animals	3	6	6		
Mandible	21.2 ± 1.33	16.9 ± 0.54	12.1 ± 0.14	80	57
Femur	21.6 ± 0.41	13.8 ± 0.23	9.9 ± 0.46	64	46
Pelvis	15.7 ± 0.42	10.0 ± 0.24	6.1 ± 0.09	64	39
Tarsus	11.7 ± 0.41	6.8 ± 0.37	5.1 ± 0.09	58	44
Collagen (Hydroxyproline)(mg):					
No. of animals	6	6	5		
Mandible	0.91 ± 0.044	0.77 ± 0.045	0.59 ± 0.035	85	65
Femur	1.52 ± 0.068	1.02 ± 0.046	0.92 ± 0.065	67	61

Table 32-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Collagen (Hydroxyproline)(mg) contd.					
Pelvis	1.05 ± 0.053	0.79 ± 0.032	0.61 ± 0.015	75	58
Tarsus	1.07 ± 0.052	0.82 ± 0.066	0.72 ± 0.057	77	67
Total MPS (Hexosamine)(mg):					
No. of animals	4	6	6		
Mandible	0.19 ± 0.025	0.17 ± 0.023	0.15 ± 0.006	89	79
Femur	0.64 ± 0.015	0.51 ± 0.041	0.52 ± 0.008	80	81
Pelvis	0.37 ± 0.014	0.27 ± 0.007	0.30 ± 0.021	73	81
Tarsus	0.38 ± 0.015	0.30 ± 0.012	0.26 ± 0.028	79	68

* (1) Control - Mothers fed on a 20% protein diet (Vit.A⁺) ad libitum.
(2) Vit.A⁻ - Mothers fed on a 20% protein, vitamin A deficient diet.
(3) LL - Mothers fed on a 20% protein diet (Vit.A⁺), but the litter size increased to 16.

** Practically the same as dry weight.

For statistical significance of differences see Table 32-b.

Table 32-b: Statistical significance of differences between the groups in Table 32-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	p less than		
Body weight	0.001	0.001	0.010
Fresh weight:			
Mandible	0.001	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Dry weight:			
Mandible	0.010	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Fat-free dry weight:			
Mandible	-	-	-
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Ash:			
Mandible	0.010	0.001	0.010
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001

Table 32-b continued

	Significance of differences between means		
	1 & 2	1 & 3	2 & 3
	p less than		
Collagen (Hydroxyproline):			
Mandible	0.050	0.001	0.010
Femur	0.001	0.001	N.S.
Pelvis	0.010	0.010	0.001
Tarsus	0.050	0.001	N.S.
Total MPS (Hexosamine):			
Mandible	N.S.	N.S.	N.S.
Femur	0.050	0.001	N.S.
Pelvis	0.001	0.050	N.S.
Tarsus	0.050	0.010	N.S.

Table 33-a: The effects of maternal vitamin A deficiency during gestation and lactation on the chemical composition of bones in the progeny.

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Ash (mg):					
Mandible	21.2 ± 1.33	16.9 ± 0.54	12.1 ± 0.14	80	57
Femur	21.6 ± 0.41	13.8 ± 0.23	9.9 ± 0.46	64	46
Pelvis	15.7 ± 0.42	10.0 ± 0.24	6.1 ± 0.09	64	39
Tarsus	11.7 ± 0.41	6.8 ± 0.37	5.1 ± 0.09	58	44
Calcium (mg):					
Mandible	7.9 ± 0.34 (37.2)**	6.3 ± 0.13 (37.6)	4.5 ± 0.08 (37.4)	80	57
Femur	8.0 ± 0.29 (35.5)	5.4 ± 0.17 (39.0)	3.4 ± 0.11 (36.1)	68	43
Pelvis	5.6 ± 0.12 (35.7)	3.8 ± 0.09 (37.4)	2.2 ± 0.09 (36.5)	68	39
Tarsus	4.1 ± 0.15 (35.4)	2.5 ± 0.10 (38.0)	1.9 ± 0.05 (36.9)	61	46

Table 33-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Phosphorus (mg):					
Mandible	4·1 ± 0·21 (19·6)	3·3 ± 0·10 (19·3)	2·3 ± 0·09 (18·9)	80	56
Femur	4·4 ± 0·10 (20·5)	2·9 ± 0·09 (20·8)	1·7 ± 0·06 (18·5)	66	39
Pelvis	3·0 ± 0·05 (19·3)	2·0 ± 0·07 (19·6)	1·2 ± 0·02 (18·6)	66	40
Tarsus	2·2 ± 0·14 (18·8)	1·4 ± 0·06 (19·8)	0·9 ± 0·04 (18·3)	64	41
Chondroitin sulphate (Uronic acid)(mg):					
No. of animals	4	6	6		
Mandible	0·08 ± 0·006	0·06 ± 0·010	0·07 ± 0·004	75	88
Femur	0·57 ± 0·010	0·38 ± 0·034	0·40 ± 0·034	67	70
Pelvis	0·31 ± 0·020	0·19 ± 0·008	0·19 ± 0·025	61	61
Tarsus	0·29 ± 0·008	0·21 ± 0·016	0·23 ± 0·017	72	79

Table 33-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	mean ± s.e.				
Ca/P ratio:					
Mandible	1.90 ± 0.020	1.95 ± 0.026	1.99 ± 0.028	103	105
Femur	1.80 ± 0.060	1.91 ± 0.036	1.97 ± 0.040	106	109
Pelvis	1.86 ± 0.016	1.92 ± 0.040	1.95 ± 0.039	103	105
Tarsus	1.86 ± 0.044	1.93 ± 0.015	2.03 ± 0.050	104	109
A/R ratio:**					
Mandible	1.13 ± 0.021	1.10 ± 0.014	0.98 ± 0.010	97	87
Femur	0.61 ± 0.010	0.54 ± 0.012	0.53 ± 0.013	88	87
Pelvis	0.66 ± 0.021	0.58 ± 0.015	0.57 ± 0.007	88	86
Tarsus	0.60 ± 0.042	0.53 ± 0.019	0.48 ± 0.010	88	80

Table 33-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
Uronic acid / Hexosamine ratio:					
Mandible	37	33	47	89	127
Femur	89	76	77	85	87
Pelvis	89	70	63	79	71
Tarsus	76	70	88	92	116
Hexosamine / Hydroxyproline ratio:					
Mandible	0.21	0.22	0.25	105	119
Femur	0.42	0.50	0.57	119	136
Pelvis	0.35	0.35	0.49	100	140
Tarsus	0.36	0.37	0.36	103	100

Table 33-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
Calcium / Hydroxyproline ratio:					
Mandible	8•7	8•2	7•6	94	87
Femur	5•3	5•2	3•7	98	70
Pelvis	5•3	4•8	3•6	91	68
Tarsus	3•8	3•0	2•6	79	68

* (1) Control - Mothers fed on a 20% protein diet (Vit.A⁺) ad libitum.
(2) Vit.A⁻ - Mothers fed on a 20% protein, vitamin A deficient diet.
(3) LL - Mothers fed on a 20% protein diet (Vit.A⁺), but the litter size increased to 16.

** Values in parentheses are per cent of ash.

*** Ratio of ash to non-ash components in the fat-free dry bone for femur, pelvis and tarsus, and in the dry bone for mandible.

For statistical significance of differences see Table 33-b.

Table 33-b: Statistical significance of differences between the groups in Table 33-a.

	Significance of differences between means		
	1 & 2	1 & 3	2 & 3
	p less than		
Ash:			
Mandible	0.010	0.001	0.010
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Calcium:			
Mandible	0.010	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Phosphorus:			
Mandible	0.010	0.001	0.001
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.001
Uronic acid:			
Mandible	N.S.	N.S.	N.S.
Femur	0.001	0.010	N.S.
Pelvis	0.001	0.010	N.S.
Tarsus	0.010	0.050	N.S.

Table 33-b continued

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Ca:P ratio:			
Mandible	N.S.	0.050	N.S.
Femur	N.S.	0.050	N.S.
Pelvis	N.S.	0.050	N.S.
Tarsus	N.S.	0.050	N.S.
A:R ratio:			
Mandible	N.S.	0.001	0.001
Femur	0.010	0.010	N.S.
Pelvis	0.010	0.010	N.S.
Tarsus	N.S.	0.050	0.050

as observed in earlier studies in this laboratory for femur (Dave, 1976). But the decrease in mandible and tarsus was not significant (Table 33-a and b).

Although the Ca:P ratios were generally greater than in controls and the increases were significant in undernourished rats, they were not significant in vitamin A deficient rats (Table 33a and b).

As in undernutrition, ash content and concentration (Tables 34-a and b) were found to be reduced. However, in the mandible the percentage of ash was affected in undernutrition but not in vitamin A deficiency. The deficits were progressively less in the order mandible, femur, pelvis and tarsus and less in all cases than in undernourished animals (Tables 33-a and 34-a). This is in agreement with the finding of Harris and Navia (1977) who reported a decreased calcium content in newly formed bone from vitamin A deficient guinea pigs compared with controls.

The amounts of collagen (hydroxyproline) were significantly decreased in the bones of the vitamin A deficient rats (Tables 32-a and b). But the concentration of hydroxyproline, however, was not affected (Tables 34-a and b). Similar observations have been made in vitamin-A deficient chicks (Havivi and Wolf, 1967). According to earlier studies in this laboratory, the concentration of total nitrogen was increased (Dave, 1976) suggesting variations in the ratio of

collagen:non-collagen nitrogen and an increase in proteins other than collagen.

The amount of total mucopolysaccharide (Tables 32-a and b) was significantly reduced in the bones of the vitamin A deficient rats, but concentration was not affected (Table 34-a) although it was significantly increased in the femur and pelvis of undernourished rats, suggesting the immaturity of these two bones compared to the mandible and tarsus.

The amount of chondroitin sulphate, measured as uronic acid (Tables 33-a and b) and its concentration (Tables 34-a and b) were significantly decreased in all the bones except the mandible in which case the difference was in the same direction but fell short of significance. The decrease in chondroitin sulphate may be related to the lack of adequate vitamin A. Vitamin A is known to be associated with the metabolism of sulphated glycosaminoglycans (mucopolysaccharides) in the epithelial tissue (Wolf and Varandani, 1960) and chondroitin sulphate is closely involved in the process of mineralization (Dorey and Bick, 1977). Whether the decrease in chondroitin sulphate is due to a failure of synthesis or due to an increased degradation is not known.

In the epiphyseal cartilage an increase in the concentration of chondroitin sulphate was found by Havivi and Wolf (1967) while a lowered uptake of sulphate, both *in vovo* and *in vitro*, by epiphyseal cartilage (Dziewiatkowsky, 1954;

Table 34-a: The effects of maternal vitamin A deficiency during gestation and lactation on percentage composition of the bones in the progeny.

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	g per 100 g fresh bone mean ± s.e.				
Moisture:					
Mandible	50·9 ± 0·61	51·8 ± 1·05	52·9 ± 0·30	102	104
Femur	61·2 ± 0·38	62·5 ± 0·26	65·5 ± 0·26	102	107
Pelvis	58·2 ± 0·37	60·2 ± 0·60	63·0 ± 0·55	103	109
Tarsus	53·2 ± 0·70	56·7 ± 0·53	62·0 ± 1·01	107	117
Total lipid:					
Femur	2·9 ± 0·29	3·0 ± 0·19	3·0 ± 0·27	103	103
Pelvis	2·7 ± 0·27	3·1 ± 0·22	3·6 ± 0·23	115	133
Tarsus	7·9 ± 0·50	9·0 ± 0·26	8·2 ± 0·27	115	104
Ash:					
Mandible	26·1 ± 0·68	25·6 ± 0·23	23·5 ± 0·25	98	90
Femur	13·4 ± 0·22	12·5 ± 0·19	11·2 ± 0·10	93	84

Table 34-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A⁻ (2)	LL (3)	Vit.A⁻	LL
	g per 100 g fresh bone mean ± s.e.				
Ash: (contd.)					
Pelvis	15.7 ± 0.10	13.7 ± 0.22	12.4 ± 0.19	87	79
Tarsus	14.6 ± 0.50	11.8 ± 0.37	10.0 ± 0.45	81	68
Collagen (Hydroxyproline):					
Mandible	1.26 ± 0.076	1.29 ± 0.025	1.09 ± 0.053	102	87
Femur	0.94 ± 0.038	1.00 ± 0.024	0.84 ± 0.005	106	89
Pelvis	1.05 ± 0.053	1.20 ± 0.080	0.90 ± 0.020	114	86
Tarsus	1.24 ± 0.041	1.29 ± 0.087	1.16 ± 0.029	104	94
Total MPS (Hexosamine):					
Mandible	0.24 ± 0.020	0.25 ± 0.015	0.27 ± 0.012	104	113
Femur	0.43 ± 0.012	0.48 ± 0.041	0.60 ± 0.012	112	140
Pelvis	0.38 ± 0.013	0.42 ± 0.012	0.59 ± 0.028	111	155
Tarsus	0.49 ± 0.018	0.50 ± 0.030	0.54 ± 0.050	102	110

Table 34-a continued

	Group*			Values as % of control values	
	Control (1)	Vit.A$^-$ (2)	LL (3)	Vit.A$^-$	LL
	g per 100 g fresh bone mean ± s.e.				
Uronic acid:					
Mandible	0·10 ± 0·005	0·09 ± 0·011	0·13 ± 0·008	90	130
Femur	0·38 ± 0·011	0·33 ± 0·017	0·44 ± 0·032	87	116
Pelvis	0·33 ± 0·016	0·28 ± 0·009	0·35 ± 0·037	85	106
Tarsus	0·39 ± 0·003	0·33 ± 0·019	0·42 ± 0·025	85	108

* (1) Control - Mothers fed on a 20% protein diet (Vit.A$^+$) ad libitum.
(2) Vit.A$^-$ - Mothers fed on a 20% protein, vitamin A deficient diet.
(3) LL - Mothers fed on a 20% protein diet (Vit.A$^+$), but the litter size increased to 16.

For statistical significance of differences see Table 34-b.

Table 34-b: Statistical significance of differences between the groups in Table 34-a.

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	g per 100 g of fresh bone p less than		
Moisture:			
Mandible	N.S.	0.050	0.010
Femur	0.050	0.050	0.001
Pelvis	0.050	0.050	0.010
Tarsus	0.050	0.050	0.001
Total Lipid:			
Mandible	-	-	-
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	0.050	N.S.
Tarsus	N.S.	N.S.	N.S.
Ash:			
Mandible	N.S.	0.010	0.001
Femur	0.050	0.010	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.010	0.001	0.010
Collagen (Hydroxyproline):			
Mandible	N.S.	N.S.	0.010
Femur	N.S.	0.050	0.001
Pelvis	N.S.	0.050	0.050
Tarsus	N.S.	N.S.	N.S.

Table 34-b continued

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	g per 100 g of fresh bone p less than		
Total MPS (Hexosamine):			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	0.001	0.050
Pelvis	N.S.	0.001	0.001
Tarsus	N.S.	N.S.	N.S.
Chondroitin sulphate (Uronic acid):			
Mandible	N.S.	N.S.	0.050
Femur	0.050	N.S.	0.010
Pelvis	0.010	N.S.	N.S.
Tarsus	0.010	N.S.	0.050

N.S. Not Significant

Table 35-a: The effects of maternal vitamin A deficiency during gestation and lactation on the percentage composition of bones.

	Group*		
	Control (1)	Vit.A⁻ (2)	LL (3)
	g per 100 g dry bone mean ± s.e.		
Total lipid:			
Femur	8.1 ± 0.54	8.5 ± 0.72	9.6 ± 0.21
Pelvis	7.2 ± 0.67	7.9 ± 0.44	9.5 ± 0.61
Tarsus	18.7 ± 0.91	21.7 ± 0.61	21.1 ± 0.58
Ash:			
Mandible	52.9 ± 0.50	52.2 ± 0.32	49.8 ± 0.52
Femur	35.0 ± 0.71	31.6 ± 0.52	31.5 ± 0.47
Pelvis	37.3 ± 0.53	34.3 ± 0.37	33.2 ± 0.37
Tarsus	30.6 ± 1.10	27.3 ± 0.81	25.9 ± 0.54
Collagen (Hydroxyproline):			
Mandible	2.43 ± 0.158	2.57 ± 0.083	2.12 ± 0.087
Femur	2.46 ± 0.063	2.57 ± 0.048	2.46 ± 0.016
Pelvis	2.55 ± 0.130	2.68 ± 0.117	2.45 ± 0.026
Tarsus	2.85 ± 0.088	2.97 ± 0.192	2.72 ± 0.026
Total MPS (Hexosamine):			
Mandible	0.49 ± 0.042	0.53 ± 0.034	0.58 ± 0.020
Femur	1.21 ± 0.034	1.26 ± 0.097	1.68 ± 0.037
Pelvis	0.96 ± 0.044	0.99 ± 0.029	1.67 ± 0.117
Tarsus	1.17 ± 0.040	1.18 ± 0.092	1.35 ± 0.150

Table 35-a continued

	Group*		
	Control (1)	Vit.A⁻ (2)	LL (3)
	g per 100 g of fat-free dry bone mean ± s.e.		
Ash:			
Femur	38.4 ± 0.48	35.7 ± 0.46	35.2 ± 0.41
Pelvis	40.5 ± 1.30	37.4 ± 0.27	36.9 ± 0.29
Tarsus	37.7 ± 1.41	34.5 ± 1.17	32.8 ± 0.57
Collagen (Hydroxyproline):			
Femur	2.61 ± 0.080	2.83 ± 0.062	2.68 ± 0.028
Pelvis	2.68 ± 0.140	2.90 ± 0.113	2.65 ± 0.021
Tarsus	3.28 ± 0.130	3.92 ± 0.231	3.36 ± 0.081
Total MPS (Hexosamine):			
Femur	1.29 ± 0.039	1.38 ± 0.088	1.85 ± 0.030
Pelvis	1.04 ± 0.051	1.15 ± 0.038	1.84 ± 0.135
Tarsus	1.39 ± 0.032	1.42 ± 0.095	1.56 ± 0.140

* (1) Control - Mothers fed on a 20% protein diet (Vit.A⁺) ad libitum.
(2) Vit.A⁻ - Mothers fed on a 20% protein, vitamin A deficient diet.
(3) LL - Mothers fed on a 20% protein diet (Vit.A⁺), but the litter size increased to 16.

For statistical significance of differences see Table 35-b.

Table 35-b: Statistical significance of differences between the groups in Table 35-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g dry bone		
	p less than		
Total lipid:			
Femur	N.S.	0.05	N.S.
Pelvis	N.S.	0.05	N.S.
Tarsus	0.05	0.05	N.S.
Ash:			
Mandible	N.S.	0.01	0.01
Femur	0.01	0.01	0.05
Pelvis	0.01	0.001	N.S.
Tarsus	0.05	0.01	N.S.
Collagen (Hydroxyproline):			
Mandible	N.S.	N.S.	0.01
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS (Hexosamine):			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	0.001	0.01
Pelvis	N.S.	0.001	0.001
Tarsus	N.S.	N.S.	N.S.

Table 35-b continued

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	g per 100 g fat-free dry bone p less than		
Ash:			
Femur	0.01	0.001	N.S.
Pelvis	0.05	0.05	N.S.
Tarsus	N.S.	0.05	N.S.
Collagen (Hydroxyproline):			
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	0.05	N.S.	N.S.
Total MPS (Hexosamine):			
Femur	N.S.	0.001	0.001
Pelvis	N.S.	0.010	0.001
Tarsus	N.S.	N.S.	N.S.

N.S. Not Significant

Wolf and Varandani, 1960) and by costal cartilage (Mohan and Rao, 1980) by others. The concentration of total MPS has not been found to be reduced in the skin (Mohanram *et al.*, 1976).

Thus contradictory reports have been made about the incorporation of ^{35}S-sulphur in the bone in vitamin A deficient conditions. These differences may be due to the difference in their experimental models. Dziewiatkowski (1954) reported a decrease in ^{35}S-sulphur incorporation into the mucopolysaccharide fraction of the whole skeleton in rats whereas Harris and Navia (1978) found in *in vitro* and *in vivo* studies of bone tissue an increased ^{35}S-sulphur intake. In the latter studies, the authors do not rule out the possibility of an increased sulphation of the glycosaminoglycans (mucopolysaccharides) molecules rather than an overall increase in the glycosaminoglycans (mucopolysaccharides) present.

The moisture content was greater in both the groups. However, the increase in moisture was not significant in the mandible (Table 34-a).

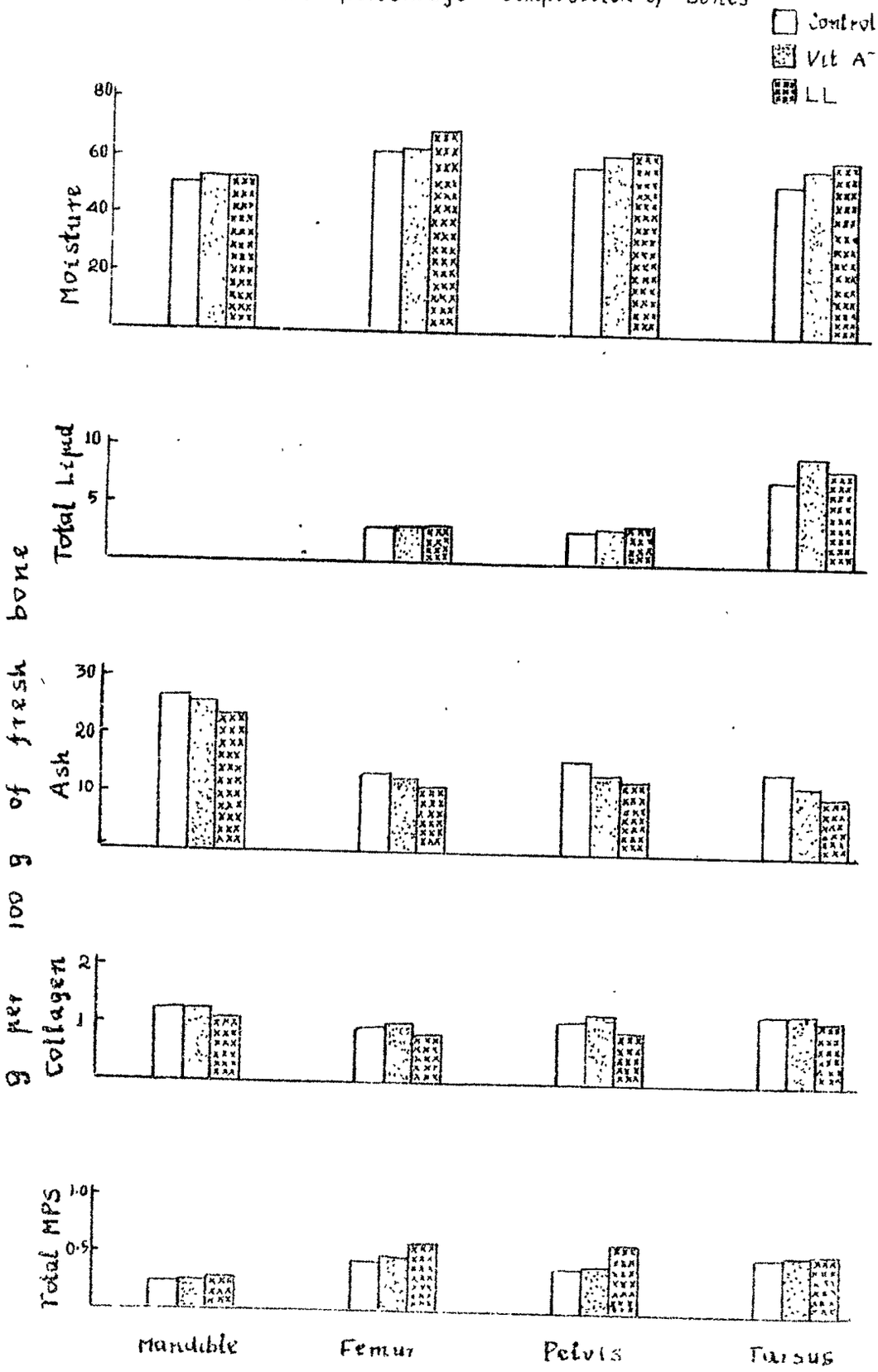
Although mean values for total lipid were numerically greater in the stressed groups in the case of all the bones, showing a consistent trend, the differences were not significant , because of the wide variation in the lipid content (Table 34-a).

In conclusion, deficiency of vitamin A in the preweaning period is associated with an increase in the concentration of moisture, a reduction in the concentration of ash and chondroitin sulphate and no variation in the concentration of total lipid, collagen and total MPS (Figure 16).

The object of these investigations was to study the effects of vitamin A deficiency in the suckling period. But to achieve this, the maternal diets had to be made marginally deficient in vitamin A during gestation. However, the effects are observed to be mainly due to deficiency in the suckling period since no differences were evident in the birth weight. In vitamin A deficiency the bones are less affected than the undernourished animals regarding the various parameters and differ from them in having a reduced level of chondroitin sulphate.

OK

Figure · 16 The effects of maternal Vitamin A deficiency on the percentage composition of bones



Experiment-V

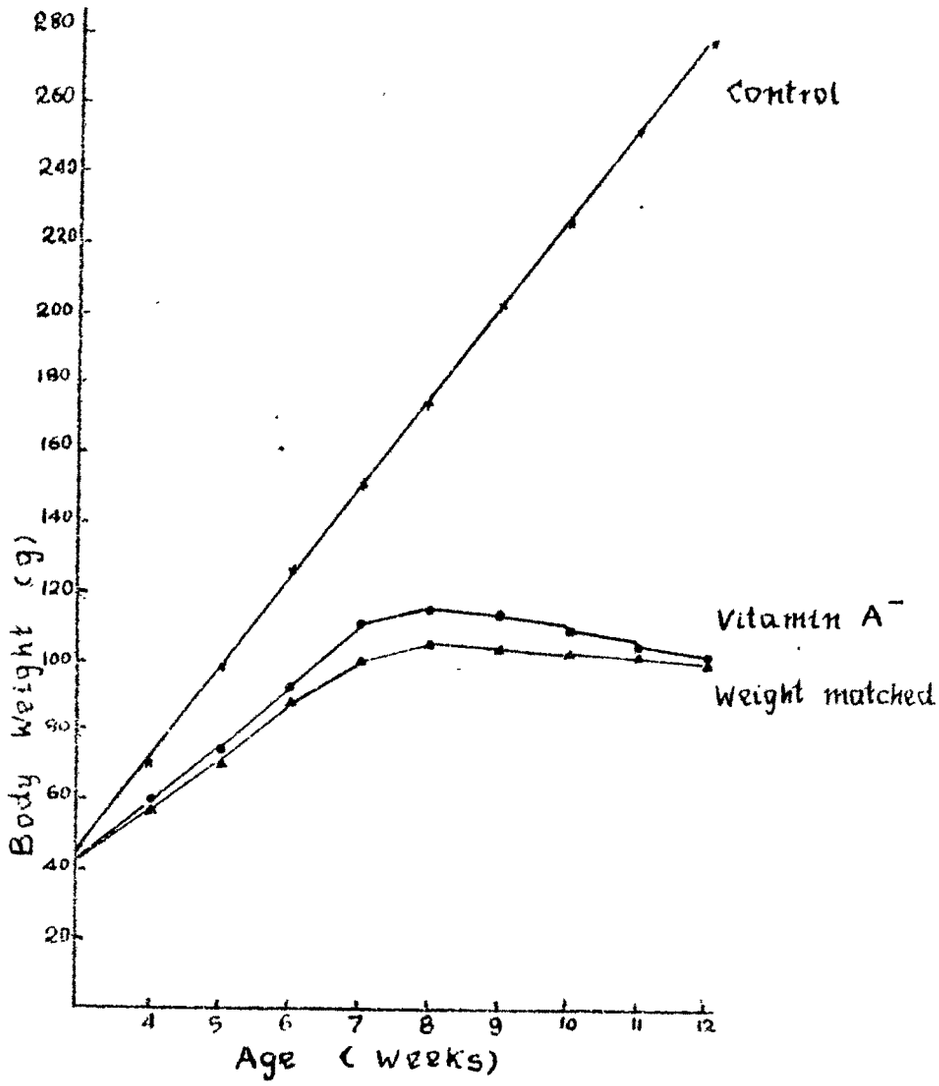
THE EFFECTS OF VITAMIN A DEFICIENCY DURING THE POSTWEANING PERIOD

Studies described previously were concerned with the effects of vitamin A deficiency during the suckling period on bone growth and composition. The present studies were concerned with the effects of deficiency after weaning.

To induce vitamin A deficiency during postweaning period, groups of weanling rats of standard weights (40-45 g) were fed either the standard diet or the same free from vitamin A till deficiency symptoms were observed in the experimental group. A weight matched group was fed the 20% protein diet with vitamin A in restricted amount so that body weights were comparable to those of the vitamin A deficient rats (Figure 17). Deficiency symptoms such as corneal xerophthalmia and hind limb paralysis generally appeared in the eighth week and the animals were killed soon after, that is in the ninth week. Liver vitamin levels in the vitamin A deficient rats were found to be reduced to 8% of control values at this point (Table 36-a).

Inflamed eyelid margins, low weight, loss of appetite and pale white incisor are some of the vitamin A deficiency symptoms reported in rats (Beresford, 1969). Limb paralysis characterized by incoordination of movement in dogs maintained on diets deficient in vitamin A was reported by Mellanby (1931)

Figure : 17 The effects of vitamin A deficiency during the postweaning period on the body weights of rats



Control : Pups fed on 20% protein diet ad libitum

Weight matched : Pups fed on 20% protein diet in restricted amount to match the weight of the vitamin A⁻ rats

Vitamin A⁻ : Pups fed on 20% protein, vitamin A deficient diet during postweaning period ad libitum

and he related the condition to lesions of the central nervous system caused by pressure from malformed bone. Howell et al. (1969) related the central nervous system compression to the increased periosteal deposition of bone in vitamin A deficient animals. Increased periosteal bone formation was also shown to result in the narrowing of the auditory canal in vitamin A deficient rats (Chole and Quick, 1976). The increased bone formation may be due to altered bone growth with increase in osteoblastic activity and unchanged osteoclastic activity (Gallina et al., 1970). Irving (1949) found in vitamin A deficient rats neither altered endochondral growth nor decreased osteoclastic activity. However he reported a marked and uncontrolled increase in osteoblastic activity.

The results of these studies are given in Tables 36-a to 40-b and the bones studied are shown in Figure 18. As in other studies, the body weights which were about 35% in the experimental groups were affected more than the weight and size of the bones (Table 36-a and b and Figure 19).

In the case of linear bone measurements, the deficits were less in the vitamin A deficient animals except for femur length than in the undernourished animals. However, the length of tarsus, remained unaffected in vitamin A deficiency (Table 36-a). The reduction in length seems to be relatively more in the femur than in the other bones in vitamin A deficiency. This may be because of the change

Figure: 18 Effects of postweaning vitamin A deficiency

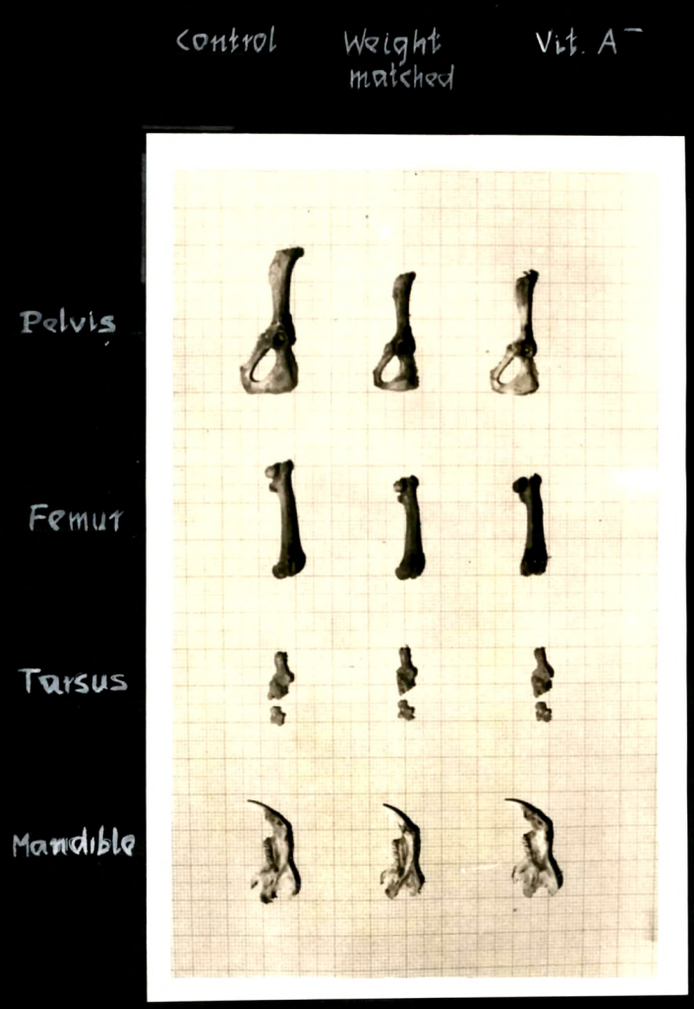


Table 36-a: The effect of vitamin A deficiency during the postweaning period on bone size and morphology.

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
No. of animals	6	6	6		
Body weight (g)	281 ± 18.3	99 ± 1.2	100 ± 2.3	35	36
Vitamin A level in the liver (μg/g liver)**	135	N.D.	11	-	8
Fresh weight of bones (mg):					
Mandible	241 ± 20.4 (0.86)***	188 ± 1.7 (1.90)	213 ± 5.8 (2.10)	78	88
Femur	652 ± 40.2 (2.32)	400 ± 9.4 (4.04)	451 ± 27.1 (4.51)	61	69
Pelvis	525 ± 20.1 (1.87)	275 ± 3.8 (2.78)	354 ± 18.2 (3.53)	52	67
Tarsus	172 ± 6.0 (0.61)	142 ± 2.2 (1.43)	133 ± 5.9 (1.34)	83	77

Table 36-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
Bone measurements (cm):					
Length:					
Mandible	2·60 ± 0·035	2·30 ± 0·010	2·38 ± 0·016	88	92
Femur	3·26 ± 0·065	2·83 ± 0·012	2·74 ± 0·048	87	84
Pelvis	3·83 ± 0·077	3·25 ± 0·030	3·32 ± 0·048	84	87
Tarsus	1·23 ± 0·015	1·16 ± 0·014	1·23 ± 0·017	94	100
Width:					
Mandible	1·37 ± 0·035	1·14 ± 0·005	1·23 ± 0·026	83	90
Femur	0·69 ± 0·017	0·66 ± 0·024	0·67 ± 0·020	96	97
Pelvis	1·49 ± 0·026	1·24 ± 0·024	1·30 ± 0·033	83	87

Table 36-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
Width/Length:					
Mandible	0.53	0.50	0.52	94	98
Femur	0.21	0.23	0.24	110	114
Pelvis	0.39	0.39	0.39	100	100
Comparison of bone lengths:					
Mandible/Femur	0.80	0.81	0.87	101	109
Pelvis/Femur	1.17	1.13	1.21	96	103
Tarsus/Femur	0.38	0.41	0.45	108	118

* (1) Control - Pups fed on 20% protein diet ad libitum.

(2) Weight matched - Pups fed on 20% protein diet in restricted amount to match the weight of the Vit.A⁻ rats.

(3) Vit.A⁻ - Pups fed on 20% protein, vitamin A deficient diet during postweaning period of ad libitum.

Period of treatment 9 weeks.

** Average for 2 rats,

*** Values in parentheses are bone weight expressed as mg per g of body weight.

N.D. - Not determined.

For statistical significance of differences see Table 36-b.

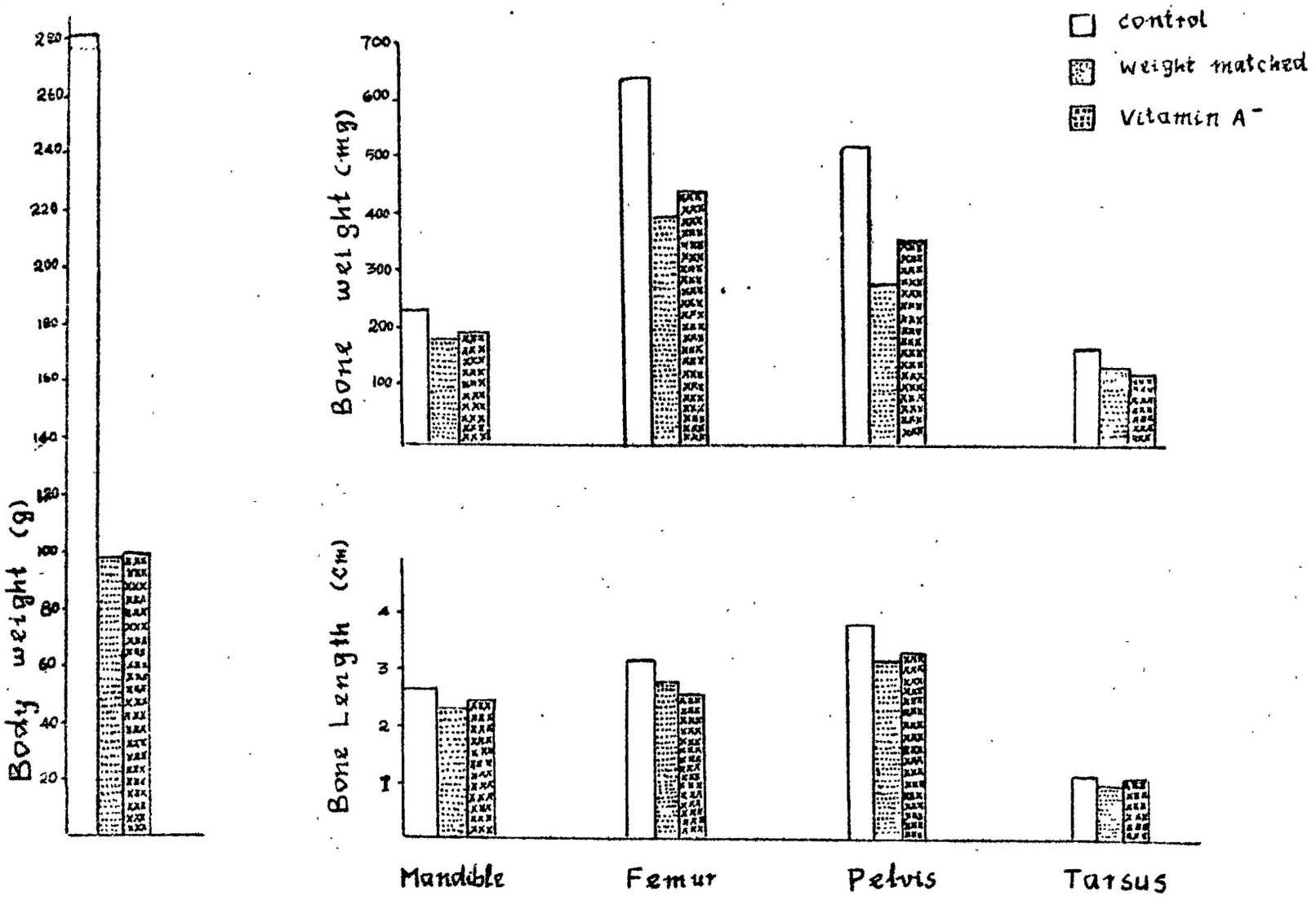
Table 36-b: Statistical significance of differences between the groups in Table 36-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
Body weight:	0.001	0.001	N.S.
Fresh weight of bones:			
Mandible	0.001	0.001	0.010
Femur	0.001	0.001	0.010
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	0.050
Bone measurements:			
Length:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	N.S.	N.S.	N.S.
Width:			
Mandible	0.001	0.001	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	0.001	0.001	N.S.

Table 36-c: Per cent increment over values for weanling rats.

	Group			Values as % of control values	
	Control	Weight matched	Vit.A⁻	Weight matched	Vit.A⁻
Body weight:	558	132	134	24	24
Fresh weight:					
Mandible	196	131	158	67	81
Femur	297	144	174	48	59
Pelvis	422	173	251	41	59
Tarsus	124	85	75	69	60
Bone measurements:					
Length:					
Mandible	25.6	11.1	15.0	43	59
Femur	76.2	53.0	48.1	70	63
Pelvis	82.4	54.8	58.0	67	70
Tarsus	12.8	6.4	12.8	50	100
Width:					
Mandible	45.7	21.3	30.9	47	68
Femur	27.8	22.2	24.1	80	87
Pelvis	58.5	31.9	38.3	55	65

Figure:19 The effects of Vitamin-A deficiency during postweaning period on Body weight and Bone size



in the shape of the femur which appears bulky and slightly bent in vitamin A deficient animals.

The mean values for fresh weight, dry weight and fat-free dry weight were significantly less in the two experimental groups as compared to the controls. The deficits were relatively smaller in the vitamin A deficient group with reference to the fresh and dry weight but greater with reference to fat-free dry weights. In vitamin A deficiency the fresh weight seems to be less affected because of greater moisture content and dry weight because of greater lipid content (Tables 37-a and b).

The content and concentration of ash (Tables 38-a and b, 39-a and b) were also significantly less in both the test groups, the deficits being greater in 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 calcium and phosphorus 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. Consequently the Ca:P ratios were significantly less in the vitamin A deficient rats than in the age matched controls, but not in the undernourished group (Tables 38-a and b). The deficits in calcium were relatively

Table 37-a: The effect of vitamin A deficiency during the postweaning period on the chemical composition of selected bones in rats.

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
No. of animals	6	6	6		
Body weight (g):	281 ± 18.3	99 ± 1.2	100 ± 2.3	35	36
Fresh weight of bones (mg):					
Mandible	241 ± 20.1	188 ± 1.7	213 ± 5.8	78	88
Femur	652 ± 40.2	400 ± 9.4	451 ± 27.1	61	69
Pelvis	525 ± 20.1	275 ± 3.8	354 ± 18.2	52	67
Tarsus	172 ± 6.0	142 ± 2.2	133 ± 5.9	83	77
Dry weight (mg):					
Mandible	179 ± 4.1	138 ± 1.7	148 ± 5.5	77	83
Femur	414 ± 28.1	238 ± 6.8	242 ± 10.9	57	58
Pelvis	326 ± 14.7	165 ± 3.9	179 ± 10.5	51	55
Tarsus	123 ± 5.0	99 ± 2.4	91 ± 5.2	80	74

Table 37-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
Fat-free dry weight (mg):		mean ± s.e.			
Mandible**					
Femur	402 ± 27.2	220 ± 6.7	207 ± 14.6	55	51
Pelvis	316 ± 14.7	152 ± 4.5	152 ± 11.1	48	48
Tarsus	117 ± 5.6	90 ± 2.7	82 ± 5.7	77	70
Ash (mg):					
No. of animals	6	3	6		
Mandible	112.6 ± 2.63	89.0 ± 0.41	83.3 ± 5.50	79	74
Femur	236.7 ± 17.89	114.3 ± 5.76	93.0 ± 7.60	48	39
Pelvis	176.2 ± 9.97	76.7 ± 4.60	66.2 ± 5.27	44	38
Tarsus	65.5 ± 2.97	48.3 ± 2.04	35.7 ± 2.89	74	55
Collagen (Hydroxyproline)(mg):					
No. of animals	6	3	6		
Mandible	4.08 ± 0.169	2.84 ± 0.298	3.80 ± 0.248	70	93
Femur	7.81 ± 0.480	4.22 ± 0.656	5.74 ± 0.439	54	73
Pelvis	6.88 ± 0.300	3.27 ± 0.252	4.86 ± 0.411	48	71
Tarsus	3.60 ± 0.099	2.95 ± 0.426	3.38 ± 0.190	82	94

Table 37-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
Total MPS (Hexosamine)(mg):					
No. of animals	6	3	6		
Mandible	0.48 ± 0.035	0.37 ± 0.072	0.48 ± 0.009	77	100
Femur	1.03 ± 0.042	0.79 ± 0.058	1.03 ± 0.058	77	100
Pelvis	0.81 ± 0.027	0.57 ± 0.080	0.82 ± 0.043	70	101
Tarsus	0.39 ± 0.034	0.36 ± 0.067	0.40 ± 0.037	92	102

* (1) Control - Pups fed on 20% protein diet ad libitum.
(2) Weight matched - Pups fed on 20% protein diet in restricted amount to match the weight of the Vit.A⁻ rats.
(3) Vit.A⁻ - Pups fed on 20% protein, vitamin A deficient diet during postweaning period ad libitum.

** Not determined.

Period of treatment 9 weeks.

For statistical significance of differences see Table 37-b.

Table 37-b: Statistical significance of differences between the groups in Table 37-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	p less than		
Body weight	0.001	0.001	N.S.
Fresh weight:			
Mandible	0.001	0.001	0.010
Femur	0.001	0.001	0.010
Pelvis	0.001	0.001	0.001
Tarsus	0.001	0.001	N.S.
Dry weight:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Fat-free dry weight:			
Mandible	-	-	-
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	N.S.
Ash:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	0.010

Table 37-b continued

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
Collagen (Hydroxyproline):			
Mandible	0.010	N.S.	N.S.
Femur	0.010	0.010	N.S.
Pelvis	0.001	0.010	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS (Hexosamine):			
Mandible	N.S.	N.S.	N.S.
Femur	0.050	N.S.	0.050
Pelvis	0.050	N.S.	0.050
Tarsus	N.S.	N.S.	N.S.

N.S. Not Significant.

Table 37-c: Per cent increment over values for weanling rats.

	Group			Values as % of control values	
	Control	Weight matched	Vit.A⁻	Weight matched	Vit.A⁻
Body weight	588	132	134	24	24
Fresh weight:					
Mandible	196	131	158	67	81
Femur	297	144	174	48	59
Pelvis	422	173	251	41	59
Tarsus	124	85	75	69	60
Dry weight:					
Mandible	349	246	281	70	81
Femur	608	307	310	50	51
Pelvis	734	317	365	43	50
Tarsus	281	207	185	74	66
Fat-free dry weight:					
Mandible	-	-	-	-	-
Femur	630	299	272	47	43
Pelvis	754	297	292	39	39
Tarsus	337	236	202	70	60

Table 37-c continued

	Group			Values as % of control values	
	Control	Weight matched	Vit.A⁻	Weight matched	Vit.A⁻
Ash:					
Mandible	436	324	297	74	68
Femur	1060	460	356	43	34
Pelvis	1052	401	333	38	32
Tarsus	542	374	250	69	46
Collagen (Hydroxyproline):					
Mandible	356	219	327	62	92
Femur	428	185	288	43	67
Pelvis	568	217	372	38	65
Tarsus	236	177	216	75	92
Total MPS (Hexosamine):					
Mandible	129	76	129	59	100
Femur	58	22	58	38	100
Pelvis	113	50	116	44	102
Tarsus	18	9	21	50	116

Table 38-a: The effect of vitamin A deficiency during the postweaning period on the chemical composition of bones in rat.

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
Ash (mg):					
No. of animals	6	3	6		
Mandible	112.6 ± 2.63	89.0 ± 0.41	83.3 ± 5.50	79	74
Femur	236.7 ± 17.89	114.3 ± 5.76	93.0 ± 7.60	48	39
Pelvis	176.2 ± 9.97	76.7 ± 4.60	66.2 ± 5.27	44	38
Tarsus	65.5 ± 2.97	48.3 ± 2.04	35.7 ± 2.89	74	55
Calcium (mg):					
Mandible	42.7 ± 1.20 (37.9)**	33.4 ± 0.54 (37.7)	32.0 ± 2.08 (38.4)	78	75
Femur	87.0 ± 5.97 (36.8)	43.9 ± 2.26 (38.4)	35.7 ± 3.23 (38.2)	50	41

Table 38-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
Calcium (mg) contd.					
Pelvis	64.6 ± 3.77 (36.5)	29.5 ± 1.52 (38.5)	25.2 ± 1.75 (38.2)	46	39
Tarsus	25.0 ± 1.10 (38.2)	19.3 ± 0.86 (39.9)	14.1 ± 1.16 (39.6)	77	56
Phosphorus (mg):					
Mandible	20.3 ± 0.46 (18.1)	16.3 ± 0.29 (18.3)	16.3 ± 0.83 (19.7)	80	80
Femur	42.3 ± 2.46 (18.0)	21.8 ± 0.04 (19.6)	18.6 ± 1.44 (20)	53	44
Pelvis	31.7 ± 1.79 (18.0)	14.6 ± 1.06 (19.0)	12.9 ± 0.88 (20.2)	46	41
Tarsus	11.7 ± 0.55 (18)	9.2 ± 0.67 (19.0)	7.3 ± 0.55 (19.7)	78	62

Table 38-a

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	mean ± s.e.				
Ca:P ratio:					
Mandible	2·10 ± 0·018	2·05 ± 0·065	1·97 ± 0·043	98	94
Femur	2·08 ± 0·018	2·01 ± 0·031	1·91 ± 0·050	97	92
Pelvis	2·04 ± 0·013	2·02 ± 0·046	1·89 ± 0·022	97	93
Tarsus	2·14 ± 0·041	2·10 ± 0·053	1·94 ± 0·026	98	91
A:R ratio:***					
Mandible	1·74 ± 0·044	1·71 ± 0·025	1·28 ± 0·090	98	74
Femur	1·42 ± 0·060	1·03 ± 0·020	0·82 ± 0·034	73	58
Pelvis	1·26 ± 0·039	1·03 ± 0·040	0·77 ± 0·024	82	61
Tarsus	1·30 ± 0·040	1·07 ± 0·081	0·81 ± 0·050	82	62

Table 38-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
Calcium/Hydroxyproline ratio:					
Mandible	10.5	10.5	8.4	100	80
Femur	11.1	10.4	6.2	94	56
Pelvis	9.4	9.0	5.2	96	55
Tarsus	6.9	6.5	4.2	94	61
Hexosamine/Hydroxyproline ratio:					
Mandible	0.12	0.13	0.13	108	108
Femur	0.13	0.19	0.18	146	138
Pelvis	0.12	0.17	0.17	142	142
Tarsus	0.11	0.12	0.12	109	109

* (1) Control - Pups fed on 20% protein diet ad libitum.
(2) Weight matched - Pups fed on 20% protein diet in restricted amount to match the weight of the Vit.A⁻ rats.
(3) Vit.A⁻ - Pups fed on 20% protein, vitamin A deficient diet during postweaning period ad libitum.

** Values in parentheses are as per cent of ash.

*** A:R ratio: Ratio of ash to non-ash components in the fat-free dry bone for femur, pelvis and tarsus and in the dry bone for mandible.

For statistical significance of differences see table 38-b.

Table 38-b: Statistical significance of differences between the groups in table 38-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
Ash:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.001	0.001	0.010
Calcium:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.010	0.001	0.010
Phosphorus:			
Mandible	0.001	0.001	N.S.
Femur	0.001	0.001	N.S.
Pelvis	0.001	0.001	N.S.
Tarsus	0.050	0.001	N.S.
Ca:P ratio:			
Mandible	N.S.	0.050	N.S.
Femur	N.S.	0.010	N.S.
Pelvis	N.S.	0.001	N.S.
Tarsus	N.S.	0.010	N.S.
A:R ratio:			
Mandible	N.S.	0.001	0.010
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.050	0.001	0.050

more than in the case of phosphorus. Similar observations were made by Harris and Navia (1977) in the newly formed bone of vitamin A deficient animals.

A decreased calcium content in vitamin A deficient implant was reported by Harris and Navia (1977). In support of this their histological studies revealed the presence of less mature bone spicules in the vitamin A deficient tissue implants (Harris et al., 1978). These spicules were reported to contain more cells and to be less organized. In this context it may be noted that reorganization of the cancellous to compact bone was reduced and in the most severe cases was absent in vitamin A deficient animals (Mellanby, 1947). Posner et al. (1954) proposed the existence of a series of apatite molecules deficient in calcium but containing normal amounts of phosphorus. The lack of vitamin A could in some way disrupt the normal addition of calcium ions to the less mature apatite forms.

In vitamin A deficiency the activity of alkaline phosphatase which is known to be involved in bone calcification has been reported to be decreased in both bone (Ludwig, 1953; Zile et al. 1973) and plasma (Zile et al. 1973). On the basis of studies on rats the secretion and physiologic action of PTH are not impaired in vitamin A deficiency suggesting perhaps that vitamin A is not needed for the mobilization of calcium.

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. A reduction in A:R ratio in the femur of vitamin A deficient rats was observed in previous studies in this laboratory (Dave, 1976). In this context it may be noted that in vitamin A deficient calves the amount of osteoid was found to be greater in the vertebrae and that these bones tended to contain less compact bone (Gallina et al. 1970).

Collagen content (Tables 37-a and b) and concentration (Table 39-a) were 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, relatively larger bone size and the greater osteoid content.

In this connection, decrease in hydroxyproline content in the proximal part of tibia in vitamin A deficient rat had been reported by Firschein (1970). However, in the vitamin A deficient chick Havivi and Wolf (1967) found no deficit with reference to concentration of collagen. Cousins (1969) reported an increase in the hydroxyproline concentration of the duramater in vitamin A deficiency. The duramater forms an internal periosteum of bones of the skull and also forms an outer envelope for the brain (Sisson and Grossman, 1953).

In spite of bone size being smaller the amount of total mucopolysaccharide (hexosamine) in the two groups were not

Table 39-a: The effect of vitamin A deficiency during the postweaning period on the percentage composition of bones in rats.

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	g per 100 g of fresh tissue mean ± s.e.				
Moisture:					
Mandible	25.8 ± 0.88	26.6 ± 0.29	30.2 ± 1.91	103	117
Femur	36.6 ± 0.62	40.5 ± 1.14	46.8 ± 1.80	111	128
Pelvis	37.9 ± 0.45	40.0 ± 0.71	49.4 ± 2.05	106	130
Tarsus	28.5 ± 1.22	30.6 ± 0.73	31.8 ± 1.59	107	112
Total lipid:					
Femur	1.8 ± 0.09	4.4 ± 0.30	7.0 ± 0.98	244	388
Pelvis	2.0 ± 0.20	4.7 ± 0.35	7.8 ± 0.95	235	390
Tarsus	4.8 ± 0.28	6.3 ± 0.59	8.5 ± 0.96	131	177

Table 39-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	g per 100 g of fresh tissue mean ± s.e.				
Ash:					
Mandible	47.0 ± 0.55	46.7 ± 0.19	39.1 ± 2.15	99	83
Femur	36.2 ± 0.80	29.0 ± 1.00	20.7 ± 0.98	80	57
Pelvis	34.1 ± 0.80	27.8 ± 0.67	18.6 ± 0.78	82	55
Tarsus	38.1 ± 1.05	33.4 ± 1.00	26.7 ± 1.15	88	70
Collagen (Hydroxyproline):					
Mandible	1.53 ± 0.034	1.63 ± 0.088	1.53 ± 0.074	106	100
Femur	1.19 ± 0.028	1.03 ± 0.085	1.07 ± 0.035	87	90
Pelvis	1.32 ± 0.025	1.20 ± 0.098	1.24 ± 0.030	91	94
Tarsus	2.16 ± 0.106	2.12 ± 0.299	2.14 ± 0.065	98	99

Table 39-a continued

	Group*			Values as % of control values	
	Control (1)	Weight matched (2)	Vit.A⁻ (3)	Weight matched	Vit.A⁻
	g per 100 g of fresh tissue mean ± s.e.				
Total MPS (Hexosamine):					
Mandible	0.20 ± 0.009	0.22 ± 0.045	0.24 ± 0.012	110	120
Femur	0.18 ± 0.010	0.20 ± 0.015	0.23 ± 0.011	111	127
Pelvis	0.16 ± 0.005	0.20 ± 0.026	0.24 ± 0.011	125	150
Tarsus	0.25 ± 0.018	0.27 ± 0.051	0.29 ± 0.025	108	116

* (1) Control - Pups fed on 20% protein diet ad libitum.

(2) Weight matched - Pups fed on 20% protein diet in restricted amount to match the weight of the Vit.A⁻ rats.

(3) Vit.A⁻ - Pups fed on 20% protein, vitamin A deficient diet during postweaning period ad libitum.

For statistical significance of differences see Table 39-b.

Table 39-b: Statistical significance of differences between the groups in Table 39-a.

	Significance of differences between means		
	1 ∽ 2	1 ∽ 3	2 ∽ 3
	p less than		
Moisture:			
Mandible	N.S.	N.S.	N.S.
Femur	0.050	0.001	0.010
Pelvis	0.050	0.001	0.010
Tarsus	N.S.	N.S.	N.S.
Total lipid:			
Femur	0.001	0.001	0.010
Pelvis	0.001	0.001	0.010
Tarsus	0.050	0.001	N.S.
Ash:			
Mandible	N.S.	0.010	0.010
Femur	0.001	0.001	0.001
Pelvis	0.010	0.001	0.001
Tarsus	0.050	0.001	0.010
Collagen (Hydroxyproline):			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	N.S.	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS (Hexosamine):			
Mandible	N.S.	0.050	N.S.
Femur	N.S.	0.010	N.S.
Pelvis	N.S.	0.001	N.S.
Tarsus	N.S.	N.S.	N.S.

N.S. Not Significant

Table 40-a: The effect of vitamin A deficiency during the postweaning period on the percentage composition on the basis of dry weight and fat-free dry weight.

	Group*		
	Control (1)	Weight matched (2)	Vit.A⁻ (3)
	g per 100 g of dry bone mean ± s.e.		
Total lipid:			
Femur	2·8 ± 0·18	7·5 ± 0·61	13·6 ± 2·30
Pelvis	3·1 ± 0·36	7·7 ± 0·76	14·3 ± 1·77
Tarsus	6·4 ± 0·35	9·0 ± 0·90	12·5 ± 1·34
Ash:			
Mandible	63·6 ± 0·55	63·2 ± 0·32	56·0 ± 1·65
Femur	56·8 ± 1·02	47·4 ± 0·71	38·7 ± 1·34
Pelvis	54·0 ± 0·85	46·6 ± 0·28	36·8 ± 1·19
Tarsus	53·2 ± 0·49	47·2 ± 1·32	38·4 ± 1·24
Collagen (Hydroxyproline):			
Mandible	2·06 ± 0·044	2·21 ± 0·109	2·24 ± 0·900
Femur	1·85 ± 0·040	1·72 ± 0·169	1·96 ± 0·064
Pelvis	2·02 ± 0·035	1·98 ± 0·162	2·37 ± 0·058
Tarsus	2·97 ± 0·152	2·88 ± 0·323	3·14 ± 0·113
Total MPS (Hexosamine):			
Mandible	0·26 ± 0·012	0·30 ± 0·061	0·38 ± 0·011
Femur	0·28 ± 0·015	0·33 ± 0·031	0·44 ± 0·018
Pelvis	0·25 ± 0·007	0·34 ± 0·041	0·45 ± 0·022
Tarsus	0·35 ± 0·023	0·36 ± 0·044	0·41 ± 0·027

Table 40-a continued

	Group*		
	Control (1)	Weight matched (2)	Vit.A⁻ (3)
	g per 100 g of fat-free dry bone mean ± s.e.		
Ash:			
Femur	58.7 ± 1.10	50.6 ± 0.53	44.9 ± 0.99
Pelvis	56.2 ± 0.53	50.4 ± 1.03	43.5 ± 0.83
Tarsus	57.1 ± 0.60	51.6 ± 2.00	44.7 ± 1.44
Collagen (Hydroxyproline):			
Femur	1.90 ± 0.041	1.96 ± 0.224	2.22 ± 0.084
Pelvis	2.07 ± 0.035	2.22 ± 0.193	2.62 ± 0.072
Tarsus	3.27 ± 0.159	3.30 ± 0.395	3.72 ± 0.142
Total MPS (Hexosamine):			
Femur	0.29 ± 0.014	0.37 ± 0.039	0.51 ± 0.021
Pelvis	0.26 ± 0.008	0.38 ± 0.051	0.53 ± 0.030
Tarsus	0.38 ± 0.022	0.43 ± 0.090	0.46 ± 0.034

* (1) Control - Pups fed on 20% protein diet ad libitum.
(2) Weight matched - Pups fed on 20% protein diet in restricted amount to match the weight of the Vit.A⁻ rats.
(3) Vit.A⁻ - Pups fed on 20% protein, vitamin A deficient diet during postweaning period ad libitum.

For statistical significance of differences see Table 40-b.

Table 40-b: Statistical significance of differences between the groups in Table 40-a.

	Significance of differences between means		
	1 ~ 2	1 ~ 3	2 ~ 3
	p less than		
g per 100 g dry bone:			
Total lipid:			
Femur	0.001	0.001	0.050
Pelvis	0.001	0.001	0.010
Tarsus	0.050	0.010	N.S.
Ash:			
Mandible	N.S.	0.010	0.010
Femur	0.001	0.001	0.001
Pelvis	0.001	0.001	0.001
Tarsus	0.010	0.001	0.010
Collagen:			
Mandible	N.S.	N.S.	N.S.
Femur	N.S.	N.S.	N.S.
Pelvis	N.S.	0.001	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS:			
Mandible	N.S.	0.001	N.S.
Femur	N.S.	0.001	0.050
Pelvis	N.S.	0.001	0.050
Tarsus	N.S.	N.S.	N.S.

Table 40-b continued

	Significance of differences between means		
	1 vs 2	1 vs 3	2 vs 3
	p less than		
g per 100 g fat-free dry bone:			
Ash:			
Femur	0.001	0.001	0.050
Pelvis	0.010	0.001	0.001
Tarsus	0.050	0.001	0.050
Collagen:			
Femur	N.S.	0.010	N.S.
Pelvis	N.S.	0.001	N.S.
Tarsus	N.S.	N.S.	N.S.
Total MPS:			
Femur	N.S.	0.001	0.050
Pelvis	N.S.	0.001	0.050
Tarsus	N.S.	N.S.	N.S.

N.S. Not Significant

significantly different from the controls except in the femur and pelvis of the weight matched animals (Tables 37-a and b). The concentration of the total mucopolysaccharide was increased in both groups and significantly so in vitamin A deficiency. The only exception to this statement was the tarsus with reference to which the difference was not significant. However the concentration of total MPS in the vitamin A deficient animals was not significantly different from the weight matched controls (Tables 39-a and b).

Higher concentrations of moisture as compared to controls were found in both groups, but the differences fell short of significance in the case of the mandible and tarsus (Tables 39-a and b).

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 the femur and pelvis (Tables 39-a and b).

These observations are in agreement with those of the pioneering observations of Mellanby (1950). He found long bones of vitamin A deficient dogs to be bulky with a thick cancellous cortex and an indistinct endosteal surface. The increased bulk was related to an excessive periosteal deposition of bone, the enlarged marrow cavity and the numerous interstices of the cancellous wall being filled with fatty marrow. Fat and water were increased. Reorganization of the cancellous bone was reduced. The physical

appearance of the bones suggested similar changes in bone marrow cavity in the present studies.

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. This is in accordance with the observation of Mellanby (1947) that in vitamin A deficiency the changes in mandible and long bones do not affect the animal as much as the malformation of the pelvis and of the bones adjacent to the central nervous system.

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 variation in the concentration of collagen (Figure 20).

If we compare the effects of vitamin A deficiency in the preweaning and postweaning periods, certain points emerge. With reference to the deficits in fat-free dry weight and ash (Table 41), the femur and pelvis seemed to be affected more during the postweaning period than during the preweaning period. Again these effects seem to depend on the ontogenetic patterns of maturation. Because of the longer and probably more severe deprivation, growth deficits as judged by body weight were greater in the animals

Figure 20 The effects of postweaning Vitamin A deficiency on percentage composition of bones

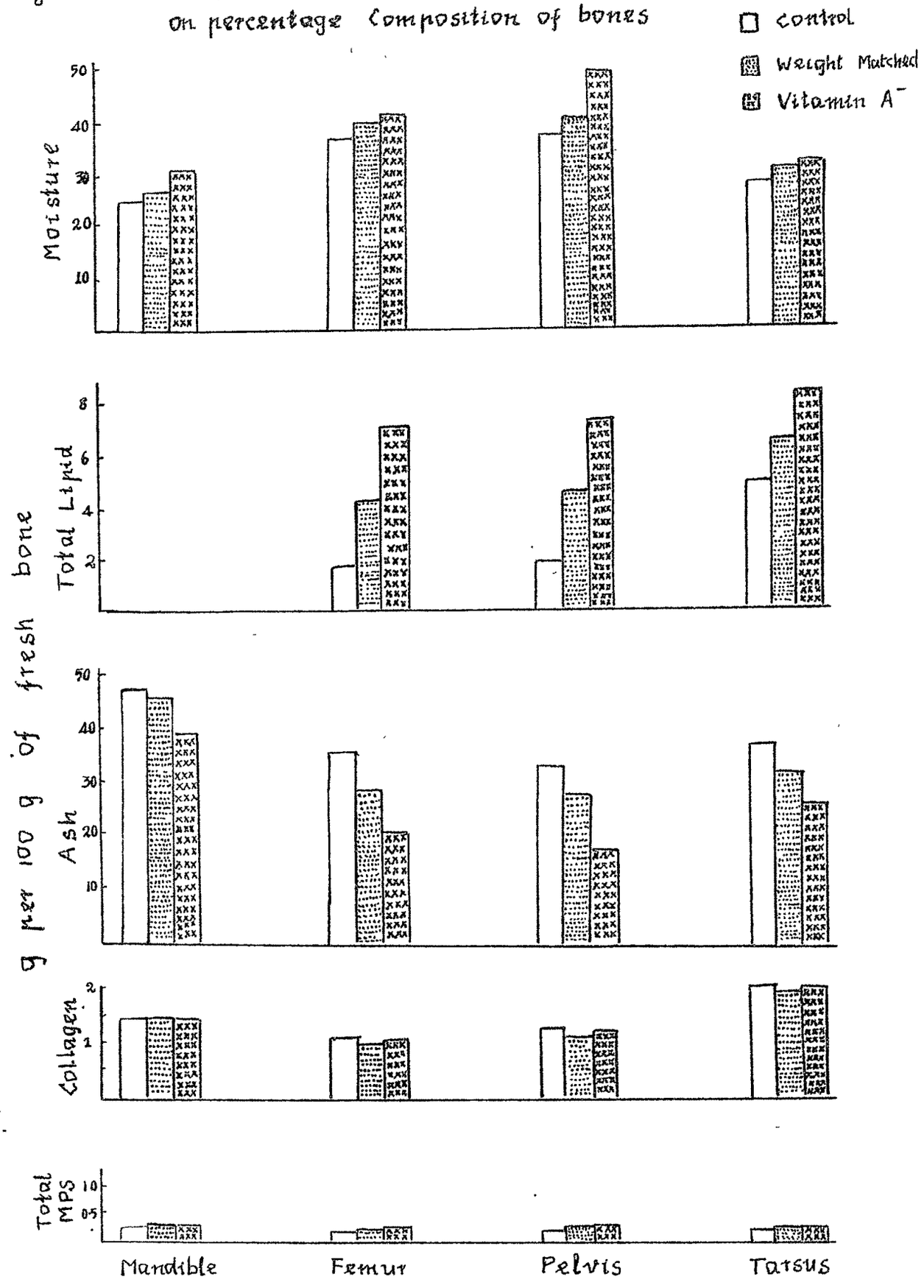


Table 41: Comparative deficits for the preweaning and postweaning Vitamin A deficiency.

	as % control values			
	Preweaning		Postweaning	
	LL	Vit.A⁻	Weight matched	Vit.A⁻
Body weight:	44	29	65	64
Fresh weight:				
Mandible	32	14	22	12
Femur	49	32	39	31
Pelvis	51	28	48	33
Tarsus	36	26	17	23
Dry weight:				
Mandible	33	15	23	17
Femur	53	32	43	42
Pelvis	56	30	49	45
Tarsus	48	33	20	26
Fat-free dry weight:				
Femur	53	33	45	49
Pelvis	58	32	52	52
Tarsus	50	36	23	30
Bone measurements:				
Length:				
Mandible	14	11	12	8
Femur	15	16	13	16
Pelvis	18	16	16	13
Tarsus	16	13	6	0
Width:				
Mandible	11	11	17	10
Femur	11	9	4	3
Pelvis	19	15	17	13

Table 41 continued

	as % control values			
	Preweaning		Postweaning	
	LL	Vit.A⁻	Weight matched	Vit.A⁻
Chemical composition:				
Ash:				
Mandible	43	20	21	26
Femur	54	36	52	61
Pelvis	61	36	56	62
Tarsus	56	42	26	45
Collagen:				
Mandible	35	15	30	7
Femur	39	33	46	27
Pelvis	42	25	52	29
Tarsus	33	23	18	6
Total MPS:				
Mandible	21	11	23	0
Femur	19	20	23	0
Pelvis	19	27	30	0
Tarsus	32	21	8	0
A:R ratio:				
Mandible	13	3	2	26
Femur	13	12	27	42
Pelvis	14	12	18	39
Tarsus	20	12	18	38

subjected to vitamin A deficiency. In spite of this, the deficits with reference to many bone parameters were comparable in both experiments suggesting that the effects of pre-weaning deficiency were more pronounced when we consider the shorter period of treatment and the more moderate nature of the deficiency.