The effect of reproduction on the interaction of dietary protein and calcium

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1. Rats were maintained from the age of 3 weeks until the end of a second reproductive cycle on diets having different protein values and Ca concentrations. 2. Reproductive performance was judged from the number of viable young, their size, body composition and rate of growth during suckling. 3. The size and quality of the bones of the mothers and offspring were assessed from radiographs, histological appearance, total weight of ash, ash/cm^3 and ash:organic matter ratios of dry fat-free bone. 4. At the end of gestation the mean numbers and total weights of foetuses were low when the mothers had received diets of low protein value: there were smaller differences in the body-weight, water, ash and N contents of the individual foetuses. 5. When 0·44 % Ca was included in the diet of low protein value, only one (three rats) of the six litters born survived beyond 1 week of age. 6. At weaning, the young born of and suckled by the protein-calorie deficient dams were only about half the weight of those from mothers receiving the high-protein diets; Ca deficiency produced relatively minor changes. 7. All the mothers lost weight during lactation irrespective of the protein value or Ca concentration of the diet; their bones had lower radiographic densities and less ash/cm^3 than is usual in non-pregnant rats of similar age. 8. The interaction of protein and Ca and their relative importance in maintaining the skeletal structure of mother and offspring during pregnancy and lactation are discussed.

In a previous publication (El-Maraghi, Platt & Stewart, 1965) it was reported that osteoporosis could be produced in the bones of rats maintained on diets which were deficient in calcium or of low protein value. The type of osteoporosis varied, diets deficient in protein leading to matrix-osteoporosis and those deficient in Ca to mineral-osteoporosis.

Either form was easily produced in young growing rats, but under the conditions tested (Ca not less than 0·11 % of the diet) only matrix-osteoporosis was found in the adult and aged rats. The adult animals were apparently able to maintain a normal skeletal structure when given diets having about one-half the protein value and one-quarter the concentration of Ca required by young growing rats for normal bone formation. It would be expected that any additional 'stress' such as reproduction would lead to frank deficiencies, and the present report deals with the effects on the bones of the mother and offspring of diets supplying only the protein and Ca requirements of non-pregnant adults.

EXPERIMENTAL

Animals and diets

Rats of a black and white hooded colony which had been maintained on the MRC cube diet no. 41 B (Bruce & Parkes, 1947) were used in all the experiments. Diets having Ca concentrations of 0·44 and 0·11 % were prepared at each of the two protein values, NDpCal% = 10·2 and 5·1. Details of the diets (A, C, G and J) are given in Table 1. Six female rats were maintained from weaning (21 days of age) on each of the four experimental diets; they were mated to stock males when 18 weeks old. Three mothers from each of the four experimental groups were killed 21 days after the appearance of a mating plug; the carcasses of the mothers and foetuses were used for the various examinations. The remaining mothers were allowed to rear their young which were killed at weaning (21 days of age). These mothers were continued on the different dietary regimens and at 28 weeks of age were again mated with normal males. The mothers and their second litters were killed when the latter were 21 days of age.

Table 1. *Composition of diets (parts by weight)*

	Diet A	Diet C	Diet G	Diet J
NDpCal %*	10·2		5·1	
Ca (%)	0·44	0·11	0·44	0·11
P (%)	0·55	0·55	0·42	0·42
Arachis oil	100	100	100	100
Mixture of B vitamins in maize starch†	10	10	10	10
Salt mixture‡	40	40	40	40
Sucrose	150	150	150	150
Cellulose	100	100	100	100
Casein	250	250	80	80
Starch	256·5	339	415	497·5
10% $CaCO_3$ in maize starch	93·5	11	105	22·5

Each animal was given 0·2 ml/week of arachis oil containing 400 i.u. retinol, 20 i.u. ergocalciferol. 0·2 mg menaphthone and 0·7 mg mixed tocopherols.

* Assayed on rats (Platt & Miller, 1959).

† Thiamine hydrochloride 30 mg, riboflavine 100 mg, pyridoxine hydrochloride 20 mg, Ca pantothenate 600 mg, biotin 2 mg, folic acid 20 mg, cyanocobalamin 0·5 mg, nicotinic acid 2 g, meso-inositol 2 g, *p*-aminobenzoic acid 6 g, choline 6 g and maize starch up to 100 g.

‡ Jones & Foster (1942) modified by replacing (w/w) $CaCO_3$ with maize starch.

Procedure

The mothers and weanling rats were killed with chloroform. Blood was removed from the heart, allowed to clot and the serum used for determining the concentration of Ca (Gran, 1960), inorganic P (Fiske & Subbarow, 1925) and protein by the Kjeldahl technique.

Radiographs were taken on non-screen film (Ilfex, Ilford, Ltd) with a Solus Eureka tube, 1·8 mm focus and a tube to film distance of 36 in to reduce errors due to parallax. Exposures were for 0·6 sec at 23 kV and 28 MA. An aluminium step wedge was included in every exposure to facilitate comparisons between different films. On the few occasions when radiographs of the left forepaw were taken during life no anaesthetic was used.

The distal part of the left femur together with the proximal part of the tibia were prepared for histological examination as described by Platt & Stewart (1962).

The right femurs were used for the determination of the A:R ratio, where A is the weight of the ash of the bone (incinerated at 700°) and R that of the dry, fat-free bone minus the ash (Chick & Roscoe, 1926; Chick, Korenchevsky & Roscoe, 1926). This ratio was determined separately for the shaft and ends of the long bones so that values for water, fat, ash and matrix of the shaft, ends or whole bone were also available. The volumes of the right femurs and second cervical vertebras were calculated from the weight of water they displaced and their ash content, expressed as mg/cm^3 of whole bone and used to supplement the other measures of bone quality. In addition, one rat of each litter was eviscerated, dehydrated in ethanol and acetone, cleared in 1% KOH and the skeletal tissues were stained with alizarin red S (Dawson, 1926).

RESULTS

Mothers killed at the end of their first pregnancy

Body-weights and food intakes

The food consumption of the mothers given the high-protein and low-protein diets increased by 25 and 18% respectively during pregnancy (Table 2). Their body-weight increased and differences in weight gain were related to the total intake and protein value of the diet, but not to the Ca concentration.

Bones

Size and radiographic density. The bones of the animals receiving the high-protein diets were slightly longer and of greater bulk than those maintained on the low-protein diets (Table 3). Radiographic density was highest in the bones of the animals receiving the high-protein, high-Ca diet and least in those of the rats given the high-protein, low-Ca diet.

Composition. The bones of the mothers given diet A (high-protein, high-Ca) had the greatest weight of ash, and reductions in the protein value or Ca concentration of the diet led to the deposition of less mineral (Table 3). The A:R ratio was affected more by the Ca concentration than by the protein value of the diet and, as found earlier by El-Maraghi *et al.* (1965), the rats receiving the high-protein, low-Ca diet had the lowest weight of ash/cm^3 of bone.

Histological appearance. The cells of the epiphysial cartilage were, as would be expected in rats aged 21 weeks, relatively inactive. The individual cartilage cells were slightly smaller and the epiphysial cartilaginous plates slightly thinner in the protein-deficient than in the well-fed rats. The dietary concentration of Ca appeared to have little or no effect on the growth and maturation of cartilage. The length of the trabeculae of the primary and secondary spongiosa was reduced in the bones of animals maintained on diets of low protein values or low Ca concentrations and the most severe effect occurred in the bones of those given the low-protein, low-Ca diet. There was some transverse trabeculation in the bones of all the animals given diets of low protein value. The thickest walls were found in the bones of rats receiving the high-protein, high-Ca diet. Reducing the protein value of the rats' diet led to the production of bones with rather thinner walls, whereas when the dietary concentration of Ca was reduced the cortices were less compact but not thinner.

Table 2. *Body-weights and food consumption of rats* during first and second pregnancies on diets of different protein values and Ca concentrations*

Diet			Initial body-wt (g)		Gain in body-wt (g)		Food intake (g/day)		Total intake of Ca during gestation period (g)		Total intake of N during gestation period (g)	
Symbol	Protein value (NDpCal %)	Ca (%)	First	Second	First	Second	First	Second	First	Second	First	Second
A	10·2	0·44	197·3	202·8	91·7	88·2	14·6	14·7	1·34	1·35	12·26	12·35
G	5·1	0·44	161·2	196·7	63·8	61·8	14·7	15·0	1·36	1·39	3·95	4·03
C	10·2	0·11	185·5	200·3	99·3	94·0	15·1	14·7	0·36	0·35	12·69	12·35
J	5·1	0·11	165·8	181·7	60·9	61·2	14·6	13·6	0·34	0·31	3·93	3·66

* Each value is the mean for three rats.

Table 3. *Measurements* of the right femur of pregnant rats (P) and rats which had reared two litters (L) on diets of different protein values and Ca concentrations*

Diet			Length (cm)		Volume (cm^3)		Weight (mg)		Total ash (mg)		A:R ratio		Wt of ash/cm^3† (mg)	
Symbol	Protein value (NDpCal %)	Ca (%)	P	L	P	L	P	L	P	L	P	L	P	L
A	10·2	0·44	3·29	3·41	0·345	0·375	473	475	229	174	1·95	1·58	663	464
G	5·1	0·44	3·14	—	0·287	—	360	—	174	—	1·85	—	606	—
C	10·2	0·11	3·27	3·31	0·357	0·387	429	416	176	93	1·53	1·02	492	241
J	5·1	0·11	3·16	3·12	0·275	0·319	357	349	159	91	1·62	1·31	577	286

* Each value is the mean for three rats.
† The standard error of the seven groups was ± 5·68.

Foetuses of first pregnancy

At the end of 21 days of pregnancy there were no obvious differences in the size and skeletal development (Pl. 1 *a–d*) of the rats born to mothers on the four diets. Body-weights showed small differences (Table 4), but the greatest variation occurred in the number of viable foetuses. It would appear that some adjustment between quality of mothers' diet and number of offspring had taken place, for resorption spots were found in the uteri of the protein-calorie deficient mothers. Nitrogen and ash, whether considered as percentages of body-weight or absolute amounts per foetus, showed little variation between the four groups. In the tibias the diaphysial centre of ossification was present and the epiphysial centres were absent in all the pups. Maturation of the cartilage cells appeared to be progressing and there were no obvious differences in the thickness or development of the cortices.

Table 4. *Body-weight, water, nitrogen and ash contents of 21-day-old foetuses from rats maintained on diets of different protein values and Ca concentrations*

Diet								Per foetus		Per litter	
Symbol	Protein value (NDpCal%)	Ca (%)	Mean no. of foetuses (range)	Mean body-wt (g)	H_2O (%)	N (%)	Ash (%)	Total N (mg)	Total ash (mg)	Total N (mg)	Total ash (mg)
A	10·2	0·44	10·3 (9–12)	4·27	86·90	1·46	1·35	62	58	644	595
G	5·1	0·44	8·7 (8–10)	4·03	88·02	1·41	1·35	57	54	492	471
C	10·2	0·11	12·0 (11–13)	4·10	87·02	1·43	1·30	59	53	704	640
J	5·1	0·11	7·7 (3–10)	4·04	87·18	1·43	1·33	58	54	443	412

The only changes which could be related to the mothers' diet were a reduced number of viable young, increased number of resorption spots in the uteri and slightly smaller foetuses in the mothers on the protein-calorie deficient diets.

Mothers during first lactation

Body-weights and food intakes

During the 3 weeks following parturition there was a great increase (74–76%) in the food consumption of the mothers on the high-protein diets; the increase was smaller (6%) when the animals were receiving diet J (low-protein, low-Ca, Table 5). The mothers receiving the low-protein, high-Ca diet are not included in this part of the report as, of the twenty-six pups born to them, only three survived beyond 1 week of age.

In spite of the increased intake of food, all the lactating rats lost weight and only when the young were removed was the loss arrested and weight regained.

Rats of first litter at 21 days of age

The pups of the rats given the high-protein diets were active, grew well and, from about 17 days of age, ate some of the solid food offered to their dams. In contrast, the offspring of the protein-calorie deficient mothers were slow-moving, grew slowly (Table 6, Pl. 1 *e–g*), were not seen to take any solid food and at 21 days of age were only about half the weight of those of the well-fed rats (diets A and C).

Table 5. *Body-weight and food consumption of lactating rats* (first and second pregnancies) on diets of different protein values and Ca concentrations*

Diet			Initial body-wt (g)		Change in body-wt during expt (g)		Food intake (g/day)		Ca intake (mg/day)	
Symbol	Protein value (NDpCal %)	Ca (%)	First	Second	First	Second	First	Second	First	Second
A	10·2	0·44	198·8	224·0	−35·8	−35·5	25·4	27·7	112	122
G†	5·1	0·44	165·0	—	−22·0	—	12·6	—	55	—
C	10·2	0·11	205·7	234·7	−27·7	−51·0	26·6	26·5	29	29
J	5·1	0·11	170·5	183·7	−46·8	−58·7	15·5	16·6	17	18

* Each value is the mean for three rats.
† Values for one rat only.

The femurs of the young of the protein-calorie deficient dams were small, their lengths being about 85% and their volumes and weights less than 50% of those of the well-nourished animals (Table 7).

Radiographic density

Radiographic density was highest in the bones of those whose mothers were given the diets with 0·44% Ca and least in those suckled by dams receiving the high-protein, low-Ca diet.

Composition

The carcass water, nitrogen and ash, when expressed as percentages of body-weight, showed only small differences; nitrogen was reduced when the mothers had been on the low-protein diets (G and J) and ash in those on the high-protein, low-Ca diet (C). The differences are marked when the total amounts are considered and the totals per litter (Table 6) are an indication of the lactational performance of the different groups of mothers.

The A:R ratios of the femurs were low and only small differences were found between the various experimental groups. The total ash content of the carcass reflected the large differences in growth. The weight of ash/cm^3 of bone was not modified by the protein content of the diet of mothers receiving 0·44% Ca but was low when the mothers had a diet of high-protein value and low-Ca concentration (Table 7).

Table 6. *Body-weight, nitrogen and ash contents of 21-day-old rats of the first and second litters of mothers on diets of different protein values and Ca concentrations*

Diet			Individual rats										Litter					
			Mean body-wt (g)		N (%)		Ash (%)		Total N (mg)		Total ash (mg)		Weight (g)		Total N (mg)		Total ash (mg)	
Symbol	Protein value (NDpCal %)	Ca (%)	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
A	10·2	0·44	29·5 (28)	35·7 (27)	2·55	2·48	2·55	2·60	752	885	752	928	275·3	321·3	7020	7968	7020	8354
G	5·1	0·44	18·2 (3)	—	2·46	—	2·62	—	448	—	479	—	54·6	—	1343	—	1431	—
C	10·2	0·11	30·3 (24)	29·2 (30)	2·50	2·42	2·21	2·20	758	707	670	642	242·4	292·0	6060	7066	5357	6424
J	5·1	0·11	15·6 (20)	14·5 (26)	2·26	1·90	2·52	2·47	353	276	393	358	104·0	125·7	2350	2388	2621	3105

Figures in parentheses indicate the number of young in each group.

Table 7. *Measurements of the femurs of 21-day-old rats of the first and second litters of mothers on diets of different protein values and Ca concentrations*

Diet			Length (cm)		Volume (cm^3)		Weight (mg)		Total ash (mg)		A:R ratio*		Weight of ash/cm^3 (mg)	
Symbol	Protein value (NDpCal %)	Ca (%)	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
A	10·2	0·44	1·51 (28)	1·67 (27)	0·084	0·103	79·6	90·8	12·8	15·7	0·59	0·66	152	153
G	5·1	0·44	1·25 (3)	—	0·039	—	31·2	—	6·0	—	0·54	—	154	—
C	10·2	0·11	1·42 (24)	1·56 (30)	0·081	0·089	76·9	81·5	9·4	9·9	0·50	0·51	116	111
J	5·1	0·11	1·25 (20)	1·06 (26)	0·037	0·034	29·3	27·7	4·9	4·4	0·49	0·49	134	129

Figures in parentheses indicate the number of young in each group.
* Ash: organic matter.

Histological appearance

The epiphysial cartilages were thinner and the trabeculae of the metaphysial region shorter in the offspring of the protein-deficient dams than in those whose mothers were given the high-protein diets. The reduction in the length of the trabeculae was most marked when the diet contained only 0·11 % Ca (diet J), and in all the animals whose mothers received the low-Ca diets (C and J), osteoclasis was prominent. The wall of the shaft was thin in all the rats, the best development being in those whose mothers received diet A (high-protein, high-Ca).

Mothers killed after second pregnancy and lactation

Body-weight and food intakes

At the beginning of their second pregnancy all the animals not only regained the weight lost during the first lactation but were heavier than at the start of their first pregnancy (Table 2). Changes in body-weight and intakes of food were similar in the second reproductive cycle to those described for the first (Tables 2 and 5) except that during the second lactation there was a greater weight loss in the rats given the high-protein, low-Ca diet.

The mammary glands of all the protein-calorie deficient mothers were small and poorly developed (Platt, Heard & Stewart, 1964), but the least well-developed glands were seen in the rats given the low-protein, high-Ca diet. These animals were able to provide very little milk for their young, none of which survived to 2 weeks of age.

Bones

Radiographic density. The femurs and vertebras of all the mothers which reared their young were rarefied; the rarefaction was severe when the protein value or Ca concentration of the diet was low, but also occurred, although to a lesser extent, when the high-protein, high-Ca diet was eaten (Pl. 2).

Composition. The A:R ratio of the femurs was lower than in the rats on similar diets which had been killed at the end of their first pregnancy (Table 3). Diets with a low concentration of Ca led to the development of bones with low weights of total ash and ash/cm^3 and the latter effect was intensified if the diet also had a high protein value. Even when the diet having both a high protein value and a high Ca concentration was given, the animals were unable to maintain the total ash content and quality of their skeletal tissues (Table 3).

Histological appearance. The bones of the animals given the low-protein diets showed changes in the epiphysial cartilage and metaphysial region similar to those described earlier (p. 735). There was also a marked reduction in the thickness of the cortices of the long bones. Pl. 3*c* illustrates the severe loss of mineralized tissue which had occurred when the animal's diet had a high-protein and low-Ca content. Diets with a similar concentration of Ca but lower protein values caused less loss (Pl. 3*d*), but even in the animals given the high-protein, high-Ca diet there was less bone than in those on a similar diet killed at the end of their first pregnancy (Pl. 3*a* and *b*).

Osteoclasis and osteoblastosis were seen in the bones of all the animals given the high-protein diet. The activity was greatest in the bones from animals with the lower intake of Ca (diet C) and in these specimens the newly formed uncalcified borders were slightly wider than those in the bones of animals receiving 0·44 % Ca.

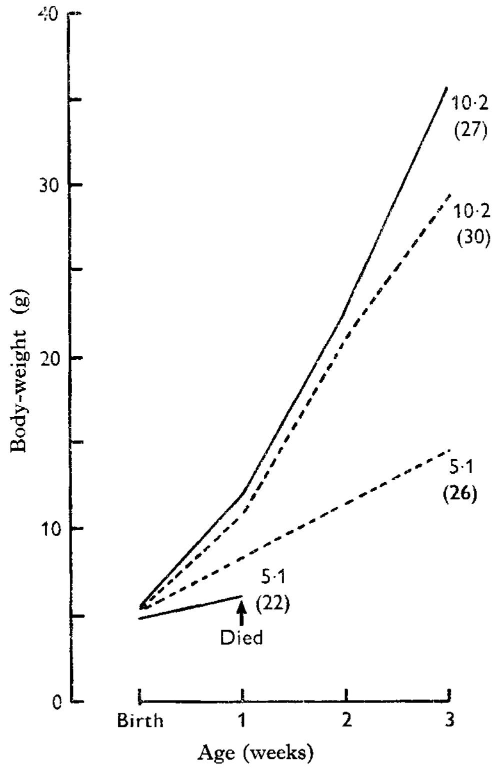


Fig. 1. Body-weight curves of the offspring (second litters) of rats maintained on diets of different protein values and Ca concentrations. The figures on the curves give the NDpCal % of the diet and (in parentheses) the number of individual rats in each group. ——, 0·44 % Ca; - - - - -, 0·11 % Ca.

Rats of second litter killed at 21 days of age

When first weighed, 10–12 h after birth, these animals were heavier than those of primigravidae on similar diets and the differences between individuals born of mothers on high- or low-protein diets were also greater. At 21 days of age the young from mothers given diet A (high-protein, high-Ca) were larger than those of the first litters. During suckling the rats behaved very like those of the first litters. All the rats born of the low-protein, high-Ca dams died; those from the other low-protein mothers survived but were sluggish and grew slowly (Fig. 1). When the protein value of the mother's diet was high and the Ca concentration low (0·11 %) there was a slight but definite retardation in the growth of the offspring compared with that of the high-protein, high-Ca animals.

Composition

The values for nitrogen and ash of the carcass (Table 6) and the size, ash content, A:R ratio and weight of ash/cm^3 (Table 7) of the femur were similar to those of the young of the first litter. Values which were low in the young of the first litter of mothers maintained on low-protein and low-Ca diets were also low and to a more marked extent in the second litters.

Histological appearance

This was essentially the same as for the first litter except that, in keeping with the results of chemical analyses, changes due to protein and Ca deficiencies appeared to be slightly more marked.

Serum analysis

The pooled serums from the three groups of mothers who raised a second litter and from the three corresponding groups of litters were analysed for protein, Ca and inorganic P (Table 8). When mothers were maintained on diets of low protein value (diet J) the concentration of protein was low in the serum of both mother and offspring. Low intakes of Ca (diets C and J) led to low values for Ca in the serum of the mother irrespective of the protein value of the diet, but in the offspring the value was reduced only if the protein value of the mother's diet was high.

Table 8. *Concentration of protein, Ca and inorganic P in the serums of rats* (mothers and offspring) maintained on diets of different protein values and Ca concentrations*

Diet			Serum							
	Protein value of mother's diet	Ca	Protein (g/100 ml)		Ca (mg/100 ml)		Inorganic P (mg/100 ml)		Ca × P	
Symbol	(NDpCal %)	(%)	Mother	Offspring	Mother	Offspring	Mother	Offspring	Mother	Offspring
A	10·2	0·44	6·28	5·88	10·4	10·5	7·2	10·5	75	110
C	10·2	0·11	6·33	5·63	7·2	9·2	8·1	10·2	58	94
J	5·1	0·11	5·65	4·90	7·4	10·0	8·5	9·8	63	98

* Values from pooled serum at the end of the second pregnancy and lactation.

All the values for protein and Ca were lower than those found in the serum of young (NDpCal% = 10·2) and non-pregnant adult (NDpCal% = 6·0) rats receiving diets containing 0·44% Ca.

DISCUSSION

The four diets used in the above tests have been described and discussed in a previous paper (El-Maraghi *et al.* 1965). The protein values are such that the higher values are equivalent to those recommended (FAO, 1957*a*, *b*) for pregnant and lactating women, whilst the lower values, although adequate for non-pregnant adults (women or rats), fall well short of the requirement during reproduction. The higher Ca concentration, 0·44% of the diet, is less than the 0·49% recommended by Cox &

Imboden (1936) for reproduction in the rat, but had the advantage of facilitating comparisons between non-pregnant (previous report) and the present pregnant and lactating rats.

Although the number of foetuses per litter was low when the rats were given diets of low protein value, the body-weight and composition of the individual 21-day-old foetuses of the first litter were only slightly affected. Larger differences were observed when the first weights of the second litters were compared but this may be due, at least in part, to the availability and amount of milk ingested before they were weighed.

Venkatachalam & Ramanathan (1964) maintained stock rats on a diet of low protein value (calculated from their results to be about NDpCal% = 3) during gestation and found that the body-weight and composition of the newborn rats were practically normal: there were, however, fewer foetuses per litter (their table 2). When diets of similar low protein value were fed during the development of the mother as well as during gestation (Ambegaokar & Chandran, 1959) there was a severe reduction both in the weight of the newborn and in the number of pups per litter. However, in both investigations the diets, in contrast to those described above (diets G and J, Table 1) were probably inadequate for maintenance of the normal adult.

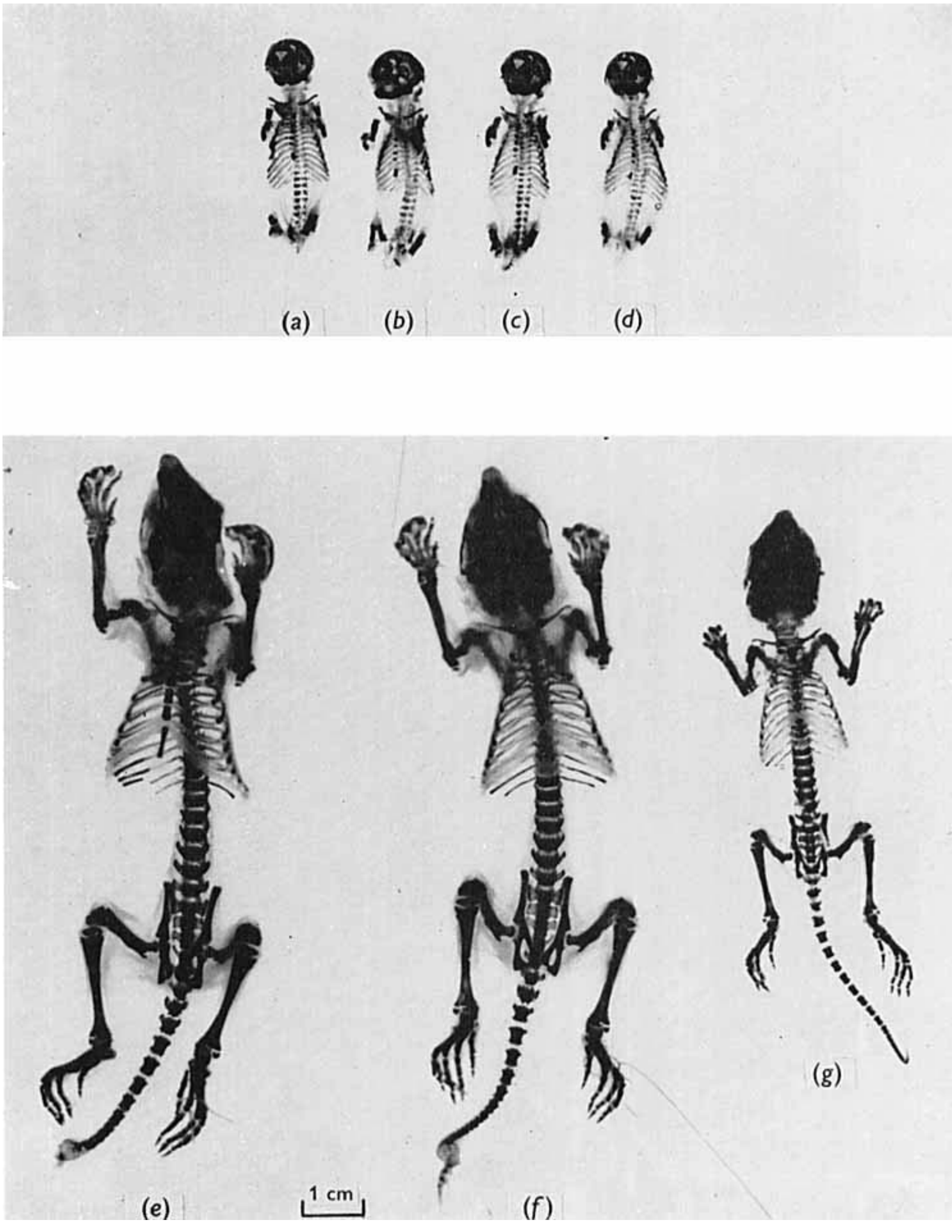
The mammary glands of the protein-calorie deficient mothers were small, poorly developed and exhibited many of the changes described by Platt *et al.* (1964). The weanlings of the mothers given the low-protein, low-Ca diet all survived to 3 weeks of age but weighed only about half as much as those from rats on the diets of high protein value. The concentration of protein was low in their blood serum and carcass, whilst their bones showed chemical and histological evidence of matrix-osteoporosis. A deficient supply of milk probably accounts for most of the changes, and when suckled by normal mothers there is a great improvement in development although they remain abnormal and somewhat smaller than normal weanlings (Payne & Stewart, 1966 in preparation). When the low-protein diet contained 0·44% Ca the ill effects were more marked. Only one (three rats) of the six litters produced from the first and second matings survived beyond 1 week of age. Greig (1952) and Richards & Greig (1952) reported that the high death rate occurring amongst the offspring of mice maintained on diets containing a high proportion (1·1%) of Ca was prevented by reducing the Ca or increasing the iron of the mother's diet. The concentration of Ca was much lower in the present experiments but was accompanied by a protein-calorie deficiency which is known to enhance the deleterious action of Ca on trace minerals (Frankul, 1964; El-Maraghi, 1964). It is probable therefore that the deaths of the offspring of the rats given the low-protein, high-Ca diet were an example of an imbalance between two dietary constituents leading to a deficiency of a third.

Reducing the Ca content without altering the protein value of the mother's diet produced relatively little change in the foetus. However, similar changes in the diet of the nursing mother led in the 21-day-old weanling to reductions in the percentage of ash in the carcass, the total ash of the femur and the weight of ash/cm^3 of bone. The differences brought about by reducing the Ca were less marked when the diets were of low protein value and the growth of the offspring thereby restricted. The changes due to a deficiency of Ca were intensified in the second litter and in addition to the carcass and bone changes there was a reduced concentration of Ca in the serum and some retardation of growth.

Diets of high protein value containing 0·44% of Ca did not prevent a loss of mineral from the bones of the mothers during pregnancy and lactation. When adult virgin female rats were maintained on diets of adequate protein value with either 0·11 or 0·44% Ca their right femurs contained 243–256 mg of mineral ash (651–679 mg/cm^3) with A:R ratios of 1·98–2·08 (El-Maraghi *et al.* 1965). Corresponding bones of animals which had produced and reared two litters on the high-protein diet with 0·44% Ca contained only 174 mg of ash (464 mg/cm^3) and the A:R ratio was reduced to 1·58. The bones showed slight but definite histological evidence of mineral-osteoporosis and there is no doubt that the mothers' diet contained insufficient Ca to meet the combined requirement of the mother and offspring; the deficit, however, was partially met by mobilizing mineral from the mother's bones. The loss of mineral was even more marked when diets of high protein value containing only 0·11% Ca were eaten, the right femurs containing only 93 mg of ash (241 mg/cm) with an A:R ratio of 1·02. These bones showed a frank mineral-osteoporosis but, although over 50% of the mineral had been released from the mother's bones, this did not fully protect the offspring, which also suffered from some degree of Ca deficiency. The reduction of the A:R ratio to 1·58 when the diet contained 0·44% Ca can probably be related to the destruction of older bone to provide additional mineral, leading to a relative excess of newly formed, less well calcified bone. The marked reduction of the A:R ratio to 1·02 when the diet provided only 0·11% Ca may be a more severe instance of imbalance between older and younger bone. There is evidence, however (see Stewart, 1965), that when Ca supplies are very low the requirement for vitamin D may be increased. The amount of vitamin D supplied in these experiments was adequate for young, adult and aged rats on diets having protein values of NDpCal% from 4·5 to 10·2 and Ca concentrations of from 0·11 to 0·44%, but the low A:R ratio in the bones of mothers given a high-protein diet with only 0·11% Ca may indicate a need for more vitamin D.

A recent paper by Campbell & Douglas (1965) also illustrates this interaction of mineral and vitamin D requirements. Diets containing adequate mineral and only a trace of vitamin D (in addition to body reserves) protected the animal against the development of rickets. When, however, the mineral concentrations were low, even 100 i.u. vitamin D/kg body-weight did not give complete protection.

A deficiency of Ca in the mother's diet did not have as adverse an effect on the growth of the offspring as a deficiency of protein. It is clear that during gestation the mother can, by producing fewer young and mobilizing her own tissues, give some protection to the offspring against deficiencies in her own diet; during lactation the demands of the offspring increase, supplies from the mother's tissues become exhausted and dietary deficiencies are shared between mother and offspring.



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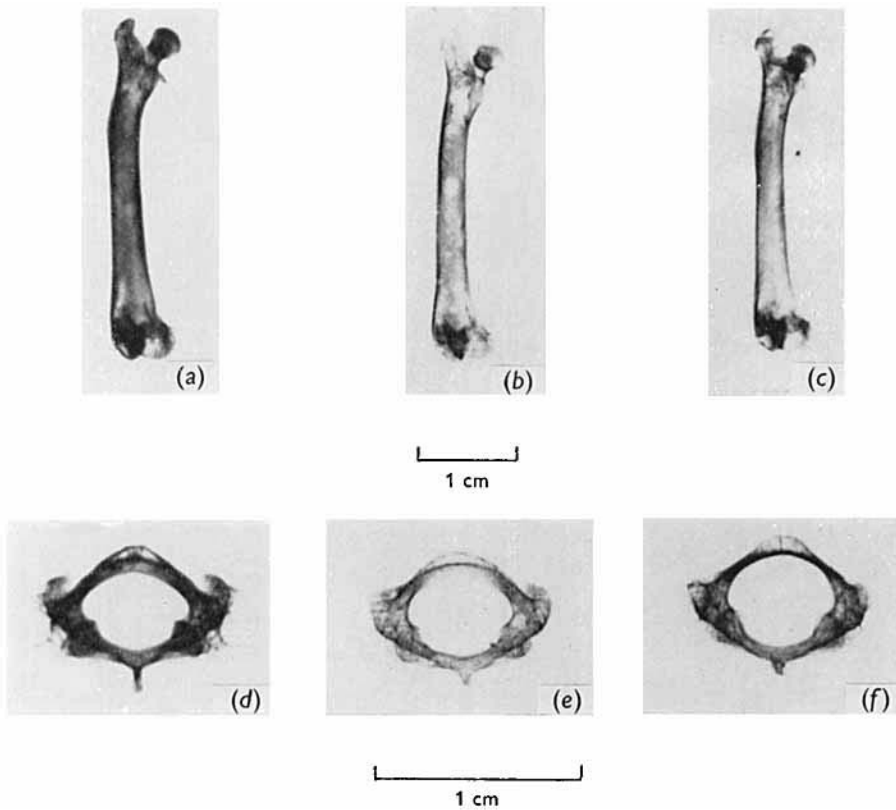

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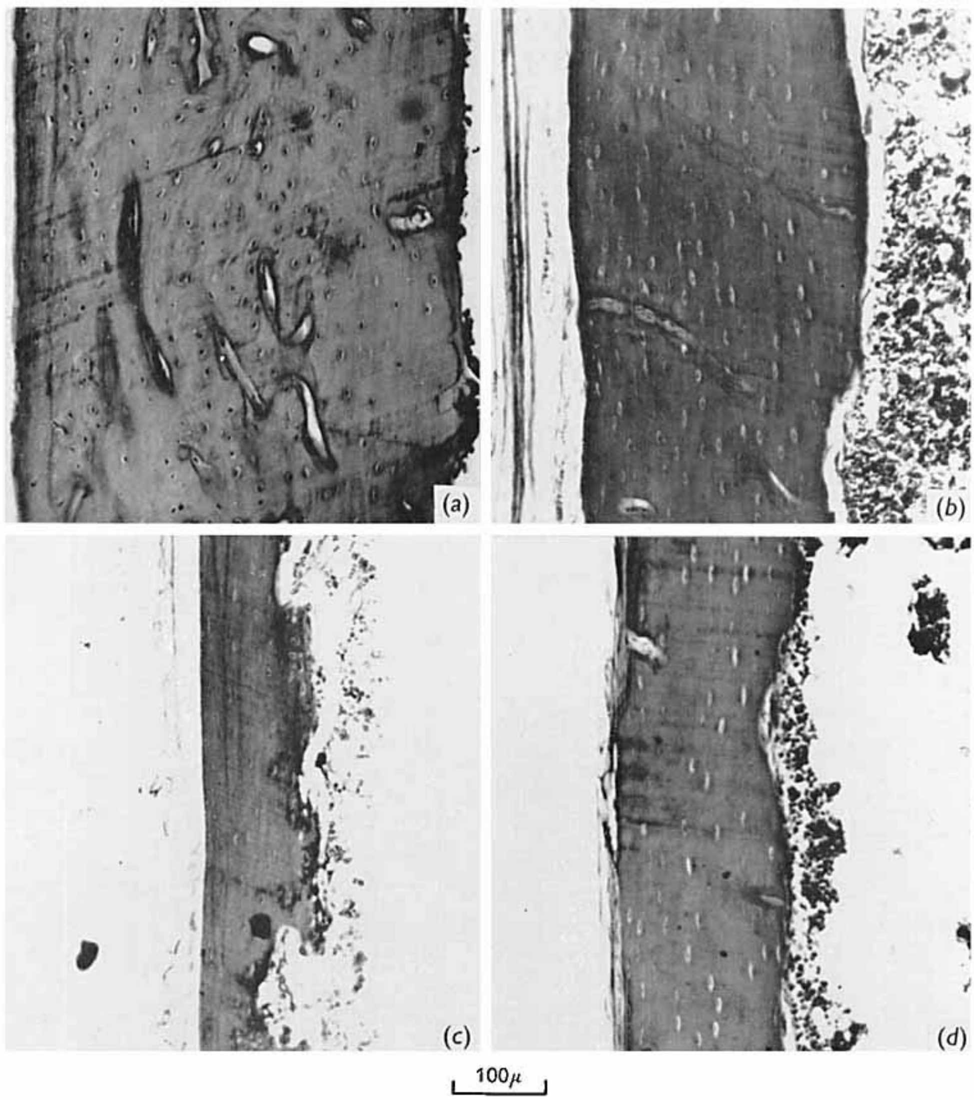

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EXPLANATION OF PLATES

Plate 1

Photographs showing the relative size and skeletal development (alizarin stained) of the offspring of rats maintained on diets of different protein values and Ca concentrations. (*a*)–(*d*) at 21 days gestation, (*e*)–(*g*) at weaning (21 days of age). The diets of the mothers were (*a*) and (*e*) NDpCal % = 10·2, Ca 0·44 %; (*b*) and (*f*) NDCal % = 10·2, Ca 0·11 %; (*c*) NDpCal % = 5·1, Ca 0·44 %; (*d*) and (*g*) NDpCal % = 5·1, Ca 0·11 %.

Note: (1) Lack of differences at end of gestation (*a*)–(*d*); (2) marked effect of differences in the protein value of the mother's diet during lactation (*f*) and (*g*) and similarity of (*e*) and (*f*), although the Ca contents of the mothers' diets were 0·44 and 0·11 % respectively.

Plate 2

Radiographs of the femurs and first cervical vertebras of rats maintained on diets of different protein values and Ca concentrations during two reproductive periods. (*a*) and (*d*) NDpCal % = 10·2, Ca 0·44 %; (*b*) and (*e*) NDCal % = 10·2, Ca 0·11 %; (*c*) and (*f*) NDpCal % = 5·1, Ca 0·11 %.

Note: marked rarefaction in (*b*), (*c*), (*e*) and (*f*).

Plate 3

Photomicrographs of the wall of the shaft of the tibia of rats maintained on diets of different protein values and Ca concentrations during pregnancy only (*a*) or two periods of pregnancy and lactation (*b*)–(*d*). (*a*) and (*b*) NDpCal % = 10·2, Ca 0·44 %; (*c*) NDpCal % = 10·2, Ca 0·11 %; (*d*) NDpCal % = 5·1, Ca 0·11 %.

Note: (1) reduced thickness of the wall of the shaft in the rat which reared two litters—cf. (*a*) and (*b*); (2) in animals given diets of high protein value the greater mobilization of bone occurs when the diet has a low Ca concentration—cf. (*b*) and (*c*); (3) when the Ca concentrations of the diets are low the thicker wall is found in the rat given the diet of lower protein value—cf. (*c*) and (*d*).

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