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STRENGTH AND SIZE OF BONE IN RELATION TO CALCIUM INTAKE

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Bone serves three main functions in the body: (1) it gives rigidity to the skeletal framework thus supporting the soft tissues and facilitating movement; (2) it acts as a readily available source of minerals; and (3) it has a haematopoietic action. We are here concerned with the first two functions only.

Fairbanks & Mitchell [1936] have raised the question as to whether a very rapid rate of bone calcification is a benefit. They express the view that benefits accruing from increased rates of calcification of the skeleton only occur up to a point probably considerably below complete saturation. On the other hand, they hold that 'until the minimum percentage saturation of the stores compatible with maximum physiological performance has been determined it would seem to be the wiser course (certainly the safer course) to consider complete saturation of the store as the ideal condition...'. It is obvious that something more than mere chemical observations are required to supply such information.

In recent work on bony growth the quality of the bone has been judged chiefly on the basis of chemical analysis, but this seems, to the present writers, to disregard the essential function of bone as a supporting tissue. A bone must be strong enough to bear the weight of the body, to resist the pull of the muscles, and to withstand the strains and stresses of an active life. A certain amount of work has been done in estimating the strength of human bones, and this was reviewed by Disse [1896]; several estimates have also been made on the strength of the bones of swine and cattle [Becker & Neal, 1931; Becker, Neal & Shealy, 1934; Maj, 1938]. Lindsay & Howes [1931] and Lindsay [1934] have described strength tests on normal and healing fractured fibulae of rats.

None of these reports gives any information about the quality of bony material, nor is advantage taken of the methods of measuring strength of materials well known to engineers.

The experiments here to be described were made with a view to correlating functional and chemical observations. In particular, we were anxious to know how the quality of the body material was affected by a much reduced and a much increased Ca intake.

Experimental procedures

(a) *Biological*

Ninety-six recently weaned male albino rats were used in the four experiments and were fed on Rowett Institute stock diet [Thomson, 1936] until they were about 60 g. in weight. In each experiment the animals were divided up into groups of four or five; one representative group was sacrificed at the beginning of each experiment and the Ca content determined after weighing the degutted carcasses. The remaining groups were placed on diets of constant protein, carbohydrate, fat and vitamin content but of varying Ca content (see Table 1) over a period of 56 days. Each animal was housed in a separate galvanized wire cage with feeding attachments of the Hopkins pattern. The food was given ad lib. and was made up as follows:

	%
Dried egg albumin	5·0
Dried egg yolk	10·0
Potato starch	15·0
Rice starch	37·0
Cane sugar	10·0
Butter fat	10·0
Dried yeast	5·0
Cod liver oil (Seven Seas Ltd. standard potency)	3 drops daily
de Loureiro's salt mixture excluding $CaHPO_4$ [de Loureiro, 1931]	3·4
$CaHPO_4$ and $BaSO_4$ in various ratios making together	4·5
	99·9

By varying the relative amounts of $CaHPO_4$ and $BaSO_4$ it was possible to produce a range of diets with from 0·075 to 1·390 g. Ca %, the Ca contents being checked by analysis. Lower values could not be obtained because of the Ca associated chiefly with the dried egg. The basic diet, less the $CaHPO_4$, contained 0·066 % Ca and 0·223 g. % P; the Ca:P ratio of the total diets ranged from 0·33 to 1·11.

This system of altering the Ca in the food without disturbing the rest of the diet is similar to that used by Fairbanks & Mitchell [1936] and has of course the defect inherent in all such experiments of causing a variation in the Ca:P ratio. It is obvious, however, that to produce a

proportionate restriction in P when the Ca is reduced would impose effects on soft tissue growth which would complicate the issue, and further, any rachitogenic action, due to the abnormal Ca:P ratio of our diets, was presumably prevented by giving adequate amounts of vitamin D.

The first twenty animals used in Exp. I came from one dealer. The remaining seventy-six used in Exps. II–IV came from another dealer. Exp. I cannot, therefore, be directly compared with Exps. II–IV, although the results vary in the same direction. Exps. II–IV can be directly compared, and the results in any one of these three experiments can be more closely scrutinized since all the animals were of the same stock and were fed under the same climatic conditions.

The animals were weighed at weekly intervals for the 8 weeks of the experiment. During this time the total food intake was noted so that the total consumption of Ca could be calculated. Owing to spillage, inevitable in such experiments, the values are probably slightly in excess of the actual amount eaten.

At the end of the experimental period the animals were killed and then weighed, the body length measured, and after degutting they were weighed again. (This method of discounting the Ca not actually within the animal's tissues by removing the entire alimentary canal was preferred to the rather unphysiological procedure of 24 hr. starvation before killing, a process which results in a very great loss of weight with presumably a call on the reserves of Ca among other materials.) The femora were dissected out, cleaned of muscle and tendon, then skiagraphed; finally, they were weighed and measured and used for the mechanical tests. Their Ca content was estimated by analysis of the residual fragments from these tests. The Ca content of the rest of the carcass was also determined so that it was possible to estimate the retention of Ca in the whole animal (less alimentary canal) over 56 days, due allowance being made for the Ca of the femora. The percentage of Ca in the degutted rat is expressed on the basis of the degutted weight. Since drying occurs very quickly in these bones it was impossible to weigh, measure, and X-ray the bones and then carry out the mechanical tests with the bone in its fresh, moist state. The bones were, therefore, allowed to dry at room temperature for several days prior to being weighed, no further loss of weight being likely to occur in the process of mounting the bones for the mechanical tests. The fragments were of comparable dryness, i.e. in equilibrium with the air, and the weights given in Table 1 may be regarded as those of air-dried bones. It is

obvious that more drastic drying might have altered the mechanical properties of the femora. Bachmann, Haldi, Wynn & Ensor [1940] found that the percentage of Ca in the femora of male rats about the same age as ours which were fed on rations varying from 0·4 to 1·0 % Ca over a similar period of time varied from 22·3 to 23·3 % of the dry weight. This is in general agreement with the values given in our table.

The Ca was determined in the food, carcasses, and bones by appropriate modifications of Shohl & Pedley's method [1922].

(*b*) *Mechanical*

In our choice of tests we have been guided by the experience already available to engineers concerned with the determination of the strength of materials. These tests usually consist in determining the load necessary to fracture a test sample. When selecting a mechanical test for a bone it is important to simulate as closely as possible the straining actions taking place in the living animal. There are four types of straining action possible—axial compression, axial tension, bending and twisting. If a bone were tested by a crushing load applied axially at the ends failure would probably take place in the bone immediately under the loading points. Even if the bone were protected so that the fracture occurred in the shaft, it is doubtful if the breaking load observed would be a reliable index of strength. The chief difficulty with this type of test is to ensure that the load will be applied directly along the long axis of the bone—this is especially difficult when the specimens are small but is highly important, since a small eccentricity of loading may halve the strength of the bone. It is obvious from the shape of the femur that, since the line joining the head and the condyles lies mostly outside the shaft, true axial loading never occurs in the living animal. Eccentric loads produce bending in a columnar structure, and this is more severe in its effect than direct compression; it is, therefore, much more important to determine bending strength. Further, if the example of the adductor magnus muscle is considered it will be realized that muscles may produce quite severe bending actions. It will be shown later that the femur is much stronger than is necessary from considerations of axial compression alone. Bones are seldom called upon to resist axial tension; even in the highly abnormal procedure of reducing a dislocation the strain is borne chiefly by ligaments and muscles. We have confined our attention, therefore, to tests of twisting and bending which are the actions to which bone is normally subject. The actions are brought about by muscular pull, by the weight of the body and by accidental violence.

Bone is usually broken by impact, i.e. sudden application of a force, either when an external object strikes the limb or when the animal strikes the ground after a leap or fall. A test involving impact loading, however, is very difficult to evaluate with any accuracy especially when the specimens are small; hence gradual application of the load during a test until the bone breaks is a more satisfactory procedure. In testing engineering materials it is customary to place greater reliance on tests involving static loading than on impact tests even where the member tested, e.g. a piston rod, has to withstand impact.

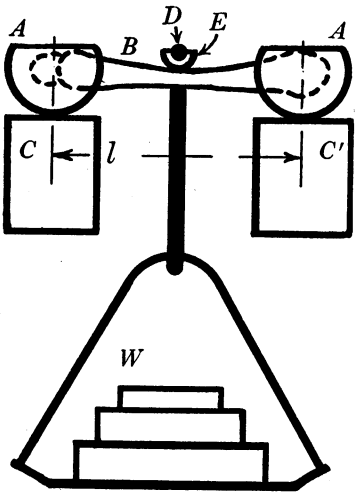

Fig. 1.

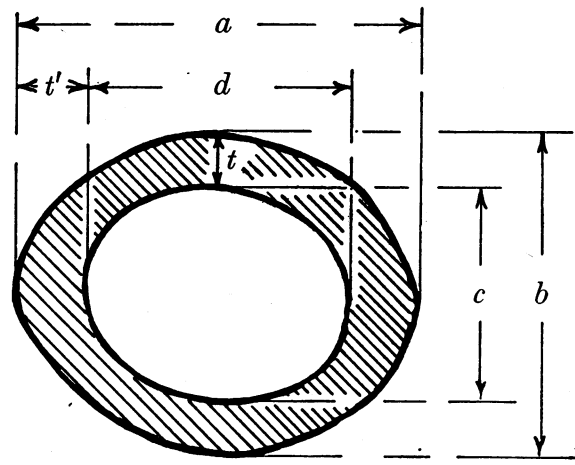

Fig. 2.

Fig. 1. Apparatus for determination of bending strength. *B*, bone; *A*, semicylindrical ends cast on to bone; *C*, *C'*, supports; *D*, wire with red fibre lining *E*; *W*, load.

Fig. 2. Diagrammatic section through the midpoint of the shaft of the femur to indicate where the measurements were made.

Bending test. Each right femur, *B* in Fig. 1, had its ends cast into short semi-cylindrical pieces, *A*, of a hard resinous substance (a cement composed of equal parts of plaster of Paris and colophony resin which, when heated up with a little tallow, forms a homogeneous mixture). This process prevented the bone from crushing at the line of contact with the supports, *C* and *C'*, provided an accurate measurement of the span, *l*, and also prevented axial rotation. For the sake of uniformity care was taken with casting on the ends to place the bone with the major axis of the central section horizontal, i.e. parallel to the axis of the cylindrical end pieces. This was accomplished by laying the bone in a little moulding machine and pouring the molten cement over and around

the ends. The span, l, was measured by vernier calipers, and the external dimensions, a and b (see Fig. 2), at the central section by a micrometer reading to 1/10,000 in. The loading W was applied by adding weights to the pan which was hung by a steel wire D, in Fig. 1, looped round the centre of the shaft. A ring of red fibre E reduced the danger of local crushing of the bone material. The load was increased by small steps until the bone broke. The actual breaking load was taken to lie between the greatest weight supported and the final weight applied; the error involved is small because the steps were a small fraction of the total weight including the pan. The thicknesses, t and t' of Fig. 2, were measured by the micrometer at the fracture site.

The weakest section of the bone is at the midpoint of the shaft where the dimensions are smallest; the bending moment is also greatest there, having a value $W/2 \times l/2 = \frac{1}{4}Wl$ as shown by Fig. 3. From this test we can calculate the bending moment M at the midpoint of the bone where it breaks. If the span l had been maintained at a constant value throughout the experiments the breaking load W would have been an index of the bending strength of the shaft of the bone. In practice it was found more convenient to allow the span to vary a little and therefore the greatest bending moment, $\frac{1}{4}Wl$, is used as the index of the strength of a bone.

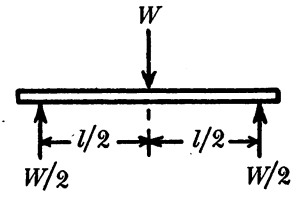

Fig. 3. To illustrate bending moment.

Twisting test. In this test hollow brass cubes were cast on the ends of the left femora by using the cement previously described. Alignment of the cubes was obtained by using a jig during the casting on process. The external dimensions, a and b, of the central section of the shaft were measured. The bone was then fitted into the machine shown in Fig. 4; end A was fixed, end B received the twisting action through the movable part comprising grip C, spindle D, and lever G. Loading W was applied gradually until the bone broke. Friction between the spindle and the supports E and F was obviated by oiling them. End B rotated through about 30° before the break occurred; the position of the lever G on its spindle was so adjusted that the bone broke when the lever was nearly horizontal. The strength of the bone in twisting is measured by the product of the breaking weight and the leverage Wl. As this product, the twisting moment or T, is constant along the bone shaft, it will cause fracture at the weakest section, usually near the centre of the shaft. In this test the line of fracture often ran obliquely or spirally

along the shaft for some distance; the thickness of the shell of the femur was measured at several places on the fracture site.

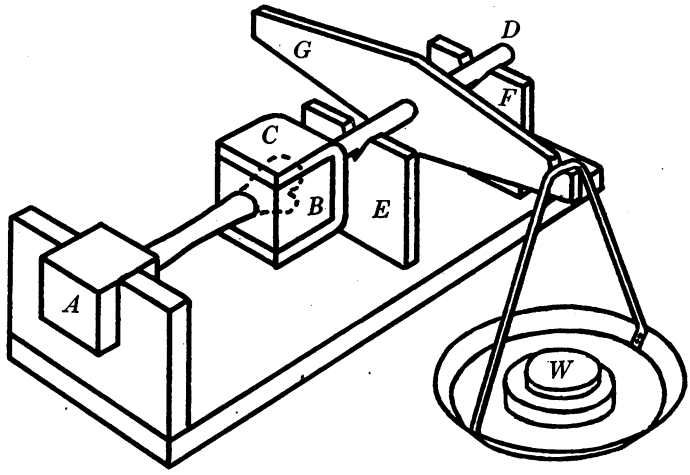

Fig. 4. Machine for measuring twisting strength. *A* and *B*, brass cubes cemented on to the ends of the femur; *C*, grip; *D*, spindle; *E* and *F*, supports; *G*, lever; *W*, load.

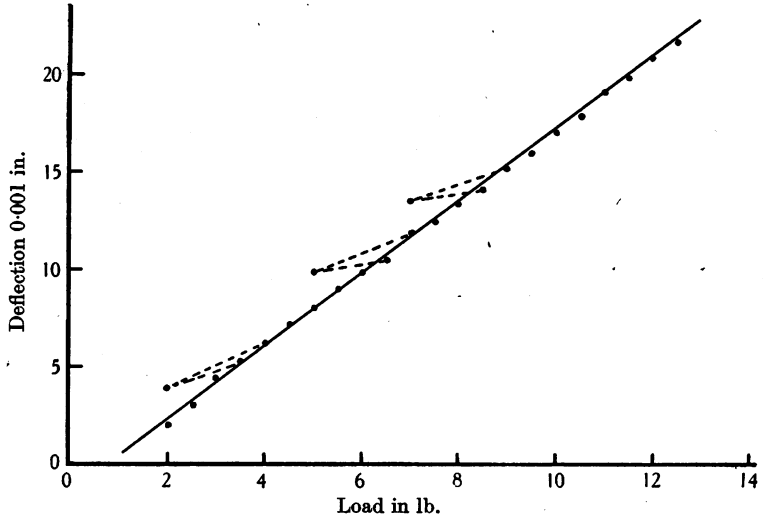

Fig. 5. Displacement of the midpoint of the shaft from its unloaded position during the bending test. The bone fractured under the conditions indicated approximately by the end of the line.

Deflexion test on bending. This test consisted in measuring the displacement of the midpoint of the shaft by means of a dial gauge resting lightly on *D* of Fig. 1 while adding weights during the bending test.

We thought it possible that some bones might yield considerably at the higher loads; this phenomenon occurs with ductile materials like steel. The graph of the relation between the deflexion and the load in our case is a straight line up to the moment of fracture (see Fig. 5); hence bone material is elastic up to fracture. The dotted lines indicate the result of reducing the load a little to a previously observed value: the deflexion was then somewhat greater than was observed originally at this loading. On increasing the load once more the deflexions fell again on the original straight line. These departures from the straight line must be due to crushing of the end mouldings on the steel supports. Since no differences were detected between the bones by this test it was carried out in a few instances only.

Breaking stress. The strength of a bone shaft depends on the shape and dimensions of the central section, and on the breaking stress of the bone substance. The latter is defined by considering the part force acting on a small area of the section; the ratio part force/small area is the stress at this point. When with increasing loads the stress on any one point on the section attains a certain value fracture occurs there and spreads immediately throughout the section. This value is the breaking stress which is a measure of the strength quality of the bone substance. From our measurements we calculated the breaking stress to determine if lack of Ca in the diet affected the quality of the bone laid down under these circumstances.

The formulae used for calculating breaking stress are

$$M = s_1 (abt), \tag{1}$$

and

$$T = s_2 (abt), \tag{2}$$

where M = bending and T = twisting moments at fracture, s_1, s_2 = breaking stress in bending and twisting respectively, and a, b, t are the dimensions of the central or fracture section as indicated in Fig. 2. These formulae do not give absolute values for the breaking stress but give values which are comparable among themselves. They assume that the section profiles of the various specimens are similar in shape with proportional dimensions. Since the section profiles of the specimens were not exactly similar an error of an unknown amount is introduced. Two additional formulae derived on a somewhat different basis were used as a check. These are

$$M = s_1 \left(\frac{\pi}{32} \frac{ab^3 - dc^3}{b}\right), \tag{3}$$

and

$$T = s_2 \tfrac{1}{2}\pi (a - t')(b - t)\, t_m, \tag{4}$$

where the dimensions are for the central or fracture section as in Fig. 2, and where t_m is the minimum thickness of the wall of the femur. Formulae (3) and (4) are based on the assumptions that the section contours are elliptical and that the material is perfectly elastic. The results indicate that there is good agreement between the simple and the more complicated formulae. Formulae (3) and (4) have additional interest since they provide absolute values for the breaking stresses, s_1 and s_2.

These formulae may be found in any standard work on strength of materials [e.g. Case, 1925].

The drying in air produced no significant alteration in the strength of the bones. Two pairs of femora from two animals were compared. Immediately after killing, one femur was removed and set up as quickly as possible for the bending test. The other bone was removed and was kept for 2 weeks in one case, and 10 months in the other. The differences in bending moments of the fresh bone and of the air-dried bone were in both cases less than 2 %.

Results

The complete data of the experiments are given in Table 1 and are graphically described in Figs. 7–9. The animals of Exp. I ate considerably more food than the other animals (Exps. II–IV) which were of a different stock. The former had heavier femora and contained more Ca than the other animals fed on similar diets. The data of Exp. I are included in Table 1 but not in Figs. 7–9; for although they demonstrate the same characteristics of structural change with increasing dietary Ca they cannot be fitted into the curves of the other experiments and will not be included in the discussion unless specifically referred to. Even though the animals of Exps. II–IV are of the same stock the data show minor variations from experiment to experiment presumably for the reasons mentioned earlier.

The percentage of Ca in the diet ranges from 0·075 to 1·390 g./100 g. dry food. This is well below and considerably above the Ca content of standard diets for the growing rat. The experimental data of Orr, Thomson & Garry [1935] and of Gaunt, Irving & Thomson [1938] show that a diet containing 0·284 g. Ca/100 g. dry matter is adequate for growth and reproduction in the rat. The total food intake remains remarkably constant in spite of the marked variation in the Ca content of the food; such small divergencies as occur do not seem to be related to the Ca content of the food. Fig. 6 shows that the relation of percentage of Ca in the diet to the total Ca intake is a straight line one. It will be seen that in Exps. III and IV the percentage retention of Ca

TABLE 1.

Experiment	No. of rats in each group	Total food intake over 56 days g.	% Ca in food	Ca intake over 56 days g.	Body wt. in g. Initial	Body wt. in g. Increase	Body length cm.	Ca content of the degutted controls g.	Total Ca retention g.	% Ca in degutted animals	% retention of Ca intake	Wt. of air dried femur g.	Length of femur cm.	Ca in femur g.
I,	4	967	0·269	2·601	59	178	19·7	0·430	1·401	0·860	53·9	0·495	3·19	0·106
7. vii. 38–	4	910	0·527	4·796	60	155	19·1		1·541	0·909	32·1	0·517	3·20	0·116
1. ix. 38	4	911	0·921	8·390	59	166	19·8		1·674	1·041	19·9	0·560	3·27	0·133
	4	898	1·303	11·701	59	161	19·4		1·759	1·118	15·0	0·553	3·17	0·126
II,	5	730	0·141	1·031	59	162	20·5	0·473	0·765	0·619	74·2	0·290	3·12	0·067
28. x. 38–	4	849	0·218	1·852	58	181	21·5		1·185	0·753	64·0	0·374	3·21	0·087
23. xii. 38	4	730	0·301	2·195	60	174	20·7		1·355	0·850	61·7	0·389	3·20	0·097
	5	811	0·365	2·960	60	174	21·1		1·469	0·905	49·6	0·407	3·19	0·102
III,	5	748	0·131	0·980	63	146	20·2	0·457	0·771	0·674	78·7	0·277	3·08	0·066
11. ii. 39–	5	718	0·144	1·034	64	141	20·0		0·876	0·733	84·7	0·289	3·08	0·066
8. iv. 39	4	686	0·203	1·393	67	144	19·9		1·150	0·878	82·5	0·327	3·08	0·083
	5	788	0·596	4·696	64	167	20·9		1·544	0·972	32·9	0·407	3·08	0·095
IV,	5	875	0·075	0·656	62	141	20·5	0·486	0·466	0·501	71·1	0·256	3·14	0·045
15. v. 39–	5	772	0·100	0·772	62	146	20·3		0·642	0·686	83·2	0·267	3·02	0·057
10. vii. 39	5	771	0·600	4·626	62	159	21·1		1·372	0·929	29·7	0·382	3·12	0·090
	5	775	1·000	7·750	62	159	21·1		1·450	0·978	18·7	0·406	3·13	0·093
	4	782	1·390	10·870	62	161	21·1		1·477	0·981	13·6	0·404	3·14	0·093

		Measurements in 0·001 in. of the femoral shaft (Fig. 2)							Breaking stress in bending and twisting calculated from formulae (1), (2), (3) and (4)			
Experiment	% Ca in femur (air dried)	a	b	t	t'	Width of marrow cavity $c=b-2t$	Bending moment M in. lb.	Twisting moment T in. lb.	(1) lb./in.² ×2100	(2) lb./in.² ×990	(3) lb./in.²	(4) lb./in.²
I,	21·4	146·5	120·0	15·3	—	89·4	4·64	2·40	17·8	9·08	—	—
7. vii. 38–	22·4	146·3	118·2	15·9	—	86·4	5·34	2·08	19·8	7·48	—	—
1. ix. 38	23·8	143·8	118·6	15·3	—	88·0	5·15	2·63	19·6	8·61	—	—
	22·8	147·5	118·6	15·5	—	87·6	5·76	2·12	18·3	6·81	—	—
II,	23·0	124·5	102·6	12·6	16·3	77·4	2·99	1·52	17·75	10·24	33,440	8,500
28. x. 38–	23·2	131·8	103·8	17·5	24·2	68·8	3·12	2·24	15·95	8·63	32,000	9,700
23. xii. 38	25·0	128·1	99·1	18·1	21·5	62·9	3·82	2·29	16·87	10·07	38,580	10,200
	25·0	132·1	105·5	18·6	23·5	68·3	4·16	2·43	15·30	9·87	33,820	9,700
III,	23·7	119·9	99·0	13·1	18·7	72·8	2·43	1·75	15·78	11·23	29,080	10,800
11. ii. 39–	22·8	117·5	97·6	13·6	15·9	70·3	2·68	1·79	16·82	11·79	33,370	10,800
8. iv. 39	25·2	115·5	98·8	16·0	20·3	66·9	3·35	2·09	17·30	12·56	36,900	11,700
	23·2	134·3	101·1	18·3	25·1	64·5	4·12	2·22	14·97	9·83	33,210	9,400
IV,	17·5	126·6	104·8	9·8	12·7	85·2	2·24	1·07	17·05	8·67	29,770	7,000
15. v. 39–	21·3	121·6	99·9	12·1	16·8	75·7	2·69	1·48	17·73	10·54	32,680	9,200
10. vii. 39	23·6	132·5	109·3	18·9	22·7	71·4	4·42	2·29	16·69	8·13	36,660	8,600
	22·9	129·6	103·6	18·9	24·4	65·8	4·21	2·07	17·42	7·87	37,840	8,700
	22·3	134·7	103·6	18·6	22·7	66·4	4·24	2·34	17·00	9·04	36,890	9,200

at the lowest levels of intake was somewhat less than at the second lowest level. With increasing intake there is a diminution in the percentage retention of Ca, but the absolute amount of Ca retained increases up to nearly 1·5 g. over 56 days when the food contains 0·36 g. Ca/100 g.; increase of dietary Ca beyond this point produces very little increase in retention. There is, therefore, little doubt that an intake above this amount is a luxus consumption.

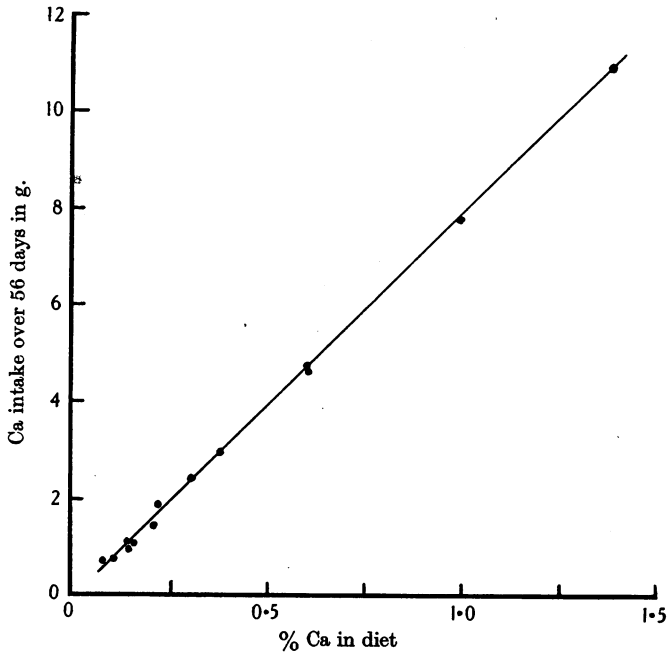

Fig. 6. The relation between the percentage of Ca in the diet and the total Ca intake.

The percentage of Ca in the animal showed the same range of values as found by Fairbanks & Mitchell [1936]—their empty weight is presumably the same as our degutted weight. The figures relating to Ca content of the degutted carcasses show interesting physiological adjustments. When the percentage of Ca in the diet was varied from 0·075 to 0·36—a ratio of 1:5—the amount of Ca in the body increased from about 0·9 to 2·0 g.—a ratio of 1:2; under the same conditions the Ca content of the femur increased in the ratio 1:2. The similarity of these ratios suggests that the Ca was distributed evenly over the skeleton on both high and low diets. The quantity of Ca in the animals at the beginning of the experiment made it necessary for the Ca retention to be trebled

in order to double the actual content of Ca in the body. A further increase in dietary Ca up to 16 times the lowest produced no further change.

Body weight

The gain in body weight is given in Table 1 and Fig. 8. The average rate of growth per day over the entire period was 2·85 g. On the higher Ca intakes the gain in body weight per gram of food eaten tended to be slightly greater than on the low Ca intakes. This is quite clearly shown in the data of Exps. III and IV, but Exps. I and II do not show this tendency. It may be—although the evidence is slight—that on the higher Ca planes body tissue is formed more economically than on the lowest intakes.

Owing to the relatively constant rate of growth of our animals we could not demonstrate the relationship shown to exist by Fairbanks & Mitchell [1936] between the percentage of Ca in the carcass and the average daily gain in body weight for several weeks before killing. Nevertheless, the percentage of Ca in the carcasses of our animals fed on the higher diets was of the same order as that found by these authors.

Skeletal dimensions

Neither restriction of Ca nor luxus consumption of Ca affects appreciably the body length (snout to anus) or the femoral length (head to condyles in the long axis of the bone). There is a slight tendency for the greater transverse diameter (*a* of Fig. 2) of the middle of the shaft of the femur to be greatest in the animals fed on the highest Ca diets. The smaller diameter, *b*, on the other hand, remains remarkably constant in all animals. On the whole the external dimensions of the femur are not altered by varying the Ca intake over the range used in these experiments. The thickness of the femoral shell, *t*, increases with rise in the percentage of Ca in the diet up to about 0·36 %; thereafter it remains practically constant (● in Fig. 7). It will be clearly seen from the column of Table 1 headed 'Width of marrow cavity' that the increase in thickness is achieved at the expense of the marrow cavity. The thickness of the femoral wall measured across the lateral edges of the shaft (*t'* of Fig. 2) is very variable indeed, but in spite of this the same general tendency is seen. There was no cancellous tissue in the middle of the femoral shaft of these animals.

Weight of femur

It will be seen from Fig. 7 that the total Ca content of the femur, the thickness of the femoral shell and the weight of the femur are very closely correlated. This is due to the fact that the bone is of practically constant

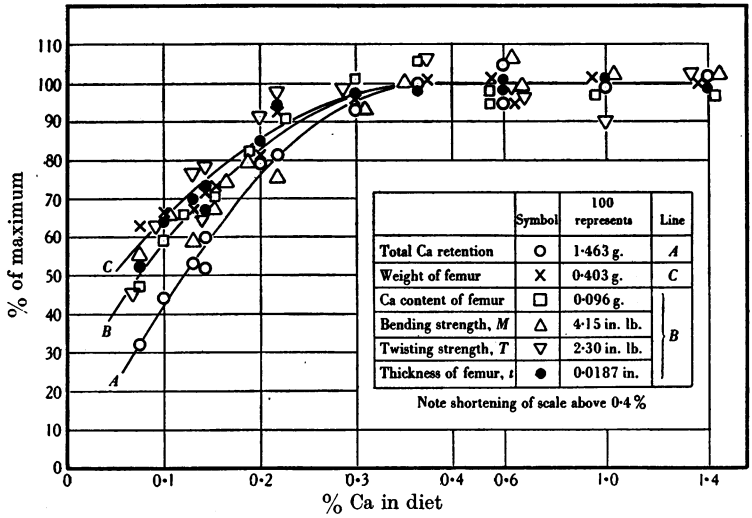

Fig. 7. Various findings, as shown in the panel in the graph, expressed as a percentage of their maximum value plotted against the percentage of Ca in the diet. (Note shortening of the scale above 0·4 %.)

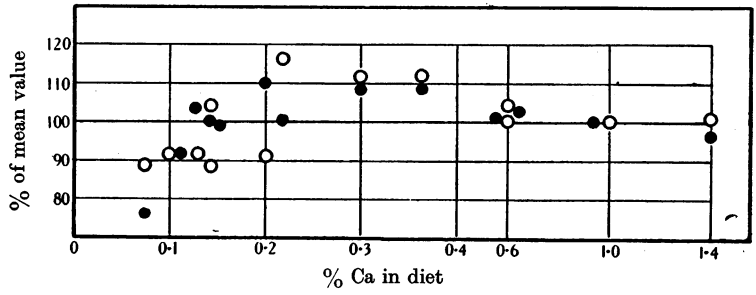

Fig. 8. Increase in weight of the whole animal and percentage of Ca in the whole femur, both expressed as a percentage of the mean value, plotted against percentage of Ca in the diet. Increase in weight, O; 100 represents 159·2 g. Percentage Ca in whole femur, ●; 100 represents 23·0 %.

chemical composition in spite of dietary variations, with the possible exception of that formed under the poorest Ca intake (see Fig. 8). The diminution of the percentage of Ca in these circumstances is most likely due to the greater proportion of periosteal and fibrous tissue on

these very thin bones. It was extremely difficult to remove this fibrous matter without damage or fracture, and thus these very fragile bones may have been less thoroughly cleaned than the sturdier ones. We are inclined to believe that the actual composition of the bony material was constant throughout the experiment.

Skiagrams

The variations in the thickness of the wall of the femoral shafts were clearly demonstrated in the skiagrams. The bones produced on the lowest Ca intake appeared very much less dense and the trabeculae in the epiphyses were much finer than in the bones produced on the higher diets; one would be inclined on the X-ray appearances to describe the former as atrophied or rarefied. The differences in appearance are due solely to the difference in thickness of the wall and not to any qualitative alteration; the terms atrophy and rarefaction should, therefore, be used very guardedly in describing X-ray appearances in bone until it has been definitely proved by some independent method that there is actually some change in the quality of the material.

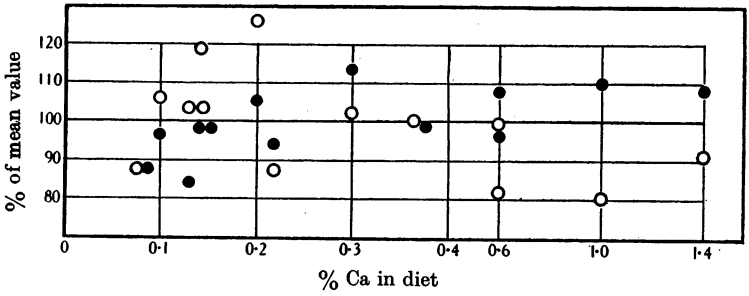

Fig. 9. Breaking stress as percentage of the mean value plotted against percentage of Ca in the diet. Breaking stress in bending, ●; 100 represents 35,000 lb./in.^{2} Breaking stress in twisting, ○; 100 represents 9500 lb./in.^{2}

Strength measurements

The values are given in Table 1, and in Figs. 7 and 9. The strength of the bones as indicated by the bending moment M, and the twisting moment T, increases with increasing Ca intake up to about 0·36 % Ca in the diet; on higher intakes the bones do not become stronger. The meaning of the various formulae used to measure the quality of the bone material has already been explained. The data for evaluation of M/abt are probably the most reliable since the bone broke at the point at which a and b were measured and because t showed less scatter than t';

it has to be noted that formula (3) includes t'. The quality of the bone as indicated by the bending tests seems to be unaffected by the amount of Ca in the diet; the slight tendency to falling off on the lowest Ca intakes is in all probability to be accounted for in the same way as the falling off in the percentage of Ca in the bones produced on the lowest diets. Formulae (2) and (4), for calculating breaking stress in twisting, are probably less satisfactory than the others because of the impossibility of measuring the bone at the actual site of the fracture owing to the spiral character of the break; there is a greater scatter of the results than in the bending tests but there is no indication that the quality is affected by the amount of Ca in the food.

When these experiments were planned it was hoped that it would be possible eventually to apply the results to human material; but since the bone quality is unaffected by the level of Ca intake it is obvious that examination of a sample of human bone would give no information of the previous level of dietary Ca. Whether rachitic changes could be measured by the methods used here has still to be investigated.

The average breaking stresses from our tests in bending and twisting are approximately 35,000 and 9500 lb./in.^{2} respectively. Similar tests on specimens of hard wood, cast iron, and mild steel would give values of 10,000, 40,000 and 70,000 for bending and 1000, 24,000 and 40,000 for twisting. Hence bone material is stronger than timber, nearly as strong as cast iron and half as strong as steel. The ratio of breaking stress in twisting to that in bending is of some interest. For isotropic material, such as cast iron and steel, the ratio is slightly more than a half —but for laminated material like wood the ratio is considerably less, about one-tenth; this is due to the weakness of the cement substance between adjacent lignified fibres. Evidently bone material, with a ratio of 0·27, has some such weakness at the interconnexions between the longitudinal fibres.

We have been able to trace only one investigator who has estimated the breaking stress of bone material, Rauber [1876]. His values are on the average lower than ours, 12 kg./mm.^{2} = 17,000 lb./in.^{2}, and are presumably for human material. (It is not to be inferred from this that rat and human bone material are different; the details of the method used by Rauber are not available to us.)

With the actual dimensions and strength of bone material before us it is now possible to consider the ability of a bone to support the body weight when acting as a simple prop. If we take the case of an animal of average weight, say 0·51 lb., on a good Ca diet, which falls to the

ground landing on its hind legs (perhaps a rather remote possibility for a four-footed animal) the impact might increase the weight effect by five times, i.e. to 2·55 lb.; each femur would receive an axial load of 1·3 lb. Taking average dimensions of the shaft section of the bone as 0·120, 0·100 and 0·018 in. for *a*, *b* and *t*, respectively, and the breaking stress of material as 35,000 lb./in.^{2}, we find that the strength of the bone as a simple prop is 180 lb. The bone is thus 140 times stronger than is necessary for this action. Our conclusion is that bones are 'made' not as simple props but rather to withstand the severer actions of bending and twisting.

It is perhaps interesting to speculate on engineering principles whether with the material available the bones could have been better designed to resist bending and twisting. A solid rod constructed out of the same amount of material would certainly be much weaker; and a bone of greater diameter with a correspondingly thinner wall would fracture readily on impact and would necessitate more cumbersome joints and more bulky limbs. The tubular bony shaft of the shape and dimensions actually found in the animal seems to be the best for withstanding the forces likely to be applied. Indeed it would be extraordinary if it were otherwise.

It is hardly necessary to apologize for the use of inches and pounds in an engineering problem in place of the more 'scientific' metric units. 1000 lb./in.^{2} equals 0·703 kg./mm.^{2}; the other conversion factors are well known.

Conclusions

Variation of the Ca intake between the limits used in these experiments did not affect the appetite nor the general condition of the rats, consequently the animals all grew to practically the same length and weight (although there was a tendency noted in some experiments for the gain in weight per gram of food eaten to be greater on the higher diets). The loss of appetite and interference with growth which results from the feeding of diets deficient in certain amino acids and vitamins is not evident in our experiments on Ca deficiency; but the more acute experiments of Kleiber, Boelter & Greenberg [1940] showed that failure of growth and appetite does occur when rats are fed on a diet containing only 10 mg. Ca/100 g. The level of Ca intake in our experiments did not determine the size of the skeleton, as judged from the length of the whole animal or the external dimensions of the femora; it would appear that these are genetically determined—this would account for the discrepancy between the measurements obtained in Exp. I and those of

the other three experiments carried out on a different strain of rats. It is obvious, however, that in a more acute Ca deficiency, e.g. produced by a Ca free diet, the initial skeletal Ca, even when redistributed according to the requirements of growth, would ultimately fail to preserve the external dimensions laid down by heredity and failure of growth would result. If the curves of Fig. 7 are extrapolated to zero percentage of Ca in the diet, the curve marked *A* (total Ca retention) would reach zero retention as would be expected; the curve marked *B* would reach 25 % of the maximum, in these circumstances the femoral wall would be reduced to between 4 and 5 thousandths of an inch, part of which would be periosteal tissue. These bones would have only one-quarter of the strength (by extrapolation) of the best bones of our series, but judging from our experience with the bones produced on the lowest Ca intake it would be almost impossible to dissect them out of the animal without fracture.

In our experiments the differences in Ca intake produced variations in the width of the marrow cavity of the bone while the external dimensions remained the same. As the result of experiments on swine Burnett [1908] concluded that no apparent increase in external measurements of the bones resulted when protein or mineral matter was added to the feed but that 'these additional nutriments...have added to the thickness of the bone walls by accretion on the inner surface of these walls, thereby reducing the marrow in the bone'. Bearing in mind the views on bone formation of Brash [1934] this finding may be expressed in a dynamic form as follows. There are two sources of Ca for the formation of bone, (1) the Ca of the food and (2) Ca reabsorbed from the walls of the medullary cavity. The new bone is deposited during growth on the periphery of the shaft of the bone while the older bone is removed from the interior of the shaft; the salts removed in this way presumably circulate and are deposited on the periphery. Ca storage is not comparable to fat storage. Fat stored is material in excess of the body's immediate metabolic needs and the limit of storage is not definitely set. But increase of Ca intake seems to spare Ca reabsorption (or endogenous Ca metabolism) and does not affect the rate of new bone deposition on the periphery because the external dimensions are unaffected by increased Ca intake and because the material of the bones (on whatever diet) is of the same chemical and physical structure. There is a limit set to this sparing action of high Ca intake, otherwise the marrow cavity would disappear entirely on very high intakes. We have no evidence as to how this control is effected, but it can be grossly upset by large doses

of oestrogens [Gardner & Pfeiffer, 1938], suggesting that it is perhaps the pituitary gland which plays a part in preventing the body using all the Ca which is presented to it. This view of the effect of high Ca intake makes it understandable why Ca is stored as living bone and not in some less elaborate form.

This interpretation of our findings is put forward with a certain diffidence; the proof of this dynamic description is beyond the present experimental technique. Further, it is possible that an animal on a low Ca diet followed by a high Ca diet might lay on bone peripherally as well as centrally provided that it is still growing. This question and others might perhaps be studied by the use of isotopes of Ca and P.

It is natural to enquire if we can state from these experiments what constitutes an adequate ration of Ca for the growing rat. It would appear from Fig. 7 that above an intake of 0·36 g. Ca/100 g. dry matter there is no increase in the size, Ca content or strength of the bones. There is no doubt that, as far as Ca is concerned, an intake above this is a luxus intake; but it may be assumed that an animal can carry out its ordinary activities on lower intakes. On the other hand, a greatly increased Ca intake seems to do no harm because of the limit set to bone formation. If further increase in thickness had occurred it would have provided very little increase in strength at the expense of increase of skeletal weight—although this is not so important to the rat as to man where the skeleton forms a larger fraction of the body weight. When the marrow cavity is greatly encroached on, as in the experiments reported by Gardner & Pfeiffer [1938], the haematopoietic function is interfered with.

Summary

Seventy-seven male albino rats were subjected to diets varying only in respect of their Ca content (and also in P, though to a somewhat different extent) from 0·075 to 1·390 %, this variation being brought about by varying the proportions of $CaHPO_4$ and $BaSO_4$ in the salt mixture.

The reasons for the choice of bending and twisting tests for measuring the strength of bone are given, and the apparatus is described in detail. From the results of these tests the breaking stress, a measure of the quality of the material, can be calculated.

With increase in the percentage of Ca in the diet there was (1) very little change in the total intake of food, with consequently very little variation in gain in body weight in spite of the wide range of Ca intake; (2) a progressive rise in the total Ca retention, in the weight and Ca

content of the femora, in the bending and twisting strengths of the femora, and in the thickness of the femoral shell; maximal values of these were reached on a diet containing 0·36 % Ca and further increase of Ca in the diet produced no further change in these values; (3) no change in body length, the external dimensions of the femora, the percentage of Ca in the femora, the quality of the bony material as revealed by the breaking stress in bending and twisting—the items in this group seem to be determined by heredity.

A tentative theory of endogenous and exogenous Ca metabolism as affecting bone formation is discussed. The terms rarefaction and atrophy should be used to describe X-ray appearances of bone only when there is independent evidence of change in the quality of the bone.

There is no harm done to the rat by a diet containing more than 0·36 % Ca but apparently no advantage; lower values of intake may be quite adequate.

The shape and the size of the bones, as found in the animal, are such that the best use is made of the material available to resist bending and twisting. Bone is elastic up to the moment of breaking. It is nearly as strong as cast iron.

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REFERENCES

Bachmann, G., Haldi, J., Wynn, W. & Ensor, C. [1940]. *J. Nutrit.* **20**, 145.
Becker, R. B. & Neal, W. N. [1931]. *Proc. Amer. Soc. Anim. Prod.* Nov. 1930, p. 81.
Becker, R. B., Neal, W. N. & Shealy, A. L. [1934]. *J. Dairy Sci.* **17**, 1.
Brash, J. C. [1934]. *Edinb. Med. J.* **41**, 305, 363.
Burnett, E. A. [1908]. *Nebr. Bull.* 107, 11, cited by Becker & Neal [1931].
Case, J. [1925]. *The Strength of Materials.* London: Edward Arnold.
de Loureiro [1931]. *Arch. Patol. Lisbon*, **3**, 72.
Disse, J. [1896]. In Bardeleben's *Handbuch der Anatomie des Menschen.* Jena: G. Fischer.
Fairbanks, B. W. & Mitchell, H. H. [1936]. *J. Nutrit.* **11**, 551.
Gardner, W. U. & Pfeiffer, C. A. [1938]. *Proc. Soc. Exp. Biol. Med., N.Y.*, **37**, 678.
Gaunt, W. E., Irving, J. T. & Thomson, W. [1938]. *Brit. med. J.* i, 770.
Kleiber, M., Boelter, M. D. D. & Greenberg, D. M. [1940]. *J. Nutrit.* **19**, 517.
Lindsay, M. K. [1934]. *J. Bone and Joint Surg.* **16**, 162.
Lindsay, M. K. & Howes, E. L. [1931]. *J. Bone and Joint Surg.* **13**, 491.
Maj, G. [1938]. *Boll. Soc. ital. Biol. sper.* **13**, 413.
Orr, J. B., Thomson, W. & Garry, R. C. [1935]. *J. Hyg., Camb.*, **35**, 476.
Rauber [1876]. Cited by Disse [1896].
Shohl, A. T. & Pedley, F. G. [1922]. *J. biol. Chem.* **50**, 537.
Thomson, W. [1936]. *J. Hyg., Camb.*, **36**, 24.