THE STRENGTH AND ELASTICITY OF BONE IN RATS ON A RACHITOGENIC DIET

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Since bone is primarily a supporting and protecting tissue it seems appropriate to investigate its properties in the same way that an engineer measures the characteristics of his particular structural materials, such as iron and steel. The orthopaedic surgeon, in dealing with bone, labours under certain disadvantages as compared with the engineer—he cannot ever gain complete control over living structures: yet at some time a knowledge of the strength and elasticity of bone is likely to be useful as well as interesting. The occurrence of fractures and other sudden changes is probably to be explained on the basis of static properties such as those measured by the engineer. Slower changes such as alterations in shape due to age or disease cannot be explained on this basis because bone, unlike iron, is a living material. The dynamic reactions of living bone have not yet been investigated quantitatively because suitable methods have not been found. In the meantime we must confine ourselves to static tests. The results of some of these tests in various conditions have already been presented (Bell, Cuthbertson and Orr 1941, Bell and Cuthbertson 1943, Bell, Chambers and Dawson 1947). In this paper it is proposed to deal almost entirely with the elastic properties of bone. Very little work has been published on this subject. We ourselves have made a few measurements on sheep bones (Bell and Weir 1949) and a few observations are recorded in *Tabulae Biologicae* (1925). The bones used for the work described in this paper were obtained in the course of experiments described by Bell, Chambers and Dawson (1947). Full details are provided in that paper but a summary of the experiments will be given here.

METHODS

Animals and diet—Male albino rats, after weaning at three weeks, were kept on a full diet until they reached roughly 50 g. in weight. The rats were then divided into three groups. Group R were put on a rachitogenic diet—the Steenbock diet No. 2965 (Steenbock and Black 1925); group N were given this rachitogenic diet with a supplement of vitamin D; group S were given a complete diet—Rowett Institute Stock diet (Thomson 1936). Litter mates were distributed among the three groups. The animals were killed after a period of about one month, the actual period of feeding varying from twenty-seven to thirty-six days. The femora were dissected out, cleaned carefully, and allowed to dry under ordinary room conditions before being subjected to the bending tests described below.

Bending tests—The method of carrying out a bending test, and the reasons for choosing it, are given by Bell, Cuthbertson and Orr (1941). It reproduces experimentally one of the strains to which living bones may be subjected by loads or muscular action. Moreover the bending test is a convenient method of applying a large stress (force per unit area) which at the same time gives an easily observable deformation (strain). Engineers have found it to be one of the most reliable tests of strength of a material.

The method of carrying out the test is indicated in Figure 1. The femur is laid on two supports C, C'. It is loaded at the middle by a hook D and pan carrying weights W. Semi-cylindrical end-pieces A, A' of a hard resinous substance, cast on the ends of the bone, allow an accurate measurement of the span l , and prevent axial rotation. The load W is increased gradually until the bone breaks. As the bone is loaded the centre point of the shaft sags and the deflexion y is measured on a dial gauge DG resting lightly on the hook. The external breadth B, and depth D, of the shaft at the mid-point of the bone (Fig. 2) are taken before the test. The corresponding internal dimensions b and d are obtained by subtracting the thickness of the bone-wall as measured at the fracture site.

The bending moment M , which measures the bending force on the bone as a whole, depends on the span as well as the load. At the centre of the span it is given by $M = \frac{Wl}{4}$. When the bone is loaded the upper surface is compressed and becomes concave, and the "fibres" of the lower surface are in tension and become stretched. For any given load the stress, or force per unit area on the bone substance, is greatest in the outer "fibres" at the centre of the shaft where the load is applied. It diminishes towards the ends. This value is given by:—

$$\text{Stress} = \frac{MD}{2I} \text{ or } \frac{WlD}{8I} \dots\dots\dots (1)$$

where I is the moment of inertia of the cross-section of the bone at the site of fracture. Since the cross-section is nearly elliptical, I is given by

$$I = \frac{\pi}{64} (BD^3 - bd^3) \dots\dots\dots (2)$$

As the load is increased, the stress at the centre increases until it reaches a maximum value at which the bone breaks. This maximum value is called the breaking stress on bending, and it is denoted by S_B .

The change in length per unit-length is called strain. Like the stress it is greatest in the outer "fibres" at the mid-point of the span, where it is given by

$$\text{Strain} = \frac{6yD}{l^2 \left\{ 1 - 8\left(\frac{c}{l}\right)^3 \right\}} \dots\dots\dots (3)$$

The stiffness, or resistance to deflexion, of a bone depends on the size and shape of the cross-section and on the elastic quality of the bone substance. Young's modulus of elasticity, denoted by E , is a measure of the stiffness of the bone material. The stiffness of the bone as a whole is proportional to EI , and the flexibility or ease of bending is proportional to $\frac{1}{EI}$. The accepted formula for E , for a beam loaded at the centre, is $E = \frac{Wl^3}{48Iy}$ where I is the moment of inertia of the cross-section of the beam and y is the deflexion or sag at the centre. This formula is applicable only if the cross-section of the beam between the supports is constant but it can be applied, with slight modification, to the rat femur which is in fact somewhat thicker towards the ends. If a length c , at each end of the bone, is so supported by the semi-cylindrical end-pieces, or is so stiff as not to be bent

FIG. 1

Apparatus for measuring elasticity and bending strength of bones. B, bone. A, A', semi-cylindrical ends cast on to bone ends. C, C', supports. D, hook covered by layer of fibre F. W, load. DG, dial gauge. l , span.

FIG. 2

Cross section of rat femur at the mid-point of the shaft to indicate the measurements taken.

FIG. 3

Load-deflexion diagrams for three typical bones, one from each group. Group R = •, group N = o, group S = x.

of the bone shaft was ashed in a crucible at full Bunsen heat. Estimations of ash, Ca and P, were made as already described (Bell *et al.* 1947).

RESULTS

Rats on the rachitogenic diet without supplement, group R, all showed gross evidence of rickets as judged radiographically by widening of the upper tibial epiphysis. In group N, which received vitamin D in addition to the rachitogenic diet, the epiphysal lines were quite narrow. In group S, on a good diet, the femora were nearly twice as heavy as those in group N. Group R bones were about 20 per cent. lighter than group N bones. The average percentage ash content of the femora is shown in Table I. The individual results are plotted in Figures 5 and 6. As diet improved the ash content of the bones increased. The average length of group R femora was 2.0 cm.; of group N, 2.4 cm.; and of group S, 2.7 cm. The scatter of results tended to be greater in group R, because the small size of the bones made measurements more difficult.

The load-deflexion diagrams for three representative bones, one from each group, are given in Figure 3. The curve for group S bone is very nearly a straight line, except for the last quarter when the deflexion increases rather more rapidly than the load. In the N and R specimens there is greater deviation from a straight line. Over the straight part of each curve the deflexion is directly proportional to the load; the gradient of the curve indicates the stiffness (or resistance to deflexion) of the bone as a whole. Since the bones are of different sizes these graphs cannot be compared directly and they give no indication

appreciably when the bone is loaded, then a small "end-correction" can be made and the formula becomes:—

$$E = \frac{wl^3}{48I_y} \left(1 - 8\left(\frac{c}{l}\right)^3 \right) \dots (4)$$

Even in an extreme case, where c is $\frac{l}{4}$ and all the bending takes place in the middle half of the shaft of the bone, the correction is only 12.5 per cent. Thus, when correction for the part of the bone embedded in the semi-cylindrical end-pieces is made, there can be only a small residual error due to increase in the size of the shaft towards the ends.

Chemical methods—After completion of the mechanical tests some of the femoral fragments were retained for X-ray crystallography. The rest

FIG. 4

Stress-strain diagram of the three bones of which the load-deflexion curves are given in Fig. 3. Group R = •, group N = o, group S = x. The large symbols are placed at the average values for each group of the stress and strain at the elastic limit and at the point of rupture.

of the quality of the material from which these bones are built. Direct comparisons of the bone material can however be made from stress-strain diagrams. Stress in the outer fibres is given by formula 1, and the strain by formula 3. These values have been calculated and are plotted in the stress-strain diagram Figure 4. The tangent of the angle made by the straight part of each curve (Fig. 4), and the x axis, is E or Young's modulus. The point at which the gradient begins to diminish indicates the upper limit of elasticity, beyond which plastic deformation occurs and ends in rupture. The breaking point gives the stress and strain at which the bone begins to break.

TABLE I
CHEMICAL AND PHYSICAL PROPERTIES OF BONES FROM RATS

R on a rachitogenic diet; N on a rachitogenic diet supplemented with vitamin D; and S on a complete diet

Group	No. of animals	Average ash per cent.	Average S_B 10^3 lb./in. ²	Average E 10^6 lb./in. ²	Average S_B Average $E \times 10^2$	Average $S_B/E \times 10^2$	S.D. of $S_B E \times 10^2$
R	12	37	11.9	0.64	1.85	2.04	0.65
N	19	43	18.9	0.99	1.90	1.91	0.20
S	6	59	26.9	1.56	1.73	1.74	0.11

TABLE II
PHYSICAL DATA FOR RAT BONES AT ELASTIC LIMIT AND AT RUPTURE

Group	R	N	S
Mean strain at elastic limit per cent.	1.45	1.28	1.39
Mean strain at rupture per cent.	2.41	2.18	1.84
Mean strain at elastic limit as percentage of mean strain at rupture	60	59	75
Mean stress at elastic limit 10^3 lb./in. ²	8.4	12.6	21.2
Mean stress at rupture 10^3 lb./in. ²	11.9	18.9	26.9
Mean stress at elastic limit as percentage of mean stress at rupture	70	67	79

While stress at the elastic limit is greater in bones with the higher ash content, strain is very much the same in all bones (Table II and Fig. 4). At breaking point, the stress is again greater the higher the ash content; but there is a tendency for the strain at rupture to be higher in bones produced on the poorer diets, when the percentage of ash is low. The differences which diet produces in E , S_B , and percentage ash, between the different groups of rats, are highly significant ($P < 0.001$ —Student's t test).

In order to study the relationship between breaking stress, elasticity, and chemical composition of the bone material, we have made three diagrams (Figure 5, 6, 7). Figure 5, already given by Bell, Chambers and Dawson (1947), illustrates the relationship between the breaking stress and the percentage of ash in the bones. The diagram suggests that there is an association between breaking stress and percentage ash. These are intrinsic qualities of bone material, and they are independent of the actual sizes of the individual bones. The scatter diagram relating Young's modulus to percentage ash is very similar (Fig. 6).

The relation between S_B and E was also investigated (Fig. 7). A significant correlation was found within group N and group S taken separately. The combined correlation coefficient for the three groups R, N, and S, even after elimination of the effect of diet, is $+0.63$, which is still highly significant. Elasticity and breaking stress appear to be related more closely to each other than to the percentage of ash in the bone; but this is at least partly due to elimination of a source of variation common to both S_B and E , namely experimental errors in determining the moments of inertia of the very small cross-sections of the bones. Since the value of E is closely and positively correlated with the value of S_B , it is not surprising to find that there is no significant difference in the values of S_B/E in the three groups (Table I). The standard deviation of S_B/E is shown in Table I to give an idea of the scatter.

To test the possibility that rupture of the bone depends on deformation rather than load, we have attempted to estimate the ultimate strain when the bone was beginning to break. The formula for the strain, formula 3, is accurate only to the elastic limit of the material. Although the values obtained beyond this point cannot be regarded as absolute values, it

seems justifiable to use them for comparative purposes. These values, together with the corresponding stress values, are given in Table II.

FIG. 5
This diagram shows the breaking stress S_B plotted against percentage ash in the bone. Group R = •, group N = o, group S = x. Each point refers to one bone.

CONCLUSIONS

One of the aims of this work was to find criteria by which the quality of bone as a supporting tissue might be judged. This inevitably involves discussion and, if possible, assessment, of the relative importance of the inorganic and organic material of the bone. It is relatively easy to measure the mineral content, and for that reason it has always received more than its due share of attention.

In the present experiment the composition of the ash of all bones was remarkably constant, with a Ca/P ratio of 2. Furthermore, X-ray crystallography showed that the structure of the inorganic material was

the same in all cases. The great difficulty of measuring variations in the quality of the organic material which is, of course, protein in nature makes it impossible to say how much it influences bone strength. Since at least 40 per cent. of the bone is collagen, either a quantitative or a qualitative alteration might alter bone strength. X-ray crystallography revealed no qualitative differences in the collagen material of bones of the three groups; so that for the present it would seem safer to assume that alterations in the physical properties of the bones are due to variations in the relative proportions of organic and inorganic constituents (Dawson 1946, Bell *et al.* 1947).

These experiments show that the three diets produce highly significant differences in the percentage of ash, in S_B , and in E . It is possible that some variations in the percentage of ash are due to variations in the absolute collagen (weight of collagen in unit volume of bone substance); but the range of variation in the percentage of ash leaves no reasonable doubt that differences in percentage ash between the diet groups are due essentially to differences in absolute ash. Presumably the collagen contributes something to the strength of the bone; but the indications are that it plays a minor part and that the relative weakness and flexibility of rachitic bones is due to decrease in the absolute ash content. Within any

one diet group, the relation between percentage ash and the other two variables, S_B and E , is masked by other sources of variation such as those associated with the many measurements involved; and thus the correlation between percentage ash and S_B , and also between percentage ash and E , is not significant.

At first sight, the scatter diagrams (Figs. 5 and 6) appear to indicate a correlation between ash and S_B , and between ash and E . Closer inspection shows, however, that the apparent trend is due largely to differences between the means of the diet groups, and that the points within any one group show no such obvious trend. Figure 7 shows that the position with regard to correlation between S_B and E is very different. Here there is an obvious trend within each diet group; the amount of scatter is very much less. Calculation shows that, even when the differences between the means of diet groups is excluded, there is still a significant correlation between S_B and E . The question of the correlation between the three variables is discussed more fully in the addendum to this paper.

Although the "goodness" of a bone is usually judged by its breaking stress, the experimental findings recorded above suggest that it may be assessed equally well on the basis of elastic properties as shown by Young's modulus. Normal bones, group S in these experiments, were elastic up to 79 per cent. of their breaking stress (Table II): the poorer bones of groups R and N were, however, only a little inferior in this respect. In some cases there was no apparent deviation of the load-deflexion curve from a straight line until the bone was about to break. Such a curve was published in the first paper of this series (Bell, Cuthbertson and Orr 1941), but in the light of further experience this curve is scarcely typical. The terminal falling over of the curve is illustrated in Figure 4 and is much more marked in the bones of group R.

While stress at the upper limit of elasticity varies over a wide range in the three groups (Table II and Fig. 4), the strain at this point is remarkably constant at about 1.5 per cent. This same percentage displacement must occur between the molecules of the bone material at

FIG. 6

This diagram shows Young's modulus, E , plotted against percentage ash in the bone. Group R = •, group N = o, group S = x. Each point refers to one bone.

FIG. 7

This diagram shows the relationship between breaking stress S_B and Young's modulus E . Group R = •, group N = o, group S = x.

the elastic limit—and it may be that, up to this amount of molecular displacement, the deformation is reversible; but that beyond it, plastic changes occur. We have no evidence as to whether the limiting displacement concerns mineral or protein constituents of the bone, or both.

We have already commented on the remarkable strength of bone material (Bell *et al.*, 1941). The breaking stress of normal rat bone is about the same as that of cast iron, and about half that of mild steel. Young's modulus, however, is only one-tenth that of cast iron and one-twentieth that of steel. Thus bone, despite its lightness (specific gravity about 2.5 as compared with 7.9 for iron), is remarkably strong and at the same time more flexible than might be expected. Presumably the biological advantage is that greater flexibility helps to absorb sudden impacts. It is unusual in metallic substances to find the elastic modulus proportional to the strength; this is more characteristic of materials like concrete and timber. Another remarkable property of bone is that it remains elastic up to three-quarters of the breaking stress. Most metals show considerable ductility before reaching their breaking point.

While Young's modulus is of interest, both on its own account and as an index of the quality of the bone, its close association with breaking stress suggests that it might be used to predict the maximum load which a bone can carry safely. Since E , unlike S_B , can be measured without damage, useful information might be gained by measuring the elasticity of living human bones.

ADDENDUM

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During analysis of the data presented in this paper an interesting observation was made of the effect of the combination of groups of results on the overall correlation coefficient. If the three groups of observation—R, N and S—are considered, it is found that there is no significant correlation between ash and S_B , or between ash and E , in any one separate group. If the groups were simple replicates of the same experiment, with all variables other than the two under investigation controlled, then combination of the data would be legitimate, and the overall coefficient might reach a significant level owing to the larger number of observations concerned. In this investigation, however, the three groups are not simple replicates of the same experiment but are in fact three different dietary groups. Thus an additional variable, namely diet, has been introduced between the groups.

It might be argued that diet has an effect upon only one of the variables concerned—for example ash—and does not change any other property of the bone. This, however, is not known for certain. The diet might, for example, affect the quality of collagen of the bones as well as their ash content. In this way elasticity and breaking stress could vary independently of the ash content. In the absence of any information on this question it seems highly undesirable to combine the data from the three groups without eliminating the effect of differences in diet.

Statistical calculations—The total number of pairs of observations is, in each case, thirty-seven. A combined correlation coefficient may be calculated from these figures which is appropriate to the thirty-six degrees of freedom between them. It will be seen, however, that of these thirty-six degrees of freedom, two are attributable to differences between the means of the dietary groups, and only the remaining thirty-four to total correlation when these differences are eliminated. The total correlation on thirty-four degrees of freedom is readily obtained by summing the sums of squares used for the calculation of the correlation coefficients for each group separately, the degrees of freedom associated being eighteen in group N, five in group S, and eleven in group R. This gives a total as before of thirty-four. The results of these calculations are summarized in Table III. It will be seen that there is always a highly significant correlation when the effect of diet is not eliminated, but that only S_B and E are significantly related when this correction is made.

Conclusion—These results are of general interest in that combination of data such as these may often be carried out without appropriate corrections and thus high and misleading values may be obtained for the correlation coefficient.