

## THE EFFECT OF POROSITY AND MINERAL CONTENT ON THE YOUNG'S MODULUS OF ELASTICITY OF COMPACT BONE

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**Abstract**—The Young's modulus of elasticity, the calcium content and the volume fraction ( $1 - \text{porosity}$ ) of 23 tension specimens and 80 bending specimens, taken from compact bone of 18 species of mammal, bird and reptile, were determined. There was a strong positive relationship between Young's modulus and both calcium content and volume fraction. A power law model fits the data better than a linear model. Young's modulus has a roughly cubic relationship with both calcium content and volume fraction. Over 80% of the total variation in Young's modulus in this data set is explained by these two variables.

### INTRODUCTION

The Young's modulus of bone is well known to be reduced by porosity. Carter and Hayes (1977), analysing both cancellous and compact bone, found that Young's modulus was proportional to the third power of the density (which they assumed to be the inverse of porosity). Others have found the exponent to be lower. Gibson (1985) has surveyed these relationships. There is evidence that the Young's modulus of bone is also strongly dependent on the mineral content of the bone material (Currey, 1975, 1987). Of course the mineral content of bone *tissue* is, other things being equal, inversely proportional to the porosity, but there is no particular reason to suppose that the mineral content of the bone *material* itself should be a function of porosity. This paper investigates the effects of both these explanatory variables on Young's modulus.

### MATERIALS AND METHODS

The bone specimens were taken from a variety of species, and were chosen with a view to maximising the spread in the values for Young's modulus without, however, using macroscopically cancellous bone. No specimens with a porosity greater than 40% are included, and only two with a porosity greater than 30%. Nearly all the specimens were taken from the wall of the shaft of long bones. The provenance of the specimens is given in Table 1. The bones from which some specimens were taken had been allowed to dry out for some considerable time before the specimens were prepared; others had been kept deep-frozen for some time; a few had been tested quite soon after death, but these were always deep-frozen at some stage. All specimens were thoroughly wetted before testing.

There is a copious literature on the effect of drying on bone (e.g. Evans, 1973; Sedlin and Hirsch, 1966). It has marked effects: its modulus of elasticity is in-

creased and it becomes much less tough. There seems to be little published work on whether re-wetting bone restores its pristine properties. Sedlin (1965) implies that in general bone seems to be rather unaffected by slow drying, as long as it is made thoroughly wet again before testing, but reference to his sources (Amprino, 1958; Smith and Walmsley, 1959) shows they carried out only very small-scale experiments. I evaluate this question below, statistically. There is considerable evidence (Evans, 1973) that freezing bone does not alter the mechanical properties of bone, after it has been thawed, to any considerable extent. Freeze-drying, on the other hand, has disastrous effects on strength (Pelker *et al.*, 1984).

The specimens were roughly machined with a band-saw. Tensile specimens were then shaped using a milling head guided by a pre-machined pattern. All specimens were then smoothed with increasingly fine carborundum paper. They were kept wet at all times during machining, and were not allowed to become hot. The final stage of polishing was with No. 400 grade carborundum paper.

#### *Young's modulus of elasticity*

In order to produce two more or less independent measures of the relationships between Young's modulus and the explanatory variables, the modulus of elasticity was tested in both tension and in bending. The two modes of testing needed different specimen shapes. The specimens came from the same bones.

The 80 bending specimens were loaded in three-point bending in an Instron 1122 table testing machine. The gauge length was 30 mm, and the specimens were 3.5 mm wide and 2.0 mm deep. They were cut so that their long axis was parallel to the long axis of the bone from which they came. Some specimens were not taken from long bones. In some of these: the antlers, the frontals and the ribs, the longitudinal axis of the bone was obvious. However, the bulla is shaped like a slightly-opened fist, and although specimens were taken parallel to the longest axis of the fist, it could

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Table 1. Sample sizes, means of Young's modulus (in GPa), calcium content (in  $\text{mg g}^{-1}$ ) and volume fraction, and their standard deviations. 'State': condition of the specimen; 'F': had been deep frozen; 'D': had dried out at some stage

Species	State	n	Bending		Ca	S.D.	Vf	S.D.
			E	S.D.				
Alligator Femur	F	6	12.02	2.42	252.9	13.15	0.906	0.038
Atlantic whale Rib, posterior	F	4	10.36	0.90	241.1	11.19	0.828	0.034
Atlantic whale Rib, anterior	F	4	8.80	1.86	251.8	12.99	0.767	0.062
Cow Femur, tibia	F	7	18.49	2.84	296.8	14.72	0.948	0.015
Crocodile Frontal	D	2	5.63	1.17	250.7	10.27	0.938	0.046
Donkey Radius	F	4	17.57	2.01	247.6	8.61	0.922	0.080
Fallow deer Tibia	F	4	26.84	3.68	274.1	8.25	0.962	0.007
Fin whale Bulla, white part	F	4	26.75	1.66	311.4	10.33	0.960	0.009
Fin whale Bulla, yellow part	F	3	33.65	0.547	309.1	15.53	0.981	0.005
Galapagos tortoise Femur, fibula, humerus	F	5	10.03	1.97	226.1	15.31	0.886	0.068
Grey seal Femur, humerus	F	4	16.72	1.96	270.0	6.18	0.946	0.011
Horse Femur	F	4	21.22	1.91	267.8	8.83	0.942	0.012
King penguin Humerus	F	2	19.47	0.20	270.1	1.68	0.906	0.011
Muntjac deer Antler	D	1	11.44	—	226.8	—	0.898	—
Sheep Metacarpus	F	5	18.95	2.21	251.3	7.25	0.952	0.021
Red deer Antler	D	8	6.71	5.02	208.3	19.10	0.807	0.095
Reindeer Antler	D	4	6.41	1.65	225.3	11.59	0.766	0.094
Roe deer Femur	F	2	20.21	0.20	280.4	2.89	0.894	0.038
Roe deer Antler	D	1	2.23	—	174.3	—	0.693	—
Wallaby Femur, tibia	F	4	18.70	2.68	274.1	6.02	0.955	0.011
Blackfooted penguin Radius, humerus	F	2	19.72	3.84	262.1	17.33	0.933	0.009

Species	State	n	Tension		Ca	S.D.	Vf	S.D.
			E	S.D.				
Alligator Femur	F	1	14.09	—	250.5	—	0.943	—
Atlantic whale Rib	F	1	8.41	—	260.0	—	0.690	—
Cow Femur	F	1	31.33	—	272.6	—	0.956	—
Donkey Radius	F	1	15.88	—	256.3	—	0.934	—
Fallow deer Tibia	F	1	28.25	—	255.7	—	0.976	—
Fin whale Bulla, yellow part	F	2	34.06	1.87	315.3	12.47	0.964	0.011
Galapagos tortoise Femur and fibula	F	4	13.78	2.45	244.6	16.79	0.942	0.015
Horse Femur	F	1	25.67	—	268.9	—	0.967	—

Table 1. (Contd.)

Species	State	n	Tension		Ca	S.D.	Vf	S.D.
			E	S.D.				
King penguin Humerus	F	1	22.13	—	281.8	—	0.911	—
Red deer Antler	D	6	7.27	3.92	211.5	9.71	0.866	0.072
Reindeer Antler	D	2	6.48	0.95	212.8	18.85	0.824	0.023
Roe deer Femur	F	1	19.05	—	252.1	—	0.953	—
Wallaby Femur	F	1	19.34	—	288.2	—	0.968	—

hardly be said that they came from the 'long axis' of the bone.

The greatest movement of the crosshead was about 0.15 mm, and in the stiffest specimens this produced a load of about 15 N. At this load the nominal machine and load cell deflections would produce a reduction in the calculated value of Young's modulus of at most 1%. The local deflections of the specimens under the loading points must also have been very small at such a small load. Overall, therefore, the calculated values of Young's modulus will be in error because of machine and specimen deflections to a trivial extent compared with the range of Young's modulus observed in these specimens.

The same specimens were later tested to destruction in various ways. These results will be reported elsewhere. In these latter tests we observed the deflection at which the trace departed noticeably from the linear; this was at about 0.6 mm. Therefore, the deflections imposed during Young's modulus tests, of about 0.15 mm, would not have damaged the specimens. The rate of descent of the crosshead of the testing machine was adjusted so that the time required to perform the test was 20–30 s. In a bending test the strains, and therefore the strain rates, vary throughout the specimen. The deformation rate used here produced a maximum strain rate in the specimen of about  $10^{-3} \text{ s}^{-1}$ .

The 23 tension specimens were prepared as described by Currey (1975). Briefly, waisted specimens were produced which were attached to the testing machine by pins inserted through oversized holes in the expanded ends of the specimens. A few specimens, from bones that experience showed were very weak, were clamped at the expanded ends, instead of being pinned. All these specimens had a low Young's modulus and could accommodate any slight misalignment of the clamps without suffering significant adventitious stress. The central section of uniform cross-sectional shape was 13 mm long, and had a cross-section of 1.8 mm by 1.8 mm. To this uniform section was attached an extensometer (Instron G5116M or G5117M depending on the extension to break expected). The crosshead moved to produce a strain rate of about  $0.2 \text{ s}^{-1}$ . This produced fracture in about 0.2 s.

This loading rate was considerably greater than for the bending specimens. This might be expected to produce slightly higher values for the modulus of elasticity (Carter and Caler, 1983; Currey, 1986). The output from the load cell and the extensometer went to a Tektronix D13 storage oscilloscope. Photographs were taken of the screen, and data reduced from the photographs. With such a mode of testing, in which extension is measured directly by an extensometer, machine compliance is not important. The specimens were kept wet at all times during testing.

#### Calcium content

The calcium content of the specimens was estimated using a colorimetric method. Two or three thin slices of bone, together weighing between 0.01 g and 0.015 g, were cut from just behind the fracture surface. They were shaken for about a day in a great excess of a mixture of xylene, chloroform and ethanol, in proportions 1:4.5:4.5, to remove fat. They were dried at 60–70°C for 30 min. They were crushed thoroughly in a pestle and mortar consisting of a cylindrical hemispherical-headed plunger fitted into a cylindrical hemispherical-headed cavity. The plunger was forced into the cavity by means of a vice. The fragments were weighed and the mineral dissolved for 24 h in 2 ml of normal HCl. The solution was then made slightly alkaline with normal NaOH, made up to 50 ml, and 5 aliquots tested for calcium using o-cresolphthalein, which gives a violet complex with calcium (Sarkhar and Chauhan, 1967). As will be emphasised below, this process gives an estimate of the calcium content of dry bone; no attempt was made to measure the water content of bone.

#### Porosity

A section, about 70  $\mu\text{m}$  thick, was taken from just behind the fracture surface. The section was stained with fast green to enhance the visibility of cavities. An estimate of the porosity of the specimen was made using a point-counting technique with a camera lucida. In each section 4356 points were scored as either lying over a cavity or not. 'Cavities' included blood channels and erosion cavities, but not osteocyte lacunae or canaliculi. This method of estimating porosity necess-

arily has an element of subjectivity. However, the estimates were reviewed by different observers, and the principal observer (who was responsible for all the estimates reported here) repeated her observations on some specimens months later. The repeatability was good ( $r > 0.98$ ,  $n = 11$ ). Porosity focusses attention on the holes in the bone, whereas Young's modulus is presumably a function of the amount of bone material. 'Vf' (volume fraction), as reported here, is  $(1 - \text{porosity})$ .

## RESULTS

The relationship in bending specimens between Young's modulus and calcium is shown in Fig. 1, and that between Young's modulus and Vf in Fig. 2. It is clear that there is a very strong relationship between Young's modulus and each of the two explanatory variables: calcium content (hereafter called 'calcium') and Vf. The pattern for the tensile specimens is very similar. Unfortunately, for modelling purposes, it is also true that there is a reasonably strong positive correlation between the two explanatory variables themselves ( $r = 0.69$  for the bending specimens, 0.60 for the tension specimens).

### Equations with one explanatory variable

It is usually good practice to start analysing a data set such as this to see how well it can be modelled by

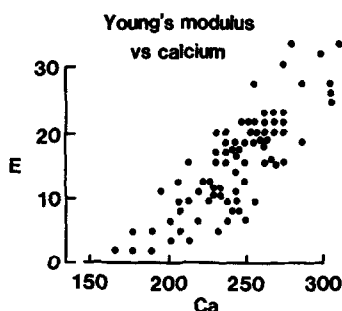


Fig. 1. Relationship, in bending specimens, between Young's modulus ( $E$ , in GPa) and calcium content ( $Ca$ , in  $\text{mg g}^{-1}$  dry mass of bone). Note that the abscissa does not start at zero.

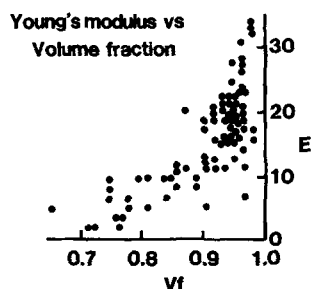


Fig. 2. Relationship in bending specimens between Young's modulus (in GPa) and volume fraction ( $Vf$ ).

a linear equation of the form:

$$Y = a + bX_1 + cX_2, \text{ etc.},$$

where  $X_1, X_2$ , etc. are different explanatory variables. It is good practice because the linear model makes the simplest assumptions about the relationships. However, it is also good practice to explore the possibility that other models may explain the data better in a statistical sense, and also make more sense biologically.

The equations derived from these data are all shown in Table 2. The linear equations, using only one explanatory variable, are numbers (1)–(4). The coefficients of the equations are quite similar, showing there is little difference in behaviour between the bending specimens and the tension specimens. As is shown by the values for  $R^2$ , these linear equations explain a reasonable amount of the total variance in Young's modulus. However, we now consider whether other models fit the data better. In particular I shall examine the power law equation of the form

$$E = aX_1^b \times X_2^c.$$

There are very good *a priori* reasons for supposing that a power law will be a better model than a linear one.

(a) Young's modulus has been shown by Carter and Hayes (1976) and by Gibson (1985) to have a power law relationship with volume fraction, admittedly at rather high porosities.

(b) It is a general finding that composites show a power law relationship between Young's modulus and fibre volume fraction, which is the equivalent of mineralization in bone (Christensen, 1983; Ishai and Cohen, 1967).

Equations of this form can be linearised by taking logarithms of the data values. Standard linear least squares regressions can then be calculated. If  $E = a \times \text{calcium}^b$ , then  $\log E = \log a + b \log \text{calcium}$ , which is a linear equation. Equations (5) and (6) show the results for bending, equations (7) and (8) the results for tension.

The power law equations for the bending and the tension specimens are quite similar, as was the case for the linear equations. It is not clear, however, that the power law equations produce a markedly better fit to the data than do the linear equations. Except in the case of equations (3) vs (7), the values of  $t$  and  $R^2$  are higher in the power law equations than in the corresponding linear equations. However, the differences are small, and certainly would not warrant the rejection of the the simpler model in favour of a somewhat more complex one.

### Equations with two explanatory variables

We have, however, more information than is contained in these eight equations, because we have both calcium and Vf values for each specimen, and we can calculate equations that take account of both. Again, we can compare the values obtained for linear equa-

Table 2. Equations of relationships between Young's modulus and various explanatory variables

Linear, bending, one explanatory variable		
(1)	$E = -35.9 + 0.204 \text{ calcium}$ ( $t = 12.9$ )	( $R^2 = 0.68$ )
(2)	$E = -48.0 + 71.0 Vf$ ( $t = 10.1$ )	( $R^2 = 0.57$ )
Linear, tension, one explanatory variable		
(3)	$E = -44.4 + 0.244 \text{ calcium}$ ( $t = 7.5$ )	( $R^2 = 0.73$ )
(4)	$E = -60.9 + 84.7 Vf$ ( $t = 4.2$ )	( $R^2 = 0.46$ )
Power law, bending, one explanatory variable		
(5)	$\log E = -8.83 + 4.15 \log \text{ calcium}$ ( $t = 13.3$ )	( $R^2 = 0.70$ )
(6)	$\log E = 1.39 + 5.34 \log Vf$ ( $t = 12.9$ )	( $R^2 = 0.68$ )
Power law, tension, one explanatory variable		
(7)	$\log E = -9.16 + 4.30 \log \text{ calcium}$ ( $t = 6.9$ )	( $R^2 = 0.69$ )
(8)	$\log E = 1.37 + 5.74 \log Vf$ ( $t = 5.7$ )	( $R^2 = 0.56$ )
Linear, bending, two explanatory variables		
(9)	$E = -52.3 + 0.143 \text{ calcium} + 35.3 Vf$ ( $t = 7.90$ )      ( $t = 5.10$ )	( $R^2 = 0.76$ )
Power law, bending, two explanatory variables		
(10)	$\log E = -4.84 + 2.55 \log \text{ calcium} + 3.13 \log Vf$ ( $t = 8.07$ )      ( $t = 7.63$ )	( $R^2 = 0.83$ )
Linear, tension, two explanatory variables		
(11)	$E = -71.2 + 0.197 \text{ calcium} + 42.5 Vf$ ( $t = 6.24$ )      ( $t = 3.08$ )	( $R^2 = 0.82$ )
Power law, tension, two explanatory variables		
(12)	$\log E = -6.30 + 3.17 \log \text{ calcium} + 3.52 \log Vf$ ( $t = 6.37$ )      ( $t = 4.79$ )	( $R^2 = 0.86$ )
Linear, bending and tension, two explanatory variables		
(13)	$E = -56.0 + 0.156 \text{ calcium} + 36.1 Vf$ ( $t = 9.95$ )      ( $t = 5.85$ )	( $R^2 = 0.76$ )
Power law, bending and tension, two explanatory variables		
(14)	$\log E = -5.22 + 2.71 \log \text{ calcium} + 3.16 \log Vf$ ( $t = 10.35$ )      ( $t = 8.99$ )	( $R^2 = 0.83$ )
Linear, bending and tension, two explanatory variables, no crocodile frontals		
(15)	$E = -57.0 + 0.152 \text{ calcium} + 38.5 Vf$ ( $t = 10.52$ )      ( $t = 6.74$ )	( $R^2 = 0.80$ )
Power law, bending and tension, two explanatory variables, no crocodile frontals		
(16)	$\log E = -5.04 + 2.64 \log \text{ calcium} + 3.32 \log Vf$ ( $t = 11.90$ )      ( $t = 11.12$ )	( $R^2 = 0.88$ )

tions and power law equations. Using forward stepwise regression we obtain equations (9)–(12).

When a second explanatory variable is used in a regression the value of  $R^2$  inevitably increases. In all the equations here the test of the amount of extra

variance explained (Cooper and Weekes, 1983) shows that the second explanatory variable is worth keeping.

Finally, equations (13) and (14) are for the linear and power law models respectively, lumping all the data from both the bending tests and the tensile tests. As

was shown above, the equations for the tension tests and the bending tests are very similar. Combining them together, one can perform a regression on both data sets, using 'mode of testing' as a dummy variable. This analysis showed that neither for the linear equation nor for the power law equation did using the dummy variable add significantly to the amount of variance explained. This shows that, even if testing the bone in different ways does have some effect on measured Young's modulus, (which it surely must have) this effect is a small one, very small compared with the other factors that we have identified as having an important effect on Young's modulus.

I stated above that there seemed to be little published information about whether dried and re-wetted bone behaved like ordinary bone. It is possible to see whether having bone of two different origins in the model is affecting the result by using 'drying' as a dummy variable. As with bending vs tension tests, the analysis showed that this variable had no significant or important effect on the equations. That is to say, adding drying as an extra explanatory variable increased the total amount of variance explained hardly at all, and the exponents for calcium and volume fraction were virtually unaltered. For present purposes, therefore, we can ignore drying as a factor affecting the model.

#### *Distribution of residuals*

Further evaluation of the linear and the power law models can be made by examining the distribution of the residuals of the equations that incorporate both explanatory variables. A regression model predicts a particular value for the explained variable at any particular combination of the explanatory variables. (A residual is the distance between a data point and the model's prediction of the value of that point: the 'fitted' value, for the particular values of the explanatory variables.) In a good model, residuals have various desirable properties (Draper and Smith, 1981). In particular, the values of the residuals should *not* be a function of the fitted values.

In the case of the tension data, with rather small numbers, there was no evidence that the residuals in either the linear or the power law model are badly behaved. However, for the bending data the situation is different. Figures 3 and 4 are the plots for the residuals vs the fitted values. The power law residuals are rather well behaved except that two values seem to be very low. These extreme values are the two specimens of crocodile frontal bone, and suggest that there is something peculiar about this particular bone. It remains to be found out what it is. The linear residuals, unlike the power law residuals, have values that seem to some extent to be a function of the fitted values. At low fitted values the residuals are higher than the mean; at intermediate fitted values they tend to be less than the mean; at high values they are evenly spread.

Since the residuals for the linear equations appear to have a curvilinear relationship with the fitted values we

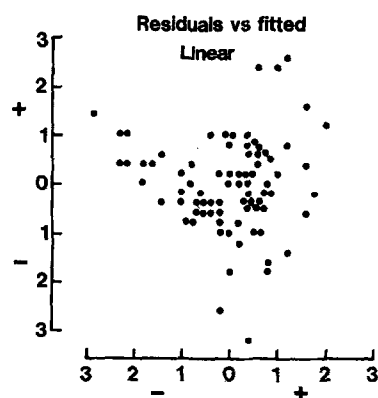


Fig. 3. Distribution of the residuals of equation (9), in which Young's modulus is regressed against calcium content and volume fraction. Ordinate: residual values in units of S.D. from a mean at zero; abscissa: fitted values in units of standard deviations.

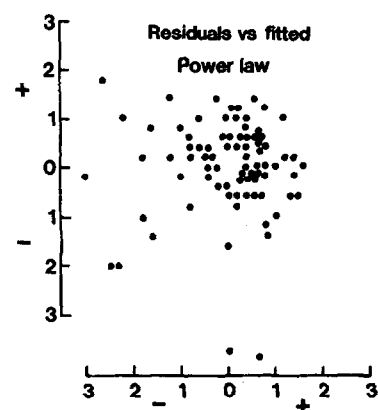


Fig. 4. Distribution of the residuals of equation (10), in which the log of Young's modulus is regressed against the log of calcium content and the log of volume fraction. Ordinate: residual values in units of S.D.; abscissa: fitted values in units of S.D. Note the two very low values, which are the specimens from the frontal bone of the crocodile.

can fit a quadratic equation. (It is inherent in the nature of residuals that the linear equation relating the residuals to the fitted values will have a slope of zero). The quadratic equation turns out to be highly significant ( $r_{78} = 0.35$ ,  $P < 0.01$ ) though the explanatory power is very small. A quadratic equation fitted to the residuals of the power law equations has no significant explanatory effect ( $r_{78} = 0.095$ ).

The examination of the residuals, therefore, by showing that the residuals of the linear equations are a function of the fitted values, while the residuals of the power law equations are not, confirms the impression, derived from the amount of variation explained, that the power law model is a better fit to the data than is the linear model.

#### *Extrapolation*

Finally, we can consider the ability of the two models to predict values outside the range of the data.

This is not perhaps a strong test, because the models are derived from the data only. However, a model which, as well as explaining the data better, is also better at prediction outside the range of data, would seem to be preferable.

First, consider the predictions of the two models when there is no calcium but the material has no porosity. This would, in effect, be tendon. The Young's modulus of tendon is, at most, 2 GPa. The linear model [equations (9) and (11)] predicts values of  $-17.0$  ( $-27.1$ ;  $-6.8$ ) GPa and  $-28.7$  ( $-46.5$ ;  $-10.9$ ) GPa for bending and tension respectively. The values in parentheses are 95% confidence intervals. The linear model does not, therefore, include the correct value within its 95% confidence interval. The power law model, by its nature, predicts a value of zero for both bending and tension. This, although wrong for tendon is, of course, far closer to the correct value.

Next, consider the predictions of the models when the bone has become apatite, with no porosity. With the stoichiometry of apatite, this occurs when the value of calcium is about 400 mg/g. The value of Young's modulus of apatite is about 115 GPa (Katz, 1985). The linear model predicts 40.3 (35.8; 44.9) GPa from the bending data and 50.0 (40.7; 59.3) GPa from the tension data. The power law model [equations (10) and (12)] predicts 62.6 (48.3; 78.8) GPa for bending and 88.8 (54.5; 139.0) GPa for tension. Taking equations (13) and (14), for bending and tension data lumped, the linear model predicts 42.5 (38.4; 46.5) GPa, whereas the power law model predicts 67.9 (54.0; 82.3) GPa. Overall, the predictions of the power law models are somewhat better than those of the linear models, although neither is a good fit at very high mineralisation.

I have also examined the possibility that an additive power law model of the form:

$$E = a + \text{calcium}^b + Vf^c,$$

should be preferred to the multiplicative power law model. Obtaining the least squares fit for this model is awkward, requiring iteration. It turned out that this model produces a lower value of  $R^2$  than the multiplicative model, though not by a large extent. This clearly does not make it a preferable model, though it may be almost as good.

For all these reasons, in the discussion I shall consider mainly the power law model.

## DISCUSSION

### *Correlation of calcium and Vf*

We now consider the biological significance of the power law equations that take account of both calcium and Vf [equations (10) and (12)]. As mentioned above, Vf and calcium are correlated with each other so it might be the case that, for instance, Vf has no effect on Young's modulus, its apparent effect coming from the effect of calcium on Young's modulus, coupled with the correlation of Vf with calcium. It seems, of course,

intuitively probable that both the explanatory variables will have an independent effect on Young's modulus. Indeed, the results of the multiple regression show that calcium and Vf do have distinguishable effects. In the case of the bending specimens, when calcium alone is used as an explanatory variable [equation (5)], 70% of the variance in Young's modulus is explained. When both calcium and Vf are used, the variance explained is increased to 83%. This is a 43% reduction in the unexplained variance, and shows that both the explanatory variables are worth keeping in the model (Cooper and Weekes, 1983, p. 192). The tension specimens are a semi-independent check on this result. (They are only semi-independent because the tension specimens come from the same bones as the bending specimens.) The variance explained increases from 69% [equation (7)] to 86% [equation (12)], a reduction of 55% in the unexplained variance.

Table 1 shows considerable variation in the mean calcium content and Vf of the bone of different species; compare fin whale bulla and cow femur and tibia on one hand, with reindeer antler and roe deer antler on the other. As mentioned above, there is a significant correlation between calcium and Vf. This raises the question of whether the relationship may be to some extent artefactual. Perhaps the slivers of more porous bone, used to calculate calcium content, had more organic material in the pores, which added to the mass, but was not truly part of the bone. One can examine this by looking at bone that takes porosity to an extreme: cancellous bone.

There is rather little information about the constitution of cancellous bone material, but such as there is seems to show that it has a mineral content not much lower than the compact bone material in the same animal. Gong *et al.*, (1964) found that the mineral content of dry cancellous bone, taken from vertebrae, in cows, dogs, humans and monkeys (*Macacus irus*) was about 96% of the mineral content of cortical bone from the femoral shaft. Using the method described above, I compared the calcium content of a specimen of cancellous and a specimen of compact bone from each of the following species: sheep, cow, red deer, fallow deer, donkey, red-necked wallaby and king penguin. The specimens were taken from the ends of long bones. The compact and cancellous specimens came from nearby places in the bone.

I found, in a result similar to that of Gong *et al.*, that the cancellous bone had, on average, 95% of the calcium content of the neighbouring compact bone. There is little evidence therefore that, within a single bone in a species, porous bone of various volume fractions, and compact bone, will be made of bone materials of very different calcium contents, or that porous bone will appear, spuriously, to have low values for calcium. Gong *et al.* did, however, find that cancellous bone contained more water than compact bone. This may well be the case, although it is in fact exceedingly difficult to partition the water in bone into

the different components that may or may not have mechanical effects. However, this difference is irrelevant here, where I am merely trying to show that the considerable differences in the measured calcium contents of the reasonably compact bone specimens discussed in the present studies, from 174 to 311 mg g<sup>-1</sup>, are not artefactual.

#### Volume fraction

Carter and Hayes (1976) suggested that 'all bone can be mechanically viewed as a single material'. By this they meant that the Young's modulus of bone could be related to its porosity by a power law, and that this power law would hold over a whole range of porosities, from very tenuous cancellous bone to fully compact bone. The empirical power law they obtained was cubic:

$$\text{Young's modulus} = k \times Vf^3.$$

There was, in their data, a rather sharp distinction in  $Vf$  between their cancellous and compact bone. The present data to a large extent bridge the gap. The present data also differ from the data used by Carter and Hayes in that they are derived from a much larger number of species, of rather wide taxonomic range and bone type.

The exponents for the effect of volume fraction in equations (6) and (8) (5.3 and 5.7) are clearly higher than those found by Carter and Hayes. However, there is the confounding effect of calcium which, in the present data is positively correlated with  $Vf$ , to consider. If this effect is removed, the exponents fall to 3.1 and 3.5 for the bending and the tension data respectively [equations (10) and (12)] and 3.2 for bending and tension considered together [equation (16)]. This is in good agreement with the findings of Carter and Hayes.

Gibson and Ashby (1982) have proposed analytical solutions, based on scaling arguments, for the  $Vf$ -dependence of Young's modulus. Unfortunately these are not very helpful because the exponent varies between one and three according to the type of structure, and also because these analytical solutions will break down as the  $Vf$  gets very high, as is the case with the specimens examined here.

#### Calcium

As interesting as the effects of  $Vf$  on Young's modulus, which are of the order one might expect, is the clear demonstration of the effect of mineral content, *per se*, on the Young's modulus of compact bone. The bone reported in this study comes from the compacta of long bones, or the equivalent part of other bones, that vary in a number of ways: their function varies, their histology varies, and, of course, their mineral content varies. It is to be expected that an increase in the mineral content of bone should, other things like porosity being equal, produce an increase in the value of Young's modulus, and this is what is found. The power law relationship that best fits the data presented here is approximately cubic.

I mentioned above that the analysis of residuals of the equations using two explanatory variables showed that the two specimens of crocodile frontal were anomalous. This analysis shows the importance of examining residuals. The plots of Young's modulus against calcium content and against volume fraction did not show any points as being conspicuously anomalous. The equations using two explanatory variables considerably increased the amount of variance in Young's modulus explained, and therefore can also be considered to be even more satisfactory. However, the examination of residuals in the multiple regression brings to light the two anomalous points, showing that the model does not take into account something that is important, for these specimens anyhow. There is, however, no *a priori* reason for excluding them, so I have so far kept them in the analysis, despite the fact that they might slightly distort the relationships for the majority of specimens.

Equations (15) and (16) are the equations for both tension and bending combined, but excluding the two crocodile frontal specimens. These equations should be compared with equations (13) and (14). The coefficients are not greatly altered but, as might be expected, the amount of variation left unexplained is reduced: from 24% to 20% for the linear model and, more importantly, from 17% to 12% for the power law model.

I have also tested the robustness of these equations by examining the change caused by the removal of other data points which calculations of Cook's distance (Cook, 1977) showed to be both influential and unusual. In no case did the exponents change much.

It is beyond the scope of this paper to examine what theoretical mechanical models might best explain the relationship between calcium and Young's modulus. However, it is very unlikely that any successful model will be a simple one because, unlike the majority of engineering composite materials, for which most modelling has been done (e.g. Christensen, 1983; Young *et al.*, 1986), bone is a three-phase composite, with the volume fraction of water decreasing as the mineral increases. Furthermore, as McCutchen (1975) and Lees and Davidson (1977) point out, the chemical interactions between the collagen and the mineral may alter as the mineral fraction increases in volume.

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