Comment on "Mechanical Resonance Spectra in Human Cancellous Bone"

Abstract. *The existence of two series of mechanical resonance frequencies in human cancellous bone is apparently supported by other results. This effect may be a microstrain phenomenon since all the experiments involved strains below 10^{-4} at the frequencies of interest.*

In the preceding report, Pugh *et al.* (*1*) describe the observation of sharply frequency dependent reductions in the dynamic mechanical modulus of human cancellous bone. These are identified as consisting of two spectral series, corresponding to the momentum wave mode spectra for atomic calcium and phosphorus, as predicted by the theory of Fitzgerald (*2*). This finding of Pugh *et al.* is apparently supported directly by previously unexplained results of Black and Korostoff (*3*) and Black (*4*) and indirectly by the data of Smith and Keiper (*5*) on human cortical bone.

In a study of eight specimens of human tibial cortical bone, tested at 37.5°C with the force parallel to the major fiber axis, I observed significant reductions in the dynamic mechanical modulus in six cases (*4*). The limiting frequencies between which the minima in the dynamic modulus occurred are given in Table 1. The frequencies I observed were more widely separated than those of Pugh *et al.*, but the data suggest that the resonance occurs at or near the phosphorus frequency $\nu_1^{P} = 203$ hertz reported by Pugh *et al.* This is despite the fact the value of the dynamic modulus at 165.8 hertz varied from 1.16×10^6 to 1.69×10^6 pounds per square inch (psi) (1 psi $\approx$ 52 torr), about 15 times the value reported for cancellous bone. The maximum reduction in modulus was 57 percent, while the strain in this frequency region was between 2×10^{-4} and 5×10^{-4}. The introduction of a reference resonance precluded the observation of ν_1^{Ca}, and ν_2^{Ca} could not be observed because of frequency limitations on the linear variable displacement transducers.

Table 1. Frequencies on the unperturbed dynamic modules curve bracketing minima observed for six specimens of human tibial cortical bone (*4*).

Samples (No.)	Frequency (hertz)	
	Lowest	Highest
2	165.8	206.2
1	165.8	223.6
2	187.2	223.6
1	206.2	239.8

In addition to the work of Jurist (*6*) cited by Pugh *et al.*, the earlier study by Smith and Keiper (*5*) seems to indicate the resonance phenomenon indirectly. In a study of the viscoelastic properties of bone, Smith and Keiper measured the dynamic modulus of human cortical bone in a direction parallel to the fiber and concluded that the elastic component of stiffness, E', for a strain of 10^{-6} was independent of frequency between 500 and 3500 hertz. However, in attempting to measure the viscous component, E'', they observed irregularities which they described as "scatter." In retrospect, I believe that they accidentally observed some of the E'' peaks predicted by Pugh *et al.*, probably those at 812 hertz (ν_2^{P}) and 1827 hertz (ν_3^{P}) on the basis of their relative magnitudes.

In conclusion, I believe that the observations reported by Pugh *et al.* are soundly grounded on previous work and are unlikely to be experimental artifacts. Whether these resonances are the momentum wave effects predicted by the theory of Fitzgerald must remain an open question until experiments can be performed on model lamellar systems of rigidly controlled purity and geometry.

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Pineal Enzymes: Regulation of Avian Melatonin Synthesis

Abstract. *Groups of 8-week-old chickens were killed at six time points in their light-dark cycle, and pineal glands were removed and assayed individually for* N-*acetyltransferase activity, hydroxyindole*-O-*methyltransferase activity, and melatonin content. The tenfold nocturnal rise in melatonin was phased identically with the 27-fold increase in* N-*acetyltransferase activity. The relatively small changes (20 percent) in hydroxyindole*-O-*methyltransferase activity did not appear important in causing the large changes in melatonin. The phase of the rhythms of* N-*acetyltransferase activity and melatonin content in chickens relative to the phase of the light-dark cycle was qualitatively similar to that of rats. In contrast, the sleep-wake cycle of chickens is about 180° out of phase with that of rats.*

Melatonin is synthesized from serotonin by *N*-acetylation, catalyzed by serotonin *N*-acetyltransferase, and *O*-methylation, catalyzed by hydroxyindole-*O*-methyltransferase (HIOMT) (*1*). Daily rhythms in pineal melatonin content, serotonin content, *N*-acetyltransferase activity, and HIOMT activity have been studied in birds; but it has not been clear which enzyme, if either, regulates the rhythms in pineal melatonin and serotonin (*2–5*). Klein and Weller (*6*) presented evidence that the melatonin and serotonin rhythms in the rat pineal gland are regulated by *N*-acetyltransferase activity. We examined the daily rhythms of pineal *N*-acetyltransferase activity, HIOMT activity, and melatonin content in an experiment in which all three determinations were made on homogenates of each pineal gland. In this experiment, *N*-acetyltransferase activity appeared to be a major regulatory factor for the avian melatonin rhythm.

We used 8-week-old chickens (White Leghorn cockerels, *Gallus domesticus*) that had been kept from the day after hatching in a light-dark cycle of 12 hours of light followed by 12 hours of dark (LD 12:12). Groups of six chickens were killed at six time points in this cycle, and the pineal glands were quickly dissected out and frozen. The glands were later thawed and individually homogenized; HIOMT activity, *N*-acetyltransferase activity, and melatonin content were measured in samples of the homogenate (*7*). In a separate series, body weights and pineal weights of eight chickens and eight rats were determined (*8*) to provide a basis for quantitative comparisons of the biochemical data from the two species.