

rate. Some of them have evidently passed a period of flowering during the intervening 19 yr, as shown by the marcescent inflorescence axes hanging down outside the dry leaf cylinder in the specimen at the scale figure and a few others.

Younger specimens may grow slightly faster—the short-stemmed specimen to the left of that measured in Fig. 1B does not appear on the earlier photograph. Presumably it was represented in 1948 by a juvenile plant which was no taller than the surrounding vegetation. Some other “new” specimens not shown in these photographs were even larger. But at any rate the age of a 2 m tall specimen like the one in the foreground of Fig. 1 must be expected to be about a century.

The slow growth rate of these plants may have practical consequences for some authorities in East Africa. The rapid increase in the tourist industry in this part of the world has implied that the number of tourists visiting the high mountains increases for each year. Tourists often like to make fires for cooking and to keep warm, and at high altitudes on these mountains the giant senecios provide the only type of firewood available. Their attractiveness as fuel is enhanced by the fact that their cylinders of marcescent leaves around the stems keep dry in all kinds of weather. But, as I have emphasized, these plants grow very slowly, so it may take more than a century to replace a grove once it has been destroyed. To preserve this “botanical big game” of the high East African mountains for the benefit of future generations, it is therefore desirable to introduce efficient protection measures as soon as possible, especially on such mountains as Kilimanjaro, where giant senecios are already scarce.

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## Effect of Humming on Vision

I WISH to comment on Rushton's article on humming and vision<sup>1</sup>. If a cathode ray oscilloscope (CRO) is operated in a free running sweep mode with a horizontal speed of about 100 cm/s, an observer will see a single horizontal straight line, as expected. If the observer coughs or clears his throat he sees a transient signal. If he sustains a “throat clearing” vibration of about 50–100 Hz, he will observe a steady sinusoidal signal on the CRO. The amplitude of the signal depends on the observer and his vocal intensity, a typical value being 5 mm at a distance of 200 cm. The amplitude increases with distance. Humming does not produce a detectable signal.

I explained this effect by assuming that the eyeballs were vibrating at the driving frequency. A further observation was that the eyeballs vibrated in the sagittal plane, normally up and down, and this can be seen when the observer tilts his head in a lateral direction. The

sinusoidal signal induced by throat clearing takes on the characteristics of a saw-tooth as the tilt is increased and finally becomes an intensity modulated line after a rotation of 90°. This direction may be fixed by the way in which the throat vibrations couple through the head to the eyes, or by some muscular constraint on the eye itself. The latter explanation seems more likely, for when a mechanical vibrator was used to shake the head in various modes, the resulting signals on the CRO were always seen as displacements in the up and down direction (with regard to the head), and lateral displacement could not be induced.

Since reading Rushton's article<sup>1</sup>, I have tried the throat clearing effect on a strobe disk and have noted that here also the polarization effect is clearly visible.

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## Temperature Dependence of the EPR Signal in Tendon Collagen

THE hypothesis of a solid-state control system governing connective tissue growth<sup>1</sup> has led us to study the electron paramagnetic resonance spectra (EPR) of non-irradiated human bone and its components<sup>2–5</sup>. We now report further results involving chiefly human tendon collagen. The tendon was clinically normal peroneus longus, and was studied in the air-dried state. The EPR spectrum of tendon collagen is a single line at  $g = 2.007 \pm 0.006$  having a width between peaks of the derivative of  $10 \pm 1$  G. The line saturates homogeneously with a weak maximum at about 10 dB, and shows no angular dependence. A resonance with the same parameters is found in dog tendon. The intensity of absorption from human tendon collagen corresponds to about  $4\text{--}15 \times 10^{16}$  spins/g. Magnetic field scans from 10–11,000 G at room temperature and at 114° K revealed no other consistently present resonances. At 114° K, the resonance is saturated even at the lowest incident microwave power level.

The trace element content of human tendon was examined by emission spectroscopy to determine the presence of iron group elements (Table 1). Most elements searched for were detected in sufficient amounts to conceivably account for the observed resonance. If it is due to a paramagnetic ion, the number of possibilities is large.

Table 1. IRON GROUP ELEMENTS FOUND IN THE HUMAN TENDON COLLAGEN BY EMISSION SPECTROSCOPY

Element	Concentration
Titanium	N.D. (50)
Vanadium	2.5
Chromium	0.6
Manganese	2.5
Iron	7.0
Cobalt	N.D. (1.5)
Nickel	2.5
Copper	9.5

Results are given in p.p.m. of air-dried tendon to an estimated accuracy of 25 per cent. For those elements not detected, the limits of detection are given. Spectrographic analyses performed by Mr J. A. Spadaro.

The temperature dependence of the EPR absorption of human tendon collagen was determined in the range 21°–95° C in atmospheres of air, pure nitrogen and pure oxygen; the results are given in Figs. 1a, c, e. Because the EPR absorption signal is relatively weak, the data in Fig. 1 were taken by scanning through each point one hundred times and summing with a time averaging

computer. There was a strong decrease in signal in the vicinity of 70° C for all three atmospheres (Fig. 1) and the original signal intensity was not regained when the sample was cooled to room temperature. No change in line width or  $g$  value was seen over the entire temperature range. The same samples were examined at room temperature at intervals of 24 h after the heating and a small daily increase in signal amplitude was seen. At the end of 2 weeks, the original amplitude was regained and the heating experiment was repeated. The results, given in Figs. 1*b*, *d*, *f*, show that the original temperature dependence is again exhibited. The entire experiment was repeated using dog tendon collagen and the results were identical. In none of the heating experiments did the collagen exhibit fibre shrinkage, as judged by visual observation only.

The role of absorbed water in the phenomenon was studied by vacuum drying samples of human tendon collagen (one week, base pressure  $10^{-6}$  torr) and then heating in a dry nitrogen atmosphere. The resulting material showed a temperature independent EPR absorption, the amplitude of which was comparable with the air-dried samples. Two other methods of drying were used: (a) long term storage over anhydrous  $\text{CaCl}_2$ , and (b) immersion in dioxane for 24 h. In both cases, the dehydrated tendon collagen gave a temperature independent EPR absorption.

The hydrothermal shrinkage phenomenon shown by collagen at 70° C suggested a comparison of the EPR intensity before and after shrinkage. Samples of human tendon collagen were immersed in water at 70° C for 10

min, by which time they had obviously undergone thermal shrinkage. After air drying, comparisons of the amplitude of the absorption signal with that from the same sample before shrinkage showed that the resonance had decreased by an average of 50 per cent. Other signal parameters remained the same. Control experiments using water at room temperature showed no change.

The temperature dependence of the collagen singlet may reflect a change at the molecular level which precedes the macroscopic effect of hydrothermal shrinkage; the partial loss of the free spin population thus being a necessary, but not sufficient, condition. The lack of a temperature dependence in dehydrated tendon collagen may indicate that water acts as an activator, lowering the amount of energy necessary for some process to occur, analogous to the role of water in the electrical conduction of proteins<sup>6</sup>. In any event, the relatively small initial spin concentration (roughly one electron/100 molecules of tropocollagen) seems to indicate that the paramagnetic centre is not a structural feature of the tropocollagen molecule.

In summary, air-dried tendon collagen is found to exhibit a narrow isotropic EPR resonance at  $g=2.007$ . The resonance shows an unusual temperature behaviour in the range 21°–90° C in the three atmospheres studied. Neither the species responsible for the resonance nor the mechanism which accounts for the observed thermal behaviour has been identified, although the importance of water and a possible correlation with the hydrothermal shrinkage phenomenon are indicated.

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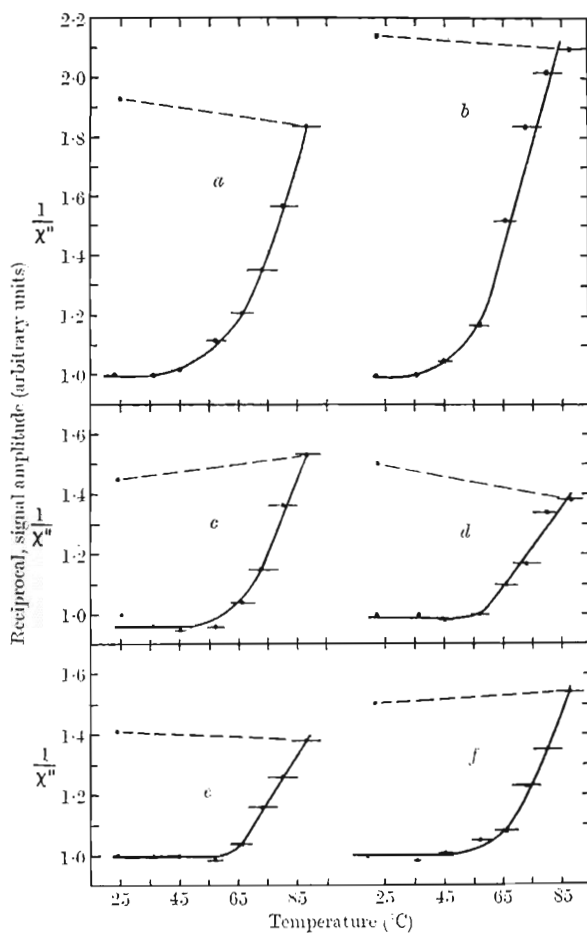


Fig. 1. Temperature dependence of reciprocal of signal amplitude from tendon collagen. *a* and *b*, in air; *c* and *d*, in nitrogen; *e* and *f*, in oxygen. The curves on the left were found initially, the curves on the right after storage for 2 weeks in the indicated atmosphere. Broken line indicates cooling cycle.

## Detection and Investigation of a New Type of ESR Signal characteristic of Some Tumour Tissues

STUDIES of the nature and role of free radicals in normal and pathological processes have been shown to be very important<sup>1</sup>. Experiments with wet, frozen and lyophilized samples have shown that there is a correlation between changes in intensity of the electron spin resonance (ESR) signal and the metabolic behaviour of biological substrates<sup>2</sup>. Clearly such ESR signals are of a biological nature.

Paramagnetic centres representing both free radicals and transition metals, as well as their complexes, have been detected in metabolic systems<sup>1</sup>. The former are characterized by a narrow, slightly asymmetric singlet with a half-width of 7–8 G for a lyophilized sample and 14–15 G for wet and frozen samples, with  $G$  values in each case close to that for pure spin. It is thought that these signals can be accounted for by free radicals of flavins, naphthaquinones, KoQ and other semiquinone compounds which are important in enzyme catalysis.

Paramagnetic complexes of iron, copper, molybdenum and manganese occur in many biological systems. In the