from leucocytes obtained from fourteen patients with CML in remission, however, the results were below the respective normal values (Table 2). A statistical analysis[15] for differences between paired observations revealed that D, the average difference between the normal and CML values, is highly significant. The intervals $1{\cdot}7 \pm 0{\cdot}9$ and $0{\cdot}84 \pm 0{\cdot}3$ are the 95 per cent confidence intervals for the estimate of the average difference of the enzyme activities between the normal and the treated CML patients expressed as units/10^8 leucocytes and units/mg protein, respectively. The 95 per cent confidence intervals for D, between the untreated patients and the normal controls, were $1{\cdot}3 \pm 0{\cdot}9$ for units/10^8 and $1{\cdot}1 \pm 0{\cdot}7$ for units/mg protein. These findings indicate that chemotherapy is not responsible for the low enzyme activity in CML leucocytes.

Table 2. PYRIMIDINE DEOXYRIBOSYLTRANSFERASE ACTIVITY IN EXTRACTS FROM LEUCOCYTES OBTAINED FROM PATIENTS WITH CHRONIC MYELOGENOUS LEUKAEMIA IN REMISSION AND FROM NORMAL CONTROLS

Normal sample No.	Enzyme activity in normals		Mature granulocytes (percentage of total leucocytes)	Patient	Enzyme activity in CML in remission		Mature granulocytes (percentage of total leucocytes)
	u/10^8 leucocytes	u/mg of protein			u/10^8 leucocytes	u/mg of protein	
				Untreated			
1	4·2	3·1	85	D. M.*	1·1	1·9	66
2	2·7	1·2	65	M. S.*	1·3	0·5	85
3	3·6	1·7	81	A. S.	3·6	1·3	60
4	4·9	2·0	45	P. S.	3·6	1·8	50
5	4·9	2·0	50	R. B.	1·6	0·84	60
6	5·0	2·1	44	W. W.	3·4	0·96	83
7	1·4	3·4	56	M. H.*	0·80	0·33	86
8	2·5	2·0	76	G. I.	1·6	0·91	55
				Patients receiving therapy†			
9	2·8	1·3	58	D. M.*	0·92	0·50	75
10	3·5	2·3	69	S. H.	1·5	1·3	78
11	4·2	3·3	84	M. S.*	1·3	2·0	76
12	3·5	2·3	74	E. M.	1·5	2·1	66
13	4·1	3·1	82	J. P.	1·9	2·3	61
14	3·6	1·6	79	J. R.	2·0	0·71	55
15	3·6	1·6	68	M. H.*	1·1	0·40	80
16	3·7	1·7	73	R. S.	1·8	0·76	80
17	2·1	1·8	61	F. J.	1·8	1·4	68
Mean values	3·5	2·2	71		1·8	1·2	70

Assays were carried out as described in Table 1. Each assay of the leucocyte extracts from CML patients was paired with an assay of normal cell extracts. For statistical analysis see text. CML, Chronic myelogenous leukaemia (all were Philadelphia chromosome positive).

* Patients studied twice at intervals of 6 months.

† All patients on therapy had received dibromomannitol at the time of study or within the previous 6 days.

Variations in the assay results in the controls (normal leucocytes) were probably caused by differences in the efficiency of homogenization secondary to variable cell sample sizes. In all cases, however, the samples of normal and CML cells were paired and treated identically.

All bone marrow preparations of the CML patients were positive for the Philadelphia chromosome (Ph^1), an abnormality present in the twenty-first chromosome of the bone marrow myeloid cells in most CML patients[16,17]. There was no clear association, however, of deoxyribosyltransferase deficiency and the Ph^1 chromosome because the enzyme activity of the one patient with CML that returned to normal was also Ph^1 positive. Preliminary results indicate that there was no apparent relationship to leucocyte alkaline phosphatase activity because histochemical measurement[18] of AP in patient G. I. was high but deoxyribosyltransferase activity was approximately 50 per cent of normal.

Because the CML leucocyte still retains significant enzyme activity, it seems that if the defect is genetic it is unlikely that a deletion or insertion mutation is responsible because such alterations should result in the complete absence of enzymatic activity or a markedly defective enzyme. The decrease to approximately 50 per cent of normal is more in keeping with a base pair switch mutation resulting in a partially defective protein.

There is evidence that this protein also contains deoxythymidine phosphorylase activity[18]. Thymine is not only a substrate but at high concentration inhibits the activity of the transferase[10,11]. In addition the enzyme is also subject to inhibition by purine bases[19]. It is possible that in CML leucocytes there is a defect at one of the active sites. An attempt to answer this question by kinetic analysis of the CML leucocyte enzyme is now in progress.

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Mechanically Induced Free Radicals in Bone

THE electron paramagnetic resonance (EPR) spectra of non-irradiated human bone and its components have been previously reported[1,2]. We wish now to report results which have been obtained from human bone at X-band.

The origin and general preparation of both powdered and whole bone samples were as previously described[1,2]. Whole bone samples were $2 \times 2 \times 20$ mm, and had a final weight of about 200 mg. The final weight of bone powder samples was 100 mg.

The EPR spectrum obtained from bone powder at room temperature is a sharp resonance at $g = 2{\cdot}008 \pm 0{\cdot}003$, of width 11 ± 1 gauss measured between peaks of the derivative. Both values are slightly greater than previously reported[2]. The resonance corresponds to a spin concentration of about $1\text{–}4 \times 10^{16}$ spins/g. As the microwave power is varied, the resonance saturates inhomogeneously. Magnetic field scans from 100 to 11,000 gauss revealed no other consistently present resonances. At 114° K, the results were identical with those at room temperature except for an increase in signal intensity.

The room temperature spectrum of whole bone is a singlet at $g = 2{\cdot}008 \pm 0{\cdot}003$, again showing a width of 11 ± 1 gauss. Other resonances, including the previously reported anisotropic resonance at $g = 2{\cdot}3$ (ref. 1), are seen occasionally. They do not seem to be an intrinsic property of the material and will not be considered here.

If the angle between the bone long axis and the external magnetic field was varied, no change in the g-value of the singlet at $g = 2{\cdot}0$ was seen. In addition, for freshly pre-

pared samples the intensity of the whole bone resonance is less than that of the powder by a factor (average) of 11·5. The bone powder resonance decays with a time constant of the order of months; during this time, whole bone resonances show no change. The whole bone resonance saturates homogeneously and shows a weak maximum at about 10 dB below the nominal klystron power output (300 mW). It shows no appreciable change in amplitude as the temperature is lowered to 114° K. At this temperature the weak maximum at 10 dB is still observed.

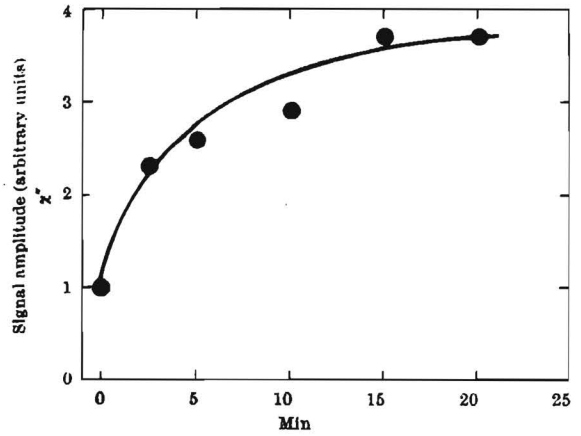

Fig. 1. Accumulation of free radicals in bone powder as a function of time of dispersion. The ordinate is the amplitude of the derivative signal.

There is a series of papers[3-7] describing the production of EPR spectra in a number of polymeric materials by the application of mechanical energy. The mechanical destruction is variously produced by filing or milling the polymer or by dispersing in a vibrating mill. Although the effect is poorly understood, the mechanical destruction is associated with the rupture of molecular chains and the formation of free radicals, including some that are stable indefinitely. Usually materials treated in this manner show EPR absorption in the vicinity of $g = 2{\cdot}0$. In particular, Ulbert[3] has obtained mechanical destruction by filing in atmospheres of air, and nitrogen with 7 per cent oxygen. Abagyan and Butyagin[4] have observed intense EPR doublets from gelatine mechanically dispersed in air and *in vacuo*. To determine if such a mechanism was responsible for the resonance at $g = 2{\cdot}008$ from bone powder, bone powder was dispersed in a vibrating mill for varying lengths of time. The results given in Fig. 1 show the accumulation of free radicals as a function of the time of dispersion. The flat portion of the curve represents the attainment of equilibrium between the rates of free radical production and decay for a given (mechanical) energy input. The last point in Fig. 1 corresponds to a spin density of about 10^{17} spins/g. With the exception of the increase in signal intensity, all signal parameters remained the same. The mechanical disruption of bone powder results in an increase in intensity of the resonance at $g = 2{\cdot}008$. It is therefore likely that the original powder resonance is a result, at least in part, of the method of preparation of the samples, namely, scraping with a glass slide. The reduced intensity of the whole bone resonance might, by itself, reflect less damage produced in the preparation of the samples. The fact, however, that the whole bone resonance does not decay, and that it shows a different saturation behaviour and temperature dependence, leads us to conclude that the resonance arises from a second magnetic species.

In summary, it is likely that two different magnetic species, both at $g = 2{\cdot}0$, are responsible for the resonances from bone, and that their relative contribution to the resonance amplitude depends on whether the sample is examined as a powder or whole. As a powder, a free radical that has been induced by damage gives the dominant contribution masking the presence of a second species. In whole bone samples, the free radical that has been induced by damage may still be present but the second species makes a significant contribution. The assumption of a second species accounts for the observed differences in intensity, stability, saturation behaviour and temperature dependence.

The nature of the magnetic species responsible for the absorption in both powdered and whole bone at $g = 2{\cdot}008$ remains unknown. The whole bone resonance at $g = 2{\cdot}008$ may represent a magnetic species that is naturally present in bone *in vivo*.

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Tissue Determined Variations of Adenylate Kinase

GENETICALLY determined isoenzymes of human erythrocyte adenylate kinase (AK), ATP : AMP phosphotransferase E.C. 2.7.4.3, have been reported[1,2]. We have characterized several forms of AK in different organs of Wistar rats, employing a mixed agarose-acrylamide gel electrophoresis[3]. The electrophoretic behaviour of AK was studied in tissues taken from the same animal. Extracts of muscle, heart, eye lens, brain, liver and erythrocytes have been compared.

Tissues were extracted by homogenization of the organs in 6 volumes of *tris*, 0·001 M glycine 0·0033 M buffer, *p*H 7·5, containing 0·03 M mercaptoethanol. The extracts were centrifuged at 4,000*g* for 15 min and the supernatants were adjusted by dilution, with the same buffer, to the enzyme activity appropriate for good electrophoresis resolution. Samples were filtered through a 'Millipore' syringe to give a clear solution. Samples (0·1 ml.) were mixed with 0·1 ml. of 1 per cent agar solution at 50° C and placed in the slots of an acrylamide-agarose (7 per cent and 1 per cent, respectively) slab, 22 × 16 cm. Electrophoresis was performed with a discontinuous buffer at 30 mA, 250 V for 4 h at +2° C (gel buffer 0·07 M in *tris* and 0·06 M in glycine, bridge buffer 0·01 M in *tris* and 0·3 M in glycine, both adjusted to *p*H 8·7 with 2 N NaOH). After electrophoresis AK activity was characterized by incubating in the dark the acrylamide slabs in a reaction mixture containing 200 ml. of 0·2 M *tris*-HCl, *p*H 7·4, 1,200 μmoles of glucose, 120 μmoles of $MgCl_2$, 240 μmoles of ADP, 48 μmoles of NADP, 16 mg of phenazine metosulphate, 16 mg of MTT tetrazolium salt, 105 U of hexokinase and 35 U of glucose-6-