ELECTRON SPIN RESONANCE IN NUCLEIC ACIDS, NUCLEOTIDES, AND THE NITROGENOUS BASES OF DNA*

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The known chemical constitution and structure of DNA gives no basis for expecting it to be paramagnetic, yet paramagnetic resonances have been observed in DNA and RNA-protein complexes by Bliumenfel'd and co-workers (1959, 1960). Similar signals have been observed in DNA by Rajewsky, Redhardt and Zahn (1960), and in both DNA and RNA in our own laboratory. The electron spin resonances are 500 - 1200 gauss broad, intense (up to 10^{20} spins/gm, for $S = \frac{1}{2}$), center at approximately $g = 2$, are asymmetric--sometimes showing a considerable amplitude at zero field--and usually have a weak, complex structure. Bliumenfel'd (1959) reports that paramagnetic elements cannot be found in sufficient quantity to account for the e.s.r. signals observed, and has proposed that the resonances are an intrinsic property of the highly ordered polymeric structure of DNA and RNA. Recently Bliumenfel'd and Benderskii (1960) have proposed that unpaired electrons arise from the formation of charge transfer complexes between bases under the influence of the electric fields associated with the intact DNA molecule.

We have found that treatments known to alter the molecular structure of DNA (boiling in aqueous solution, enzymatic hydrolysis, sonication), while changing the e.s.r. line shapes, caused no significant changes in spin resonance intensity. Moreover, it was found that, under certain conditions, single nucleotides and purine or pyrimidine bases gave e.s.r. signals very similar to the nucleic acid signals.

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Because of the possibility that these resonances are due to transition elements known to occur in nucleic acids (Fuwa, 1960) either as contaminants or constituents, all samples were examined for trace metals by X-ray fluorescence spectroscopy and by direct chemical analysis. The results are shown in Table I. In only one of these samples could the observed spin density be as much as 50 per cent accounted for by the presence of any single element shown, and because there is no correlation between the observed spin density of several nucleic acid samples and their transition element content, these elements probably play a minor role in the observed resonances. For the reagent grade bases, in which no metals have been detected, it seems highly unlikely that paramagnetic ions can contribute significantly.

When the bases are pressed into pellets (2 mm thick, 8 mm dia.) at pressures up to 15,000 Kg/cm^2, electron spin resonances are seen that resemble the signals in the nucleic acids. However, these signals are generally not seen in unpressed powders of the bases and were not seen in crystals of cytosine. Very weak signals have been seen in unpressed samples of guanine. Figure 1 shows the e.s.r. spectra of a cytosine sample as a function of pressure. Signals are also seen in mixtures of bases, two at a time, prepared from aqueous solution and pressed, and similar signals are observed with pellets of the corresponding nucleotides. Compressed ribose, glucose, starch, cholesterol, hydroquinone, and phenylalanine show no resonance absorption, indicating that the signals are not due to chance contamination from the die, nor are they a general property of compressed organic compounds.

Preparations of calf thymus DNA, salmon sperm DNA, herring sperm DNA, and yeast RNA all showed e.s.r. signals initially which were enhanced by compression. The DNA and RNA samples gave e.s.r. signals which reached a maximum at pressures of 3000 to 6000 Kg/cm^2, and further compression did not affect signal intensity. In contrast, the intensity of the signals shown in Fig. 1 increased with increasing pressure up to the maximum pressure available. Reproducibility of the pressure enhancement of signals from nucleic acids and bases is still

TABLE I

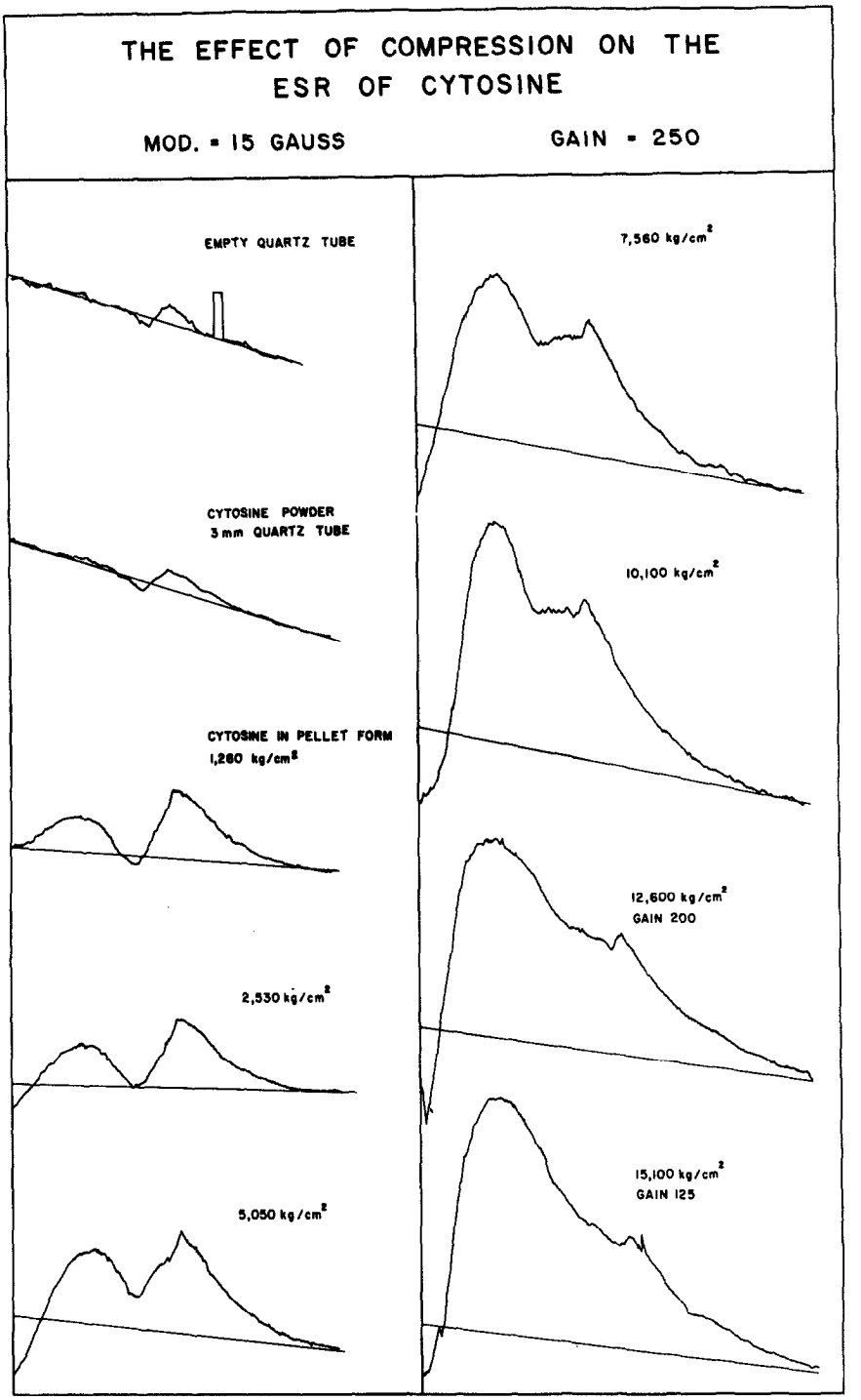
PARAMAGNETIC ELEMENT COMPOSITION OF SAMPLES

(% By Weight)

	V	Cr 0.18*	Mn 0.08*	Fe 0.08*	Co 0.20*	Ni 0.37*	Cu 1.06*	Mo 0.32*	Eu 0.21*	Gd 0.11*	Re 0.62*
Calf Thymus DNA $0.5\text{-}3.0\times10^{20}$ spins/gm	---	<.0005	<.0005	.012-.039	<.004	<.004	<.003	<.07	<.03	<.03	<.015
Salmon Sperm DNA 0.5×10^{20} spins/gm	---	<.066	<.004	.005-.012	<.004	<.004	<.003	<.07	<.03	<.03	<.015
Yeast RNA 0.5×10^{20} spins/gm	.0014	<.066	.003	.021-.028	<.004	<.004	.0015	<.07	<.03	<.03	<.015
Cytosine 1×10^{20} spins/gm	---	<.066	<.007	<.0002	<.004	<.004	<.003	<.07	<.03	<.03	<.015
Thymine 1×10^{20} spins/gm	<.001	<.066	<.007	<.0002	<.004	<.004	<.003	<.07	<.03	<.03	<.015

Notes: The numbers indicated by the asterisk (*) indicate the percentage of the listed element which must be present to produce 10^{20} spin/gm (spin $\frac{1}{2}$). The calculations were based upon the elements having ionic forms with an S-ground state and their having been previously observed at room temperature.

(-) = not sought, (<) = not detected at the indicated sensitivity



Magnetic field increasing from left to right (0-6000 gauss)

unsatisfactory; however, the roles of compression parameters, hydration, and average crystal size have not yet been investigated.

Two alternative explanations for the observed pressure enhancement are: 1) electron spin density is actually increased by pressure; 2) the electron spin density is constant at all pressures, but the resonance may depend on environmental factors leading to short relaxation times or to a highly distorted energy level system, and increasing the pressure may bring about a more favorable condition for resonance.

Since we find that degradation of the DNA--by drying the solution over a boiling water bath, by boiling and lyophilizing, by sonication, and by digestion with pancreatic deoxyribonuclease--does not significantly alter the spin density, it would seem that neither the intact double helix nor high molecular weight are essential for the observed magnetism. Upon cooling salmon sperm DNA to 77° K we did not observe the disappearance of the e.s.r. absorption reported by Bliumenfel'd. We confirm his observation that heating of dry salmon sperm and herring sperm DNA to the range 125° - 175° C results in complete disappearance of the electron spin resonance. It is possible that this heating results in severe local distortion of the structure, whereas other denaturing treatments leave the molecule locally intact. To test whether there was a relationship between the e.s.r. signal and the structure of the DNA molecule, orientation was induced by drawing fibers of salmon sperm DNA. X-ray diffraction analysis verified that there was a high degree of alignment of the DNA helix axes along the fiber axis. The e.s.r. spectrum of a bundle of parallel, straight fibers showed a considerable degree of anisotropy as the fiber bundle was rotated about an axis perpendicular to the D. C. magnetic field. The resonance spectrum has an approximate two-fold symmetry axis with respect to the fiber axis and, therefore, with respect to the molecular axis, implying that the spin system does not arise from a randomly oriented microcrystalline contaminant but is related to the DNA structure.

We propose that the origin of the unpaired electrons observed in nucleic

acids lies in electronic interactions between base molecules when the latter are strained from their normal crystallographic positions of equilibrium. Such strain is introduced artificially by compression, and may be supposed to occur naturally in the intact DNA molecule. Alterations of the e.s.r. spectra of DNA as the molecule is degraded suggest that the macromolecular structure of the molecule affects the environment of the unpaired electrons but not their occurrence.

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