SOME COMMENTS ON BROAD ELECTRON SPIN RESONANCE ABSORPTIONS OBSERVED IN NUCLEIC ACID PREPARATIONS*

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A number of recent papers have reported the existence of intense broad electron spin resonance absorptions in various preparations of nucleic acids, nucleoproteins, nitrogenous bases and bacteriophage (Bliumenfeld 1959; Bliumenfeld, Kalmanson and Shen-Pei-ken 1959; Blois and Marling 1961; and Müller, Hotz and Zimmer 1961).

It is of obvious importance to determine whether or not the signals in any given instance could be due to paramagnetic cation constituents of the sample or are due purely to organic interactions. The object of this note is to point out that the criteria for deciding this appear to be much more stringent than has apparently been realized to date.

The fact that the signals are broad (500-1500 gauss) indicates that they are not due to individual organic free radicals since such signals have line widths of less than 50 gauss or so. As has been pointed out previously by Bliumenfeld and co-workers, the breadth of the signal implies that it is due to an aggregate of coupled electrons. Such aggregates are well known in inorganic systems, e.g., in ferromagnetic materials or in superparamagnetic specimens (Bean and Livingston 1959) which are samples that contain sets of interacting paramagnetic atoms. The sets range in size from 25 Å to 125 Å and form a single paramagnetic domain. In these inorganic systems broad intense esr absorptions are also observed (see, for example, Kittel, 1956). The exact

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nature of the spectrum depends on many factors - the material studied, particle shape and size, and environment.

In estimating the strength of a spin resonance signal one customarily compares the integrated signal strength of the sample with the integrated signal due to a paramagnetic standard of known unpaired electron concentration such as diphenyl picryl hydrazyl making due allowance for differences in dial settings. For relatively narrow signals this procedure gives the correct result. For very broad spectra, however, this procedure will give incorrect results and the unpaired electron concentration may, in some cases, appear to be several orders of magnitude larger than it actually is.

In the paramagnetic standard one deals with microwave absorption between one spin state and a reversed spin state whose energy difference is small compared to thermal energy. At room temperatures and even at temperatures as low as $77^{\circ}K$ the two states are almost equally populated, as shown by a simple Boltzmann factor calculation. As is well known, the intensity of an esr signal will be determined by the difference in population between the two states.

On the other hand, in ferromagnetic or superparamagnetic samples the interactions with the applied magnetic field will be much greater than in the paramagnetic standard. One does not have the same distribution of energy levels and the Boltzmann factor enters quite differently into the equations describing the systems. As a consequence of this, if one determines the unpaired electron density simply by comparing the integrated signal strength with that of a paramagnetic standard, one seriously overestimates the unpaired electron concentration by a large factor. This factor will differ for every system being studied and cannot be stated in general. It may easily have the order of magnitude of 500, however, as may be most easily seen from the comments of Gorter when this subject was discussed at a recent meeting (Isenberg 1961, Gorter 1961). Consequently, for very broad signals it is incorrect to reason, for example, that since it would require 0.08% Fe, 0.18% Cr, etc. to account for 10^{20} spins per gm and that one has a lower paramagnetic ion concentra-

tion than this and one still obtains a signal intensity of apparently 10^{20} spins/gm, then the unpaired electrons must result from organic interactions. When one considers the correction factor that might easily be needed, to the author's knowledge there is no case reported in the literature, to date, in which the corrected spin intensity could not actually be lower than the reported paramagnetic cation concentration of the sample, assuming the ion spins were coupled in small arrays.

The observation that the signal strengths do not parallel the paramagnetic ion concentrations has no bearing on the question because the total paramagnetic cation concentration will not necessarily yield a broad signal; only that fraction of the paramagnetic cations whose spins are coupled may do so. The absence of parallelism is therefore no indication that the signal could not be due to the presence of inorganic cations.

The author has examined a commercial preparation of protamine nucleate (Nutritional Biochemical Corporation) and observed a spin resonance signal of half width 1100 gauss and an apparent integrated intensity of 3×10^{21} spins/gm. The sample had an iron content of 0.3% which, on a simple comparison basis, is apparently insufficient to account for the strength of the signal. However, when the possible correction factor described above is considered there is more than enough iron to account for the signal. When the sample was heated in vacuo to 200°C the signal disappeared irreversibly in agreement with the observations of Bliumenfeld. However, when the sample was heated very slowly, by placing the sample tube within a test tube so that an insulating air layer was between the sample and a bath at 220°C, the shape of the signal altered but the signal strength did not. This occurred in spite of severe charring of the sample. After the slow heating to 200°C the sample could be heated to over 400°C without a loss of signal. This observation agrees with the report of Blois and Marling (1961) that the signals do not necessarily disappear when the integrity of the polymer is destroyed.

Isenberg and Szent-Gyorgyi (1959) have published a preliminary report

showing the possible existence of a broad esr signal in dry adenosine triphosphate. Subsequent investigations have shown that, in some instances, when the sample was dissolved and then permitted to dry, the signal vanished. This could mean that the signal depended on the folding of the ATP molecule as originally proposed. At the present time it appears more likely, however, that small inorganic aggregates were responsible for the original signal. When the sample was dissolved and dried these did not necessarily reform into aggregates.

In spite of the precautions that have been pointed out here, it should be emphasized that there may, indeed, be many cases in which purely organic interactions lead to intense broad signals. The object of the present note has simply been to point out that the criteria for judging this has not been, to date, as rigorous as appears to be necessary. However, even should it turn out that in certain cases the signals are due to coupled inorganic cations, the results may still be of interest since the question will arise as to why these coupled systems tend to be associated with nucleic acids or nucleotides. It is possible that nucleotides may in some cases assist, or perhaps even play an essential role in the coupling of paramagnetic cation spins.

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