LETTERS TO THE EDITORS

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Paramagnetic Resonance Absorption of Ferrihæmoglobin and Ferrimyoglobin Derivatives

MAGNETIC susceptibility measurements on ferrous and ferric hæmoprotein complexes have led to the bonding of the iron being classified as 'essentially ionic' or 'essentially covalent', as with other co-ordination compounds containing elements of the first transition series¹. Ferrihæmoglobin, acidic ferrihæmoglobin and its fluoride complex are in the first category, oxy- and carbonmonoxy-ferrihæmoglobin and the azide and cyanide complexes of ferrihæmoglobin are in the second. The electronic state of the iron has accordingly been thought strictly comparable to that in the Fe^{2+} and Fe^{3+} aquo-ions and that in the $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Fe}(\text{CN})_6^{3-}$ complex ions respectively, since the corresponding magnetic susceptibilities are very similar.

Another manifestation of the magnetic properties is revealed by paramagnetic resonance absorption measurements from which g -values may be obtained, quantities related to the magnetic moment, the magnitudes of which for a compound of a first transition element depend not only upon the quantum number S but also the total orbital quantum number L and the symmetry around the ion. The ferric ion, which is in an orbitally singlet state ($L = 0$), has² one g -value of 2.0. Acidic ferrihæmoglobin and its fluoride complex, in which the iron has hitherto been assumed to be bound by essentially ionic bonds on the basis of paramagnetic susceptibility measurements, were therefore expected also to have g -values of 2.0. Using wet solid specimens, measurements at liquid-air and liquid-hydrogen temperatures have, however, given identical g -values of 5.90 ± 0.05 for both derivatives³; these are very similar to that obtained for hæmin³.

Since the observation of such high g -values in polycrystalline specimens was quite unexpected, the measurements were repeated at as many frequencies as possible in order to check that zero-field splitting was not responsible for the position of the line. Determinations were made at 3.2 cm., 1.25 cm., 1.0 cm., 8 mm. and 6 mm. wave-lengths, and the results are summarized in Fig. 1. The fact that all the points for both hæmin and acidic ferrihæmoglobin lie on straight lines passing through the origin show conclusively that true g -values are being measured. A slight asymmetry in the shape of the line was noted which indicates that the g -value spreads to somewhat lower values; but from intensity considerations this variation would appear to be small. It is hoped to carry out measurements on single crystals to obtain the precise values for the variation. It is quite clear, however, that the electronic state of the iron is entirely different in these 'ionic' complexes from that of the gaseous or aquated Fe^{3+} ion.

Measurements have also been made on the azide and cyanide complexes of ferrihæmoglobin, in which the bonding of the iron is classified as essentially covalent. A well-defined variation of g -value from 2.2 to 2.8 was obtained for the former, but the latter gave very weak absorption. No measurements have

Fig. 1. Plots of reciprocal wave-length against magnetic field for hæmin (ref. 3) (open circles) and acidic metmyoglobin (full circles), from which g -values of 6.00 ± 0.05 and 5.90 ± 0.05 respectively have been obtained

yet been made in the range $g < 2$, but the results for the azide complex show that there is a significant quantitative difference between a 'covalent' hæmoprotein complex and a 'covalent' inorganic co-ordination complex such as the ferricyanide ion, which has g -values⁴ along the three axes of 0.94, 2.18 and 2.30.

Very similar absorption was observed for the corresponding ferrimyoglobin derivatives, and identical g -values were obtained. These results show, in a more direct manner than the magnetic susceptibility values, that no appreciable intramolecular interaction, in the sense of a coupling between the magnetic moments, occurs between the iron atoms in the recrystallized ferrihæmoglobin used for these measurements.

Note added in proof. Preliminary measurements on single crystals of acidic ferrihæmoglobin, kindly supplied by Dr. V. Ingram, of the Cavendish Laboratory, indicate that the high g -value of about 6 corresponds to g_1 , that is, in the plane of the hæm. The asymmetry of the line reported above is due to a $g_{||}$ value, that is, along the axis perpendicular to the hæm, of less than 3, probably about 2.5.

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¹ Martell, A. E., and Calvin, M., "Chemistry of the Metal Chelate Compounds" (Prentice-Hall, Inc., New York, 1952).

² Herzberg, G., "Atomic Spectra and Atomic Structure" (Dover Pub., New York, 1944).

³ Bennett, J. E., and Ingram, D. J. E., Faraday Society Discussion, "Microwave and Radio Frequency Spectroscopy", April 1955 (in the press).

⁴ Bleaney, B., and Ingram, D. J. E., *Proc. Phys. Soc.*, A, **65**, 953 (1952).