Magnetic susceptibility measurements on haemoproteins

It is the purpose of this paper to call the attention of experimental workers to a small systematic error which occurs in the standard method of determination of the Bohr magneton number of the iron in haemoproteins. I refer to the technique[1] of measuring the difference of susceptibility of a haemoprotein containing a paramagnetic iron ion from that of the same haemoprotein with a diamagnetic iron ion. For example, the susceptibility due to the iron in haemoglobin is obtained by comparing it with that of an equimolar concentration of a diamagnetic compound such as oxyhaemoglobin or carboxyhaemoglobin. Clearly, one defect in this very neat method is that it is not certain that the diamagnetism of the protein is the same in the two compounds. However, it is not unreasonable to suppose that it is so and the actual results obtained by this method show that at least it is not a very bad assumption.

A second assumption implicit in the method is that the six d electrons which are paired in the "diamagnetic" comparison haemoprotein do not in fact make any paramagnetic contribution to the susceptibility. Recently, a theoretical discussion[2] was given of this problem for the analogous compounds of the cobaltic ion Co^{+++}. This ion also has six d electrons and they are paired in most of its compounds. However, experimentally there is quite a large temperature-independent paramagnetic susceptibility associated with the cobaltic ion in these compounds. Numerically it is about $150 \cdot 10^{-6}$ compared to $1270 \cdot 10^{-6}$ expected for one unpaired electron at room temperature. This is small but not negligible.

Our theory shows that this paramagnetism is due to the fact that a magnetic field polarizes the d^6 group giving an induced paramagnetism which is, therefore, independent of temperature. In the usual language, the field induces an orbital, not a spin, paramagnetism. We calculated this paramagnetism numerically and obtained reasonable agreement with experiment. Unfortunately, the calculation requires a knowledge of the position of the longest wavelength singlet-singlet absorption band of the metal ion. This was available for the cobalt compounds but is hidden below the much more intense visible bands of the porphyrin ring in haemoproteins. So it is not possible to make such a definite calculation in this case.

We can however make an estimate, using the fact that haemoproteins have a ligand field somewhere near the point where the compound changes from high-spin (or essentially ionic) to low-spin (or essentially covalent). This enables one to estimate that the hidden band should lie at about 14,000 cm^{-1}. (The energy of the transition is $A - C$ from GRIFFITH AND ORGEL[3]; we assume that A is approx. equal to Π from Table I of GRIFFITH[4], see also ORGEL[5]; C is about 3,800 cm^{-1} from CATALAN, ROHRLICH AND SHENSTONE[6].) Then equation (3) of GRIFFITH AND ORGEL[2] is

$$\chi = 4.085/E,$$

where E is the energy of the transition. Our estimate, then, is $\chi = 290 \cdot 10^{-6}$. χ probably lies between $240 \cdot 10^{-6}$ and $340 \cdot 10^{-6}$. It is not reasonably possible for it to lie outside the range $150 \cdot 10^{-6} < \chi < 400 \cdot 10^{-6}$.

So if one uses PAULING AND CORYELL's method, the resulting estimate of the susceptibility of a paramagnetic metal ion containing unpaired electrons is actually the difference between the true paramagnetism of that metal ion and the residual paramagnetism of the ion in the comparison haemoprotein. Let us call the susceptibility derived by PAULING AND CORYELL's method χ and the residual paramagnetism χ_0. Then the true susceptibility of the first ion is

$$\chi_1 = \chi + \chi_0. \tag{1}$$

As χ, χ_1 and χ_0 are all paramagnetic susceptibilities, we see that χ is an underestimate of the true susceptibility.

The results of susceptibility measurements are usually expressed in terms of effective Bohr magneton numbers μ. μ is defined in terms of χ by the relation[7]

$$\mu = \sqrt{\frac{3kT\chi}{N\beta^2}} \tag{2}$$

where k is Boltzmann's constant, T the absolute temperature, N Avogadro's number and β the Bohr magneton (the quantum unit of magnetic moment). If Curie's law is obeyed, μ is independent of temperature. Let us now rewrite equation (1) in terms of μ and let μ_0, μ_1 correspond to χ_0, χ_1 respectively. Then it follows that

$$\mu_1^2 = \mu^2 + \mu_0^2, \tag{3}$$

the other quantities having cancelled out. If we accept the estimate of $290 \cdot 10^{-6}$ for χ_0 then $\mu_0 = 0.83$ at room temperature. μ_0 is proportional to the temperature because the susceptibility χ_0, unlike susceptibilities arising from unpaired electrons, is independent of temperature.

This leads to an uncertainty in the values of magneton numbers derived from experimental

measurements on paramagnetic haemoproteins. If μ is the effective Bohr magneton number derived with neglect of this matter then it should be replaced by

$$\mu_1 = (\mu^2 + \mu_0^2)^{1/2}.$$

Thus μ also will have been an underestimate. For $\mu_0 = 0.83$, as above, Table I gives some typical corrections. In other words, a μ of 5.92 is notto be regarded as being in very good agreement with the spin-only value for five unpaired electrons and similarly for other numbers of unpaired electrons.

TABLE I

EFFECT OF CORRECTION ON BOHR MAGNETON NUMBERS AT ROOM TEMPERATURE

μ	1.52	2.70	4.83	5.86	5.92
μ_1	1.73	2.83	4.90	5.92	5.98
Correction	0.21	0.13	0.07	0.06	0.06

I might perhaps mention that it is not entirely hopeless to try to determine μ_0 experimentally, for its dependence on temperature is different from that of μ_1. μ_0 determined in this way, however, would also include any contribution from a change of diagmagnetic susceptibility of the protein.

Because the ferric ion is in a spherically symmetric ^6S state, the μ_1 for the five unpaired electrons is very likely to be close to the free-spin value over a wide range of temperature[7] and so this ion might possibly serve as an alternative comparison compound. However, in methaemoglobin, electron-resonance measurements have shown that there is a splitting of this ^6S ground term[8,9]. The effect of this on the susceptibility would be to make it obey a Curie-Weiss law

$$\sigma = \frac{35N\beta^2}{3k(T + \Delta)},$$

with Δ of the order of 20°, down to temperatures approaching $T = \Delta$.

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Reduction of cytochrome c by a resin containing a copper–amine complex

MILLS AND DICKINSON[1] have described the use of a resin containing a copper–amine complex (Duolite S-10*) for the removal of dissolved oxygen from water. In the oxidized form the resin is blue-green in colour and contains Cu^{++} complexed with the amine groups in the resin. On reduction, the resin contains metallic copper dispersed and/or absorbed in the resin matrix and is purple coloured with a metallic sheen. It has been found that a resin treated in this way can be used to prepare reduced cytochrome *c*.

A chromatographic column containing a resin bed 24 cm long and 1 cm diameter was prepared[1] and reduced by use of 0.5 *M* $Na_2S_2O_4$ in 1.25 *M* NaOH. The reduced resin was washed with glass-

* Supplied by the Chemical Process Company, Redwood City, Calif. (U.S.A.).