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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Department of Theoretical Chemistry, University of Cambridge (Great Britain) J. S. GRIFFITH

1 L. PAULING AND C. D. CORYELL, *Proc. Natl. Acad. Sci. U. S.*, 22 (1936) 210.
2 J. S. GRIFFITH AND L. E. ORGEL, *Trans. Faraday Soc.*, 53 (1957) 601.
3 J. S. GRIFFITH AND L. E. ORGEL, *J. Chem. Soc.*, (1956) 4981.
4 J. S. GRIFFITH, *J. Inorg. & Nuclear Chem.*, 2 (1956) 229.
5 L. E. ORGEL, *J. Chem. Phys.*, 23 (1935) 1819.
6 M. A. CATALAN, F. ROHRLICH AND A. G. SHENSTONE, *Proc. Roy. Soc. (London) A.*, 221 (1954) 421.
7 J. H. VAN VLECK, *The Theory of Electric and Magnetic Susceptibilities*, Chap. 11, Oxford University Press, London, 1932.
8 J. S. GRIFFITH, *Proc. Roy. Soc. (London) A.*, 235 (1956) 23.
9 J. E. BENNETT, J. F. GIBSON AND D. J. E. INGRAM, *Proc. Roy. Soc. (London) A.*, 240 (1957) 67.

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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.).

distilled water until the pH of the effluent was 7.6–7.8. We have found it convenient to use a three-way stopcock, connected to separatory funnels containing the reducing solution and glass-distilled water and to the column by short pieces of rubber tubing. The solution of cytochrome *c* was injected directly on top of the resin bed by use of a syringe with a long small-gauge needle. During injection of cytochrome *c* solutions the distilled water was left connected to the column, but flow through the column was halted by the pinchcock used to regulate effluent flow. Fractions of 0.6 ml were collected with a flow rate of about 2 ml/min.

Reduction of cytochrome *c* by the column gave 96% or more reduction in fractions 3–8. About 60% of the cytochrome *c* was present in fractions 3–5 and 20% more was present in fractions 6–14.1 ml 0.5 *M* phosphate buffer, pH 7.4, added following fraction 9, served to elute cytochrome *c* remaining on the column. The pH of the effluent rose sharply at fraction 15, as phosphate of the buffer was retained by the Cu^{+} + Cu^{++} ions of the resin complex. The use of a 1.0 ml sample of cytochrome *c* solution on a column of this size gave a five-fold dilution of the cytochrome *c* solution during the reduction.

A smaller column (6 cm long, 0.5 cm diameter) was prepared, reduced and washed with oxygen-free, glass-distilled water (from the larger sized column). 1.0 ml of cytochrome *c* was injected directly above the resin bed and 0.2 ml fractions were collected at a flow rate of 0.5 ml/min. In a typical experiment (Fig. 1), fractions 6–10 contained 60% of the cytochrome *c* added, 94% reduced and at 55% of the original concentration. Fractions 5 and 11–18 contained another 25% of the added cytochrome, leaving about 15% not eluted from the column. A column of this size can reduce approximately 10 ml of 10^{-4} *M* cytochrome *c* before requiring regeneration. Cytochrome *c* reduced by the column was tested in the manometric cytochrome *c*-oxidase assay[2] with 0.001 *M* ethylenediaminetetraacetate added and gave the same oxygen uptake as an equivalent amount of cytochrome *c* not passed through the column.

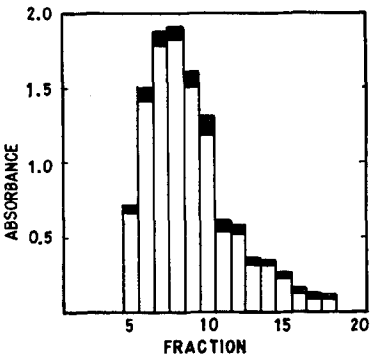

Fig. 1. The reduction of cytochrome *c* by a resin containing a copper–amine complex. The clear portion of the bars indicates the change in absorbance caused by reduction of cytochrome *c* by the column and the shaded portion indicates further change in absorbance on addition of $Na_2S_2O_4$. Measurements were made in 1 cm cells in the Uvispek spectrophotometer and absorbance was calculated for the undiluted fractions.

When the column was used for the preparation of reduced cytochrome *c*, the first colourless effluent was discarded together with the first 0.2 ml of reduced cytochrome *c*. The effluent was then collected until a slight decrease in colour intensity was noted in the outlet tube of the column (a volume of effluent equal to the volume of cytochrome solution injected was usually collected).

The results show that the reduced resin reduces cytochrome *c* and that reduced cytochrome *c* may be obtained in reasonable yield in useful concentrations by this method.

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*Laboratory of Physiological Chemistry***, *University of Amsterdam, Amsterdam (The Netherlands)*

W. B. ELLIOTT*

1 C. F. MILLS AND B. N. DICKINSON, *Ind. Eng. Chem.*, 41 (1949) 2842.
2 E. C. SLATER, *Biochem. J.*, 44 (1949) 305.

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* On leave from the Department of Biochemistry, University of Buffalo, N.Y. (U.S.A.).
** Postal address: Jonas Daniël Meyerplein 3, Amsterdam-C.