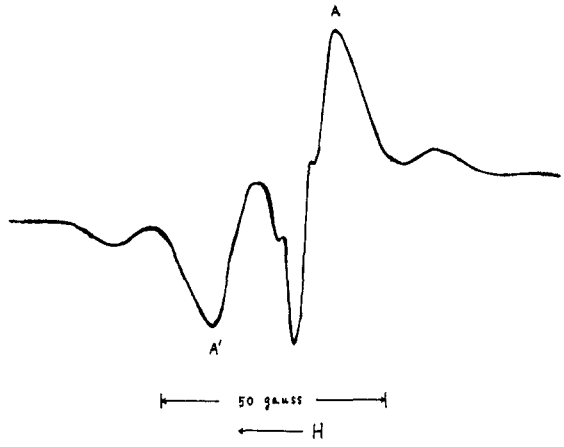

Figure 2a. Unbleached 3-methylpentane + 20 mole % vinyl acetate. The sample was irradiated and measured at 77°K. (radiation dose, 8×10^5 rads).

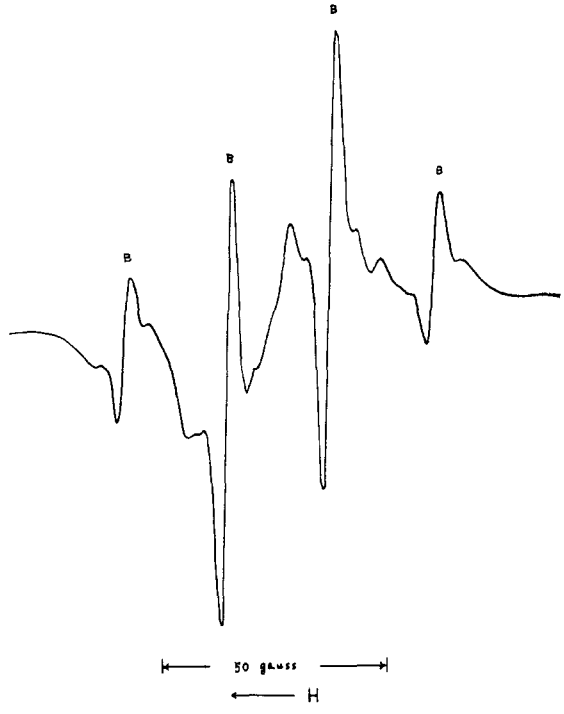

Figure 2b. Bleached 3-methylpentane + 20 mole % vinyl acetate. The sample was irradiated and measured at 77°K. (radiation dose, 8×10^5 rads).

acetate and that of the same sample bleached by visible light, respectively. The gain in the measurements is the same for Figures 1a and 1b and for Figures 2a and 2b.

In Figure 1a, a relatively narrow singlet is superimposed on a spectrum consisting of six absorption lines. In Figure 1b, only the spectrum consisting of six components in the intensity ratio 1:5:10:10:5:1 is obtained, which can be ascribed to the radical CH_3CH_2-$CHCH_2CH_3$. The methyl radical was not observed in pure 3-methylpentane under our experimental conditions. The singlet, centered at $g = 2.006$, which disappears by optical bleaching can be ascribed to trapped electrons.

In Figure 2a, an additional spectrum indicated with A and A′ is superimposed on the spectrum obtained in pure 3-methylpentane, as we can see from Figures 1a and 2a by comparing the relative intensities of the absorption lines in both figures. Bleaching causes the e.s.r. spectrum to change. The additional spectrum indicated with A in Figure 2a disappears by optical bleaching, being reasonably ascribed to the vinyl acetate ion (probably cation). In Figure 2b, the quartet indicated with B is superimposed on the multiline spectrum. The line separation of the quartet is 23 gauss in accord with that for the methyl radical obtained by Fessenden and Schuler.[7] Thus the quartet can be ascribed to the methyl radical which is produced by the neutralization reaction between the vinyl acetate cations and electrons and trapped in the special matrix (3-methylpentane–vinyl acetate system) used in the present study. The other multiline spectrum in Figure 2b consists of the absorption lines due to the radical produced from 3-methylpentane and those due to some radicals which are not identified.

A possible mechanism for the charge neutralization reaction observed in the 3-methylpentane–vinyl acetate system is given by

$$CH_2CHOCOCH_3^+ + e \longrightarrow CH_2CH + OCOCH_3$$

$$OCOCH_3 \longrightarrow CO_2 + CH_3$$

Hamill, *et al.*,[3] have suggested prompt decomposition of the acetoxy radical in explaining their experimental results. The second step shown above is consistent with their interpretation. The complicated nature of the e.s.r. spectrum of the mixture makes it difficult to identify the vinyl radical[7] definitely.

(7) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **39**, 2147 (1963).

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Evidence for a Permanent Dipole Moment in Hemoglobin from Kerr Effect Optical Dispersion

Sir:

The electric dipole moment is an important parameter in the study of protein structure in solution because it is related to charge distribution, α-helical structure, and other factors. Efforts to obtain permanent dipole moments of globular proteins from dielectric constant data have yielded uncertain results because obscuring effects such as proton fluctuations[1] and ion atmosphere polarization[2] may occur. This communication describes a preliminary investigation of the Kerr effect optical dispersion of horse hemoglobin solutions. Knowledge of the three-dimensional structure of the hemoglobin molecule[3,4] suggested the possibility of using the known orientation of the four heme groups to investigate the direction of orientation of the molecule in a field. Assuming the molecular conformation and dimensions to be the same in solution as in the crystal,

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(3) D. J. E. Ingram, J. F. Gibson, and M. F. Perutz, *Nature*, **178**, 906 (1956).
(4) M. F. Perutz, M. G. Rossmann, A. F. Cullis, H. Muirhead, G. Wells, and A. C. T. North, *ibid.*, **185**, 416 (1960).

the data are most reasonably interpreted in terms of a permanent dipole along the twofold axis of the molecule.

In general, the sign of the Kerr effect is positive if the optical polarizability along the orientation direction of a molecule in an applied field is greater than the average polarizability. Optical anisotropy may arise from nonspherical molecular shape or from anisotropic chromophores such as the heme groups in hemoglobin. To separate the shape and intrinsic effects, the wave length dependence (optical dispersion) of the Kerr effect may be measured near the absorption wave lengths of the chromophores. The change with wave length of the shape effect is small in a narrow wave length region, so that appreciable wave length dependence may be interpreted to give the relative directions within the molecule of the orienting moment and the chromophores. If one is known, the other may be deduced.

Two simple possibilities may be considered for hemoglobin: (a) orientation of the molecule with its long axis parallel to the field; and (b) orientation with the short or twofold axis parallel to the field. A theoretical calculation of the intrinsic optical anisotropy of the heme groups has been carried out for cases a and b.[5] The optical anisotropy may be defined as

$$\Delta\alpha = \alpha_{11} - {}^1/_2(\alpha_{22} + \alpha_{33}) \qquad (1)$$

where the α_{ii} are components of the optical polarizability tensor of a hemoglobin molecule in a coordinate system in which the orienting dipole is along the 1-axis. To make the calculation, it was assumed that all of the visible transitions are polarized in the planes of the heme groups. The contribution of the absorption spectrum to the in-plane polarizability of a single heme group was then calculated. The results were consistent, where comparable, with recently obtained anomalous dispersion data.[6] Using the molecular structure as determined in the crystalline state,[3,4] the intrinsic anisotropy of the four hemes per molecule was calculated for the visible region and is shown in Figure 1 for cases a and b for both met- and oxyhemoglobin. The shape effect contribution to $\Delta\alpha$ causes only a vertical displacement of the curves in Figure 1 if its wave length dependence is negligible. A realistic calculation of the shape effect has not been attempted, although one would expect it to be positive for case a and negative for case b on the basis of an ellipsoidal model.

Experimental Kerr constant increments, k, for met- and oxyhemoglobin at the three wave lengths studied (365, 436, and 546 mμ) are shown as points in Figure 1. k is defined by

$$B = B_0(1 + kc) \qquad (2)$$

where $B = (n_{||} - n_{\perp})/\lambda E^2$ is the Kerr constant of the solution and B_0 that of the solvent (water), both at 15°. $n_{||}$ and $n_{\perp}$ are the refractive indices of the solution parallel and perpendicular to the static field E at wave length λ, and c is the hemoglobin concentration in g/l. k is reported rather than B because the measurements were relative to water rather than absolute. Descriptions of the apparatus and sample preparation will be given elsewhere.[5,6] Electric dichroism experiments were also carried out on solutions of both met- and oxyhemoglobin in the Soret band using the 406 mμ mercury line, but no signal could be detected in either case under the most sensitive conditions available.

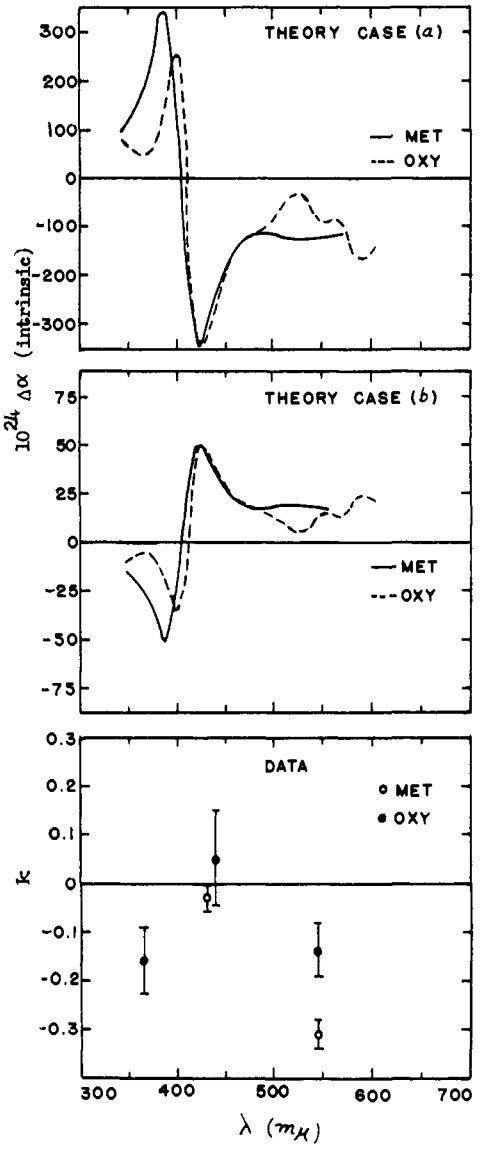

Figure 1. The calculation of the theoretical curves is described in the text. Each experimental point is the average of three or four duplicate determinations. The uncertainties shown are an average of experimental average deviations and slightly larger theoretical estimates. The concentration range and per cent transmittance of the methemoglobin were 1.1–1.4 g./l. and 37–48%, and of the oxyhemoglobin were 0.6–1.0 g./l. and 4–30%. No concentration dependence was observed. There was too little transmittance with methemoglobin at 365 mμ to obtain a point.

Comparing the wave length dependence of the experimental k's and theoretical $\Delta\alpha$'s in Figure 1, it may be concluded that case b is consistent with the data while case a is not. Although a more complicated possibility intermediate between cases a and b cannot be ruled out, it is reasonable to investigate the quantitative aspects of case b. If the only important orienting mechanism is a permanent dipole parallel to the twofold (short) axis, the Kerr effect equation described previously (eq. 12 of ref. 7) may be used to calculate $\Delta\alpha\mu_{02}^2$ for each wave length. (This equation requires B, which is obtained

(5) W. H. Orttung, to be published.
(6) W. H. Orttung and J. Warner, manuscript in preparation.
(7) W. H. Orttung and J. A. Meyers, *J. Phys. Chem.*, 67, 1911 (1963).

from eq. 2 using k and estimates of B_0.[5]) To separate the dipole moment, μ_{02}, from $\Delta\alpha$, the change with wave length of $\Delta\alpha$ was estimated from the curves of Figure 1 and divided into the corresponding change in $\Delta\alpha\mu_{02}^2$ to obtain estimates of 120 and 70 D. ($\pm 20\%$) for the permanent moments of met- and oxyhemoglobin, respectively. The shape effect contribution was then estimated as about -40×10^{-24} ml. for both molecules.

It is unlikely that an intrinsic anisotropy of static polarizability could lead to an orientation parallel to the short axis of the magnitude observed. If there is a mechanism favoring orientation parallel to the long axis of the molecule, such as anisotropy in a fluctuation dipole or in an ion-atmosphere polarization, the above analysis would then yield only a lower limit to the permanent dipole along the twofold axis. From this possibility it can be seen that if the data had suggested case a, no inference about the existence of a permanent dipole could have been made. Calculations of the electric dichroism suggest that no signal should have been observed for case b and the amount of orientation predicted by the Kerr effect, so that this experiment was consistent with the interpretation of the Kerr effect measurements.

It is therefore reasonable to conclude that the above experiments and analysis are evidence for a permanent dipole along the twofold axis in hemoglobin at neutral pH. The suggestion of a greater dipole moment for methemoglobin relative to oxyhemoglobin is consistent with the dielectric increment data,[8] although dipole moments estimated from dielectric increments (400–500 D.) apparently include contributions that are not effective in orientation, such as a randomly oriented fluctuation dipole or an ion-atmosphere polarization about an almost spherical molecule.

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The Formation of Iron(IV) in the Oxidation of Iron(II)[1]

Sir:

The oxidation of Fe_{aq}^{2+} by 1-equiv. oxidants such as hexachloroiridate(IV), tris(1,10-phenanthroline)iron(III), manganese(III), and cobalt(III) proceeds in a single step. These reactions are of the type

$$Fe^{2+} + Ox_1 \overset{\text{1 equiv.}}{\rightleftharpoons} Fe^{3+} + Red_1 \qquad (1)$$

They are generally rapid and their rates are first order with respect to the ferrous ion and the oxidant concentrations.[2-4] On the other hand, the oxidation of Fe_{aq}^{2+} by 2-equiv. oxidants is more complex. Such reactions have to involve unstable oxidation states of the iron or of the oxidant.

We are studying the kinetics and attempting to identify the immediate products of the reaction of Fe_{aq}^{2+} with chlorine, hypochlorous acid, hydrogen peroxide, and ozone. In these studies, the concentrations of Fe_{aq}^{2+} and of the oxidizing agents vary from 10^{-4} to 10^{-1} M and from 5×10^{-5} to 5×10^{-4} M, respectively. The perchloric acid concentration varies from 0.1 to 3.0 M. The ionic strength is 3.0 M and the temperature 25.0°. Equal volumes of two solutions containing the Fe_{aq}^{2+} and the oxidant are mixed on the flow apparatus which has been described previously,[5,6] and the formation of iron(III) and the dissociation of any $FeCl^{2+}$ and $(FeOH)_2^{4+}$ produced in the reaction are followed at 240 to 280, 336, and 335 mμ, respectively.[7-10] Although $FeCl^{2+}$ and $(FeOH)_2^{4+}$ have similar absorption spectra, they may readily be distinguished on the basis of their rates of dissociation. The rate of dissociation of $(FeOH)_2^{4+}$ increases with increasing acidity while the dissociation of $FeCl^{2+}$ decreases with increasing acidity.[11,12] Some preliminary results of these studies are presented in Table I. Because of the rapidity of the reaction

$$FeOH^{2+} + H^+ \rightleftharpoons Fe^{3+} + H_2O \qquad (2)$$

we cannot determine whether $FeOH^{2+}$ is formed as a primary product in the oxidations.

Table I. Rate Constants and Distribution of the Products of the Oxidation of Fe_{aq}^{2+} by H_2O_2, Cl_2, HOCl, and O_3 (Temperature 25.0°; Ionic Strength = 3.0 M)

Oxidant	k, M^{-1} sec.$^{-1}$ [a]	Yields, %[b] Fe^{3+} + $FeOH^{2+}$	$FeCl^{2+}$	$(FeOH)_2^{4+}$
H_2O_2	65 ± 5[c]	>99	..	<1[e]
Cl_2	80 ± 5	<30	>70[d]	<5[d]
HOCl	(3.2 ± 0.4) × 10^3	~80	≤5[d]	~15[f]
O_3	(1.7 ± 0.4) × 10^5	~60	..	~40[g]

[a] The iron(II) was present in large excess. k, the over-all rate constant, was calculated from $0.693/t_{1/2}$[Fe(II)] where $t_{1/2}$ is the half-time for the formation of iron(III). [b] The yields of $FeCl^{2+}$ and $(FeOH)_2^{4+}$ are based on extinction coefficients of 1.73×10^3 at 336 mμ and 3×10^3 at 335 mμ for these species, respectively. [c] This value is in satisfactory agreement with the rate constant determined under slightly different conditions by C. F. Wells and M. A. Salam [Nature, **203**, 751 (1964)]. [d] 3.0 M $HClO_4$. [e] 0.2 M $HClO_4$. [f] 0.1 M $HClO_4$. The yield of the dimer produced in this reaction decreased with increasing perchloric acid concentration. [g] 1.0 M $HClO_4$.

The formation of $(FeOH)_2^{4+}$ in the HOCl and O_3 oxidations is of particular interest, since it suggests that iron(IV) is an intermediate in these reactions. The kinetic data are consistent with the dimer being formed as shown below.

$$Fe^{2+} + Ox_2 \xrightarrow{\text{2 equiv.}} Fe(IV) + Red_2 \qquad (3)$$

$$Fe(IV) + Fe^{2+} \xrightarrow{\text{rapid}} [Fe(III)]_2 \qquad (4)$$

(1) Research performed under the auspices of the U. S. Atomic Energy Commission.
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