dasycarpidone and epidasycarpidone show the same mass spectrum.[2] Professor J. A. Joule has kindly informed us that he and his coworkers have synthesized (±)-dasycarpidone and (±)-epidasycarpidone by another route. A sample of (±)-epidasycarpidone provided by Professor Joule shows the same behavior on tlc as our material.

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Paramagnetic Proton Nuclear Magnetic Resonance Shifts of Metmyoglobin, Methemoglobin, and Hemin Derivatives[1]

Sir:

We present here preliminary results of studies of paramagnetic proton nmr shifts in hemin and related compounds, chlorohemin, chloromesohemin, chlorodeuterohemin, metmyoglobin,[2] and methemoglobin. Our results suggest that the isotropic hyperfine interaction is transmitted principally through the σ-bond system of the porphyrin ring. Moreover, a comparison of the metmyoglobin and methemoglobin spectra may indicate the effect of nonequivalent heme groups in hemoglobin.

The 100-MHz proton nmr spectra of the hemin derivatives are shown in Figure 1; those of metmyoglobin and methemoglobin are shown in Figure 2. Spectra were measured both at 60 MHz, with a Varian DP-60 spectrometer, and also at 100 MHz, with a Varian HA-100 spectrometer modified to give the required large frequency-sweep ranges and sweep offsets in a field-frequency controlled mode.[3] Samples of the hemin derivatives, taken as ~0.06 *M* solutions of the free acid in DMSO-d_6, and those of methemoglobin and metmyoglobin, taken as ~0.01 *M* solutions in 0.1 *M* deuterated phosphate, pD 7,[3] were run at ambient temperature (27.0 ± 0.5°).

The spectra in Figures 1 and 2 show lines shifted far downfield (30–90 ppm) from the normal range of chemical shifts in diamagnetic porphyrins.[4–6] No lines were observed shifted upfield by comparable amounts. A comparison of line positions and relative intensities in these spectra leads to the tentative assignments for the chlorohemin derivatives shown in Table I, and the numbering scheme is shown in Figure 3. The large downfield shifts of lines assigned to the 2a,4a methylene protons in mesohemin and to the 2,4 protons of deuterohemin suggests that the isotropic hyperfine interaction is transmitted through the σ-bond system. Were the unpaired electron spin in the porphyrin ring system distributed over π-type orbitals in a strictly planar ring system, one would expect the isotropic hyperfine constants for the 2a,4a methylene protons in mesohemin to be of opposite sign to those for the 2,4 protons in deuterohemin.[7,8]

Several of the lines in the methemoglobin and metmyoglobin spectra may arise from histidine and/or water, bonded to the heme group in the fifth and sixth coordination positions. Further, there are large differences in relative intensities and line widths, the lines of the metmyoglobin spectra generally being sharper. The greater line widths of the hemoglobin spectra may be due to a nonequivalence of the four heme groups or to the larger rotational correlation time of the methemoglobin molecule.

We have taken the contribution of the pseudo-contact term in the isotropic hyperfine interaction to be negligible, as indicated by calculations to the second

Table I. Assigned Line Positions for Paramagnetic Proton Nmr Shifts in Chlorohemin Derivatives at 100 MHz and 300°K[a]

	Assignments[b]					
Compound	$H_{\alpha,\beta,\gamma,\delta}$	CH_3(1a,-3a,5a,8a)	CH_2-(2a,4a)	CH_2-(6a,7a)	-CH=CH_2 (2a,4a)(2b,4b)	H(2,4)
Chlorohemin	62.61[c]	50.75		46.80	57.53	
				40.51	54.23	
				37.18	44.51	
Chloromesohemin	61.96	50.15	44.02	44.02		
	60.17	49.05	39.59	39.59		
Chlorodeuterohemin	64.42	49.34		41.92		
	61.18	46.98		39.68[d]		72.29[e]
	60.01					
	57.44					

[a] The numbering scheme used is shown in Figure 3. [b] Shifts are in parts per million downfield from DMSO; the precision of the measurements is better than ±0.05 ppm, or better than the line width (~2–3 ppm). [c] A slight splitting was observed here, ~0.06 ppm. [d] Broadening, indicating the presence of additional lines, can be observed here. [e] This shift is approximately the same as that found for pyrrole protons in $\alpha,\beta,\gamma,\delta$-tetraphenylporphyriniron(III) chloride (D. R. Eaton and E. A. LaLancette, *J. Chem. Phys.*, **41**, 3534 (1964)).

(1) This paper was presented in part to the 12th Annual Meeting of the Biophysical Society, Feb 19–21, 1968, Pittsburgh, Pa.

(2) A. Kowalsky (*Biochemistry*, **4**, 2382 (1965)), in a related proton nmr study of cytochrome *c* and heme polypeptides, has mentioned the occurrence of large downfield shifts in the proton nmr spectra of metmyoglobin; however, no values were cited for these shifts or spectra given.

(3) Details of this modification as well as the preparation and sources of materials will be published elsewhere.

(4) R. J. Abraham, A. H. Jackson and G. W. Kerner, *J. Chem. Soc.*, 3468 (1961).

(5) E. D. Becker, R. B. Bradley, and C. J. Watson, *J. Am. Chem. Soc.*, 83, 3473 (1961).

(6) W. S. Caughey and W. S. Koski, *Biochemistry*, **1**, 923 (1962).

(7) D. R. Eaton and W. D. Phillips, *Advan. Magnetic Resonance*, **1**, 119 (1965).

(8) A. Forman, G. N. Murrell, and L. E. Orgel, *J. Chem. Phys.*, **31**, 1129 (1959).

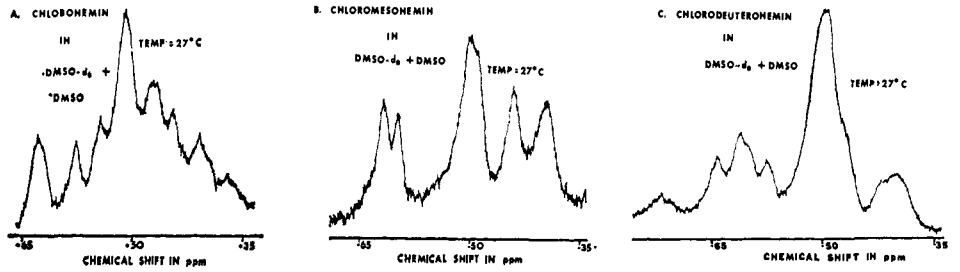


Figure 1. Nmr spectra (100 MHz) of (a) chlorohemin, (b) chloromesohemin, (c) chlorodeuterohemin. The frequency scale is in part s per million downfield from DMSO. For other conditions, see text and Table I.

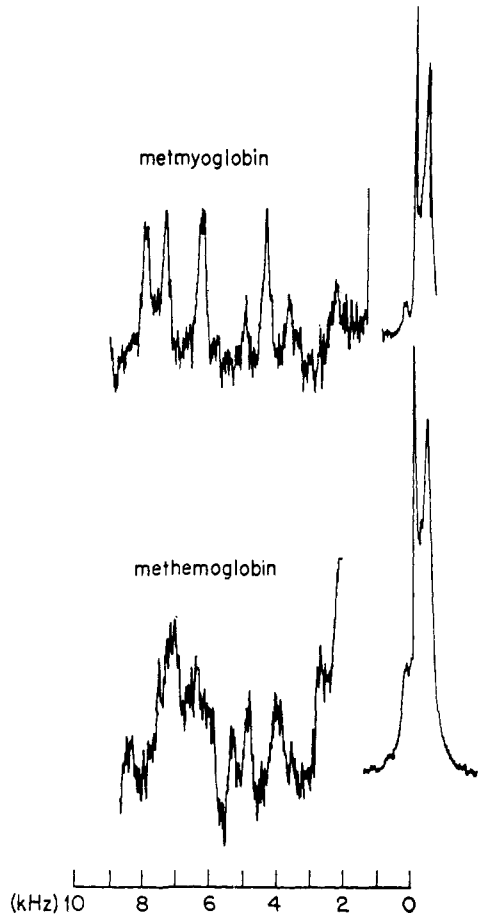
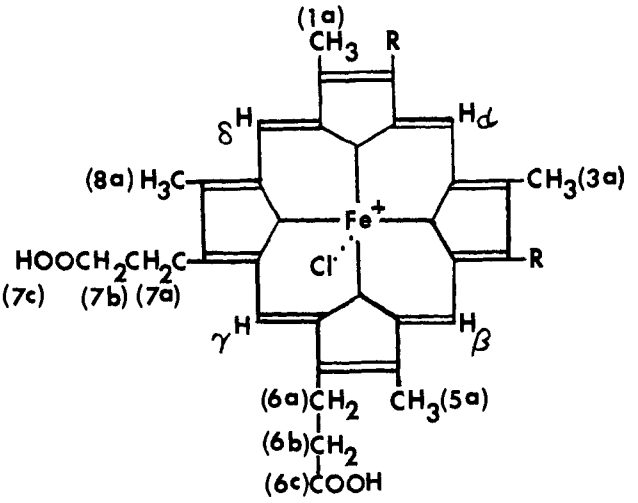


Figure 2. Nmr spectra (100 MHz) of metmyoglobin and methemoglobin. The frequency scale is in kilohertz downfield from the HDO impurity line at "0." Note the change in gain from the region (~0–1 kHz) containing normal diamagnetically shifted lines of the protein to that for the paramagnetically shifted line (~1.5–9 kHz).

order in the applied field of the susceptibility components and the condition that the zero-field splitting parameter, D, is much less than the thermal energy, kT.[9,10]

Variable-temperature studies of these compounds are in progress to gauge the extent of the pseudo-contact interaction. Additional studies are also planned on complexes of the chlorohemin derivatives with nitrogen bases, to aid in the assignment of the lines observed in the metmyoglobin and methemoglobin spectra. Although no concentration dependence of the paramagnetic shifts was found (over a tenfold range), we plan to extend the range of these concentration studies in order to rule out conclusively association effects.



chlorohemin, R = $_{H}>C=C<^{H(2b',4b')}_{H(2b'',4b'')}$ (2a,4a)

chloromesohemin, R = $-CH_2CH_3$(2b,4b) (2a,4a)

chlorodeuterohemin, R = -H(2,4)

Figure 3. The structural formulas for hemin derivatives.

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(9) R. Karplus and J. Schwinger (*Phys. Rev.*, 73, 1020 (1948)) give an appropriate formula for this calculation.

(10) The zero-field splitting constant has been shown both by direct measurements (P. L. Richards, W. S. Caughey, H. Eberspaecher, G. Feher, and M. Malley, *J. Chem. Phys.*, 47, 1187 (1967)) and indirect measurements (P. Eisenberger and P. S. Pershan, *ibid.*, 45, 2832 (1966)) to be of the order 10 cm^{-1}, compared to a thermal energy at room temperature of about 200 cm^{-1}.