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Nuclear hyperfine interaction in ferrihemoglobin hydroxide: Theory

R. R. Sharma

Department of Physics, University of Illinois, Chicago, Illinois 60680
and Solid State Science, Argonne National Laboratory, Argonne, Illinois 60439

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Expressions appropriate for the evaluation of the hyperfine splitting in ferric-hemoglobin hydroxide have been derived in the Hartree-Fock approximation using "giant" multielectron molecular-orbital wave functions formed as linear combinations of atomic orbitals of the constituent atoms and the molecular orbitals of the OH^- complex incorporating overlap effects. Core orbitals as well as valence orbitals have been considered. The relevant multicenter matrix elements have been evaluated accurately by means of the analytical expressions which we have derived by employing a general closed-form expression developed previously for the coefficients of the expansion of a Slater orbital from one center onto the other. Various calculated electric field gradient components have been analyzed separately in terms of the valence and the core orbitals of the central ion (iron) interacting with the orbitals of the other constituent atoms and the complex OH^-. One finds that the "local" and "distant" parts of the field gradient due to OH^- nearly cancel one another and, consequently, produce negligible effect on the hyperfine splitting of iron. The nitrogens of the porphyrin plane contribute dominantly to the splitting. Other surrounding atoms contribute less and their influence decreases rapidly as their distance from the central ion increases. On combining various contributions, the calculated hyperfine splitting comes out to be $1.44 + 0.16$ mm/sec, which agrees excellently with the experimental splitting 1.57 mm/sec observed by Lang and Marshall. The results from the present calculations have been compared with those obtained by Weissbluth and Maling employing semiempirical treatment on a porphyrin-hydroxide model compound. Their estimate is found to give negative sign to the splitting in contrast with our result. Sources of disagreement have been pointed out. Comparison has also been made with the previous calculations on hemin and the observed differences in the splittings in relation to the present system have been clarified. Arguments have been presented, which lead to the prediction of positive hyperfine splitting in ferric-hemoglobin compounds.

I. INTRODUCTION

Hemoglobins, because of their importance, have been extensively studied and are of great general interest. Experimental studies include magnetic susceptibilities,[1,2] Mössbauer effect,[3-8] electron-spin resonance,[9-11] absorption spectroscopy,[12-16] and x-ray diffraction.[17-21] Theoretical works have been performed by Griffith[22] and Kotani[23] with the point of view of explaining the magnetic properties of hemoproteins. Semiempirical molecular orbitals have been calculated by Ohno *et al.*,[24] Veillard and Pullman,[25] Pullman *et al.*,[26] and Zerner *et al.*[27]

Mössbauer absorption studies have been performed by Gonser and co-workers,[3] Maling and Weissbluth,[5] Karger,[6] Lang and Marshall,[7,8,28] Caughey *et al.*,[29] Winter *et al.*,[30] and others. *De facto*, the first measurement of the Mössbauer effect in a biomolecule, hemin, was made by Gonser[31] just four years after the discovery of the Mössbauer effect. Lang and Marshall[8] performed the first systematic studies of biological molecules by the Mössbauer effect. They were able to eliminate the difficulties in the measurements associated with the extreme dilution of the Mössbauer nucleus in these systems. Experimental data and their interpretations on hemoglobins and other related compounds of biological interest have been summarized by various authors.[32-37]

Weissbluth and Maling[38] have interpreted the Mössbauer data utilizing the electronic populations available from the extended Hückel calculations of Zerner *et al.* First-principles calculations[39-41] have also been performed on the isomer shift and nuclear quadrupole splitting in hemin using the molecular-orbital scheme involving the overlap covalency and induced polarizations.

In this paper we consider ferrihemoglobin hydroxide[42] (HiOH) to study the nuclear hyperfine interactions associated with its active center, the iron nucleus. Since the hyperfine interactions depend on the electronic structure surrounding the active center and because in biochemical reactions the changes which occur in the electronic environment are localized at this center, it is crucial to understand the hyperfine interactions in this system.

Though the experimental data are now available for many hemoglobin compounds, the hemoglobin hydroxide is of particular interest, since *inter alia*, the semiempirical treatment of Weissbluth and Maling[38] yielded the sign of the hyperfine splitting in ferrihemoglobin hydroxide opposite to that of other hemoglobin compounds. This raises the question whether the hemoglobin hydroxide is basically different from all hemoglobin compounds or it is the semiempirical treatment which brings about this difference. Other point of special concern is the large observed value[8] of the nuclear hyperfine splitting in ferrihemoglobin hydroxide. The large value could not be accounted for in terms of the usual (though faulty) assumption[43] that the d-electron charge distribution of a high-spin ferric system possesses spherical symmetry and that the contribution from the ligands and remote atoms are small.

In our calculations we have used the Hartree-Fock approximation in the molecular-orbital scheme. The multielectron molecular-orbital wave functions have been constructed by means of the Hund–Mulliken–Van Vleck[44] molecular orbital linear combination of the orbitals of the atoms and complexes constituting the system considering Pauli overlap effects. This is an extension of the procedure adopted previously for evaluation of the electric field gradients in nonmetals.[45-48] A similar procedure has been successful in accounting for the Mössbauer quadrupole splitting in ferric hemin.[41] There are certain advantages associated with such a procedure. Firstly, no uncertain parameters are involved in the theory. This eliminates the fitting procedure so essential in semiempirical treatments, such as in the extended Hückel approximation, where the parameters in the theory are derived by fitting[27] with the optical spectra or, in general, any experimentally observed quantities. Secondly, in the present procedure the core as well as the valence orbitals of the atoms and complexes in the system can be incorporated adequately in forming the multielectron molecular orbitals. The core states—particularly the p states of Fe—are found to give dominant contributions[40,41,45-49] and therefore, required to be included in the theory. Such states have not been given consideration in earlier semiempirical treatments.[38] Thirdly, our procedure is a practical one for understanding the complicated systems since the relative cost of calculations is not prohibitively high.

The present system (hemoglobin hydroxide) is more complicated than hemin since a larger number of atoms are involved in the former. In hemoglobin hydroxide the fifth ligand site of iron is occupied by nitrogen of hystidine, whereas it is empty in hemin and at the sixth ligand site an OH^- complex is attached instead of a single atom or iron as Cl^- in hemin. The contributions from these ligands must be incorporated appropriately so as to obtain a reliable estimate of the hyperfine interaction. In the semiempirical treatment of Weissbluth and Maling[38] the nitrogen of hystidine seems to have been completely ignored. For estimating the contributions from the complexes one necessitates the evaluation of various multicenter matrix elements accurately. This is essential since, otherwise, large errors in the calculated hyperfine splitting occurs as a result of mutual cancellations of the electronic and the nuclear contributions and also as a result of the enhancement due to the Sternheimer antishielding factor.

In Sec. II we describe breifly the theory adopted here and present various simplified expressions. The details of the calculations and the results for the hemoglobin hydroxide have been given in Sec. III and the comparison with the experimental results has been made. Section IV consists of discussion and conclusion.

II. THEORY

Main points of the theory of electric hyperfine interactions involving molecular orbitals have previously been outlined.[41,45-48] For hyperfine interactions one requires to evaluate the electric-field gradients (EFG's) at a required site due to the electronic and nuclear charge distributions in a given system. For the nuclear charge distributions one first assumes the positions of the nuclei to be fixed and then calculates the relevant EFG's in a straightforward manner. However, the problems arise when one evaluates the EFG's from the electronic charge distributions. One usual way to obtain the electronic EFG's is by means of the molecular orbitals of the system which are taken as linear combinations of atomic orbitals. If Ψ_i are the molecular orbitals then

$$\Psi_i = \sum_j C_{ij} \chi_j , \tag{1}$$

where C_{ij} are the coupling coefficients for the linear combination of the atomic orbitals χ_j. For complicated systems the coefficients C_{ij} are determined approximately. Zerner *et al.*[27] used the extended Hückel approximation to adjust the values of C_{ij} to analyze the optical properties of metalloporphyrins. These values of the coefficients C_{ij} were also utilized by Weissbluth and Maling to study the hyperfine interactions in hemoglobin compounds. In these molecular orbitals the core orbitals of the atoms involved were ignored and, consequently, the pertinent contributions to EFG's (particularly, from the $3p$ orbitals of iron which are found to be important) were lost.

Since in complicated systems the cost of calculations of the wave functions is high and the labor involved is enormous, we search for a method which is not very costly, does not require formidable calculations and incorporate important mechanisms and interactions which lead to correct

EFG's. An appropriate method known is the one based on the Hund–Mulliken–Van Vleck linear combination of atomic orbitals involving overlap effects. This method has also the provision of including the valence and the core states; we intend to apply it in our calculations.

We have pursued the Hartree-Fock (HF) formalism which is expected to yield correct results for the EFG in hemoglobin hydroxide as our experience shows in case of hemin. This is more so because the core p-electron wave functions which are close to the iron nucleus produce the dominant EFG. In the Hartree-Fock method since the corefunctions are appropriately incorporated, and because the core functions are not expected to change very much in more accurate wave functions, we expect that the HF method should produce good results and that the results from better wave functions beyond the HF wave functions should not differ very much.

Since we are concerned at present with the hemoglobin hydroxide in which OH^- complex (besides the nitrogens from the porphyrin and histidine) is linked to iron, one requires to modify the linear combinations appropriately in the molecular orbitals in such a way that the complex OH^- is accounted for adequately. This is essential since the OH^- is more like a tight unit itself and ought to be considered as realistically as possible. In other words, the molecular orbitals of OH^- must be included explicitly in forming the molecular orbitals of the systems.

Accordingly, we form the molecular orbitals of HiOH as linear combinations of the overlapping atomic and molecular orbitals of the constituent atoms and complexes such that

$$\begin{aligned}\Psi_i \equiv \Psi^{ab}_{n'L'M'} &= N_{n'L'M'} \\ &\times\Big(\psi^0_{n'L'M'} - \sum_{gnLM} S_{g,nLM,n'L'M'}\,\chi_{gnLM} \\ &\quad - \sum_{i\gamma} S_{\mathrm{OH}^-,i\gamma,n'L'M'}\Phi_{\mathrm{OH}^-,i\gamma}\Big) \\ \Psi_i \equiv \Psi^b_{g,nLM} &= \chi_{g,nLM} \\ &\equiv \Psi^b_{\mathrm{OH}^-,nLM} = \Phi_{\mathrm{OH}^-,i\gamma}\,,\end{aligned} \tag{2}$$

where the molecular orbitals Ψ_i of the system involve explicitly the molecular orbitals $\Phi_{\mathrm{OH}^-,i\gamma}$ of the complex OH^-; $i\gamma$ specifies the molecular-orbital quantum numbers. To make distinction with the molecular orbitals such as $\Phi_{\mathrm{OH}^-i\gamma}$ we shall refer to the molecular orbitals Ψ_i of the system as "giant molecular orbitals" (GMO). In the above "ab" and "b" stand for the antibonding and bonding orbitals, respectively. $\psi^0_{n'L'M'}$ are the atomic orbitals of Fe^{3+} with $n'L'M'$ as the corresponding quantum numbers. χ_{gnLM} are the orbitals of the surrounding atoms located at site "g" with quantum numbers nLM. The parameters $S_{g,nLM,n'L'M'}$ are the matrix elements $\langle\chi_{gnLM}|\psi_{n'L'M'}\rangle$ and similarly

$$S_{\mathrm{OH}^-,i\gamma,n'L'M'} = \langle\Phi_{\mathrm{OH}^-,i\gamma}|\psi_{n'L'M'}\rangle;$$

$N_{n'L'M'}$ are the normalization constants for the GMO's Ψ_i. The GMO's of Eq. (2) are more complex in structure compared with the ones used for the nonmetals[45-48] or hemin[41] as it is evident since $\Phi_{\mathrm{OH}^-i\gamma}$ themselves are the complicated molecular orbitals.

In the present calculation we do not plan to include the induced polarization effects as were considered in case of hemin. This is because (i) the calculations without polarizations are expected to yield reasonably good results as it is manifested from our calculations in hemin, (ii) it is more instructive to, first, solve the problem without polarizations and include them later to separate the effect of the polarizations, and (iii) the induced polarizations increase the complicacy of the calculations manyfold.

Concerning the basis orbitals we take the Fe^{3+} orbitals $1s^2$, $2s^2$, $2p^6$, $3s^2$, $3p^6$, and $3d^5$ for $\Psi^0_{n'L'M'}$ in Eq. (2). As for the atomic functions $\chi_{g,nLM}$ we consider $2s^2$, $2p^3$ orbitals of the four nitrogens of the porphyrin plane and one nitrogen at the fifth ligand site from the hystidine and $2s^2$, $2p^2$ orbitals of the surrounding carbons. For the complex molecular orbitals $\Phi_{\mathrm{OH}^-,i\gamma}$ we take into account 1σ, 2σ, 3σ, 1π, and 2π molecular orbitals of OH^- which may be written

$$\Phi_{\mathrm{OH}^-,i\gamma} = \sum_{tN_tL_tM_t} d_{t,i\gamma N_tL_tM_t}\,\chi_{t,N_tL_tM_t}\,, \tag{3}$$

where t assigns the locations of O and H in OH^-; $\chi_{t,N_tL_tM_t}$ are the basis functions (with quantum numbers $N_tL_tM_t$) of O and H used to construct the linear combinations with $d_{t,i\gamma,N_tL_tM_t}$ as the coupling coefficients; $i\gamma$ assumes the values 1σ, 2σ, 3σ, 1π, 2π for various molecular orbitals of OH^-.

Now the electronic contribution to the field gradient q_{el} may be obtained from the GMO's of Eq. (2). In HF approximation,

$$q_{el} = e\sum_j \epsilon_j\langle\Psi_j|(3\cos^2\theta_j - 1)/r_j^3|\Psi_j\rangle\,, \tag{4}$$

where e is the charge of an electron including its sign and ϵ_j is the occupation number of Ψ_j.

The total contribution to the field gradient q is obtained by combining appropriately the nuclear and electronic components as

$$q = (1-R)(q'_i + q'_{nl}) + (1-\gamma_\infty)(q'_d + q'_{db} + q'_n)\,, \tag{5}$$

where $1-R$ and $1-\gamma_\infty$ are the Sternheimer shielding factors. The notations in Eq. (5) are the same as used in Ref. 48. We have adopted "primes" to denote the EFG's without shielding effects. In Eq. (5) the subscripts l, nl, and d designate, respectively, the "local", "nonlocal", and "distant" components of the EFG's from the antibonding GMO's; db and n designate the EFG's from the bonding (distant) GMO's and the nuclear charges, respectively.

The "local," etc., parts appearing in Eq. (5) may be written explicitly as follows:

$$q'_l = \sum_{n'L'M'} \epsilon_{n'L'M'} |N|^2_{n'L'M'} \langle \psi^0_{n'L'M'} | (3\cos^2\theta - 1)/r^3 | \Psi^0_{n'L'M'} \rangle , \tag{6}$$

$$\begin{aligned} q'_{nl} = - \sum_{n'L'M'} \epsilon_{n'L'M'} \Big(& \sum_{gnLM} [N_{n'L'M'} \langle \Psi^0_{n'L'M'} | (3\cos^2\theta - 1)/r^3 | \chi_{gnLM} \rangle S_{gnLMn'L'M'} + N^*_{n'L'M'} \\ & \times \langle \chi_{gnLM} | (3\cos^3\theta - 1)/r^3 | \Psi^0_{n'L'M'} \rangle S^*_{gnLM,n'L'M'}] \\ & + \sum_{i\gamma} [N_{n'L'M'} \langle \Psi^0_{n'L'M'} | (3\cos^2\theta - 1)/r^3 | \Phi_{\mathrm{OH}^-,i\gamma} \rangle S_{\mathrm{OH}^-,i\gamma,n'L'M'} \\ & + N^*_{n'L'M'} \langle \Psi^0_{n'L'M'} | (3\cos^2\theta - 1)/r^3 | \Phi_{\mathrm{OH}^-,i\gamma} \rangle^* S^*_{\mathrm{OH}^-,i\gamma,n'L'M'}] \Big) , \end{aligned} \tag{7}$$

$$\begin{aligned} q'_d = \sum_{n'L'M'} \epsilon_{n'L'M'} |N_{n'L'M'}|^2 \Big(& \sum_{gnLMg''n''L''M''} S^*_{gnLMn'L'M'} S_{g''n''L''M''n'L'M'} \\ & \times \langle \chi_{gnLM} | (3\cos^2\theta - 1)/r^3 | \chi_{g''n''L''} \rangle + \sum_{i\gamma i'\gamma'} S^*_{\mathrm{OH}^- i\gamma,n'L'M'} S_{\mathrm{OH}^- i'\gamma' n'L'M'} \\ & \times \langle \Phi_{\mathrm{OH}^- i\gamma} | (3\cos^2\theta - 1) r^3 | \Phi_{\mathrm{OH}^- i'\gamma'} \rangle \\ & + \sum_{i\gamma gnLM} [S^*_{gnLMn'L'M'} S_{\mathrm{OH}^- i\gamma n'L'M'} \langle \chi_{gnLM} | (3\cos^2\theta - 1)/r^3 | \Phi_{\mathrm{OH}^-,i\gamma} \rangle \\ & + S_{gnLMn'L'M'} S^*_{\mathrm{OH}^-,i\gamma n'L'M'} \langle \Phi_{\mathrm{OH}^- i\gamma} | (3\cos^2\theta - 1)/r^3 | \chi_{gnLM} \rangle] \Big) , \end{aligned} \tag{8}$$

$$q'_{db} = \sum_{gnLM} \epsilon_{gnLM} \langle \chi_{gnLM} | (3\cos^2\theta - 1)/r^3 | \chi_{gnL} \rangle + \sum_{i\gamma} \epsilon_{i\gamma} \langle \Phi_{\mathrm{OH}^- i\gamma} | (3\cos^2\theta - 1)/r^3 | \Phi_{\mathrm{OH}^- i\gamma} \rangle , \tag{9}$$

and

$$q'_n = \sum_g [\zeta_{ng} |e| (3\cos^2\theta_g - 1)/a^3_g] + \sum_t \zeta_{nt} [\,|e| (3\cos^2\theta_t - 1)/a^3_t] , \tag{10}$$

where the $i\gamma$ summation takes care of the contributions from 1σ, 2σ, 3σ, 1π, and 2π (molecular orbitals) of OH^-. $\epsilon_{i\gamma}$ is the occupation number of the molecular orbital $i\gamma$. $\zeta_{ng}|e|$ is the "net" charge of the ligand nucleus located at site g, which is obtained by reducing the actual charge of the nucleus by the amount equal to the total charge of those electrons which have not been considered for constructing the bonding orbitals. Similarly, ζ_{nt} represents the "net" charge of the nuclei O and H in the complex OH^-. For other symbols one may consult Ref. 48. The above expressions [Eqs. (6)–(9)] can be simplified in a manner analogous to the one adopted previously[48] which involves rotation of the wave functions with the help of the rotation groups and expansion of the functions from one center on to the other by means of the α functions.[50-52]

The simplification yields for the local component,

$$\begin{aligned} q'_l = 2e \left(\frac{4\pi}{5} \right)^{1/2} \sum_{n'L'} \left\langle \frac{1}{r^3} \right\rangle_{n'L'} \sum_{M'} \epsilon_{n'L'M'} & \\ \times \Big(\sum_{i\gamma} \Big| \sum_{tN_tL_tM_t} d^*_{ti\gamma N_tL_tM_t} b^{*(\gamma_t,-\beta_t,-\alpha_t)}_{L',M_t,M'} M^{n_tL_tM_t}_{n'L'}(t) \Big|^2 & + [M^{nLM}_{n'L'}(g)]^2 [B_{L'M'M'}(-\beta_g)]^2 \Big) \\ \times F^{0,M'}_{2,L'}(L') , & \end{aligned} \tag{11}$$

where $M^{nLM}_{n'L'}(g)$ are the radial parts of the overlap integrals between the wave functions $\Psi_{n'L'M'}$ and $\chi_{t,N_tL_tM_t}$. For the nonlocal component,

$$\begin{aligned} q'_{nl} = -4e\left(\frac{4\pi}{5}\right)^{1/2} \sum_{n'L'} \Bigg(& \sum_{i\gamma}\sum_{tN_tL_tM_t} d_{i\gamma tN_tL_tM_t} \sum_{t'N'_tL'_tM'_t} d_{i\gamma t'N_{t'}L_{t'}M_{t'}} \\ & \times M_{n'L'}^{N_{t'}L_{t'}M_{t'}}(t') \sum_{l'} \left\langle u^0_{n'L'} \left| \frac{1}{r^3} \right| \alpha'_l(N_tL_tM_t \,|\, a_t r)\right\rangle \\ & \times \sum_{M'} \epsilon_{n'L'M'} B_{L'M_{t'}M'}(-\beta_g) B_{lM_tM'}(-\beta_g) F^{-M}_{L',0}(l') \\ & + \sum_{gnLMl'} M^{nLM}_{n'L'}(g) \left\langle u^0_{n'L'} \left| \frac{1}{r^3} \right| \alpha_{l'}(nLM \,|\, a_g r)\right\rangle \\ & \times \sum_{M'} \epsilon_{n'L'M'} B_{L'MM'}(-\beta_g) B_{l'MM'}(-\beta_g) F^{-M',0}_{L',2} \Bigg). \end{aligned} \tag{12}$$

For the distant component,

$$\begin{aligned} q'_d = 2e\left(\frac{4\pi}{5}\right)^{1/2} \sum_{n'L'} \Bigg(& \sum_{i\gamma tN_tL_tM_t} d^*_{i\gamma tN_tL_tM_t} \sum_{i'\gamma' t'N_{t'}L_{t'}M_{t'}} d_{i'\gamma' t'N_{t'}L_{t'}M_{t'}} M^{N_tL_tM_t}_{n'L'}(t) M^{N_{t'}L_{t'}M_{t'}}_{n'L'}(t') \\ & \times \sum_{\tau N_\tau L_\tau M_\tau \tau' N_{\tau'} L'_\tau M'_\tau} d^*_{i\gamma\tau N_\tau L_\tau M_\tau} d_{i'\gamma'\tau' N'_\tau L'_\tau M'_\tau} \\ & \times \langle \chi_\tau N_\tau L_\tau M_\tau \,|\, Y^0_2/r^3 \,|\, \chi_{\tau' N'_\tau L'_\tau M'_\tau}\rangle \sum_{M'} \epsilon_{n'L'M'} B^{(-\beta t)}_{L'M_tM'} B^{(\beta t')}_{L',M_{t'},M'} \\ & + \sum_{n'L'M'gnLMn''L''M''} M^{n''L''M''}_{n'L'}(g) B_{2,0,M-M''}(\beta_g) \sum_{l} (-1)^l \langle v^0_{nl} \,|\, \alpha'_l(Q\,20 \,|\, a_g R) \,|\, v^0_{n''L''}\rangle F^{M-M'',M''}_{l,L''}(L) M^{nlM}_{n'L'}(g) \\ & \times \sum_{M'} \epsilon_{nL'M'} B_{L'MM'}(-\beta_g) B_{L',M'',M'}(-\beta_g) \Bigg). \end{aligned} \tag{13}$$

For the distant-bonding component,

$$\begin{aligned} q'_{db} = 2e\left(\frac{4\pi}{5}\right)^{1/2} \Bigg(& \sum_{i\gamma tN_tL_tM_t t'N_{t'}L_{t'}M_{t'}} \epsilon_{i\gamma} d^*_{i\gamma tN_tL_tM_t} d_{i'\gamma't'N_{t'}L_{t'}M_{t'}} \langle \chi_{tN_tL_tM_t} | Y^0_2/r^3 | \chi_{t'N_{t'}L_{t'}M_{t'}}\rangle \\ & + \sum_{gnLM} \epsilon_{gnLM} B_{2,0,0}(\beta_g) \sum_l (-1)^l \langle v^0_{nL} | \alpha'_l\ (Q20|gR) | v^0_{nL}\rangle F^{0,M}_{l,L}(L) \Bigg). \end{aligned} \tag{14}$$

In the preceeding expressions t (or t', τ, and τ') locates the position of O and H in OH$^-$; $v_{N_tL_t}$ is the radial part of the basis function $\chi_{t,N_tL_tM_t}$ such that

$$\chi_{tN_tL_tM_t} = v^0_{N_tL_t}(R) Y^{M_t}_{L_t}(\Theta, \Phi). \tag{15}$$

The remaining symbols carry the same meaning as in Ref. (48). One may further simplify the above expressions for particular values of the quantum numbers making use[53,54] of the appropriate rotation-group elements, in general, $B_{lmm'}(\beta)$ and the Clebsch-Gordan coefficients, in general, $F_{l,l'}(L)$.

For the wave functions located at site g the simplified expressions are similar to the one given in Refs. 41 and 48; because of the lack of space we are compelled to omit them here. As for the simplified expressions concerning the contributions from the OH$^-$, we will deal with them as follows. The "local" part of EFG arising from the interaction between the $3d$ orbitals of iron and the $i\gamma$ molecular orbits of OH$^-$ simplifies to

$$q'_l(3d - i\gamma) = \frac{4e}{7} \langle r^{-3}\rangle_{3d} \left(\sum_{i\gamma=1\sigma,2\sigma,3\sigma} \left| \sum_{tN_tL_t} d^*_{ti\gamma N_tL_t0} M^{n_tL_t0}_{3d}(t)\right|^2 + \sum_{i\gamma=1\pi,2\pi} \left| \sum_{tN_tL_t} d^*_{ti\gamma N_tL_t1} M^{n_tL_t1}_{3d}(t)\right|^2 \right), \tag{16}$$

where $M^{n_tL_t0}_{3d}(t)$ and $M^{n_tL_t1}_{3d}(t)$ represent, respectively, the appropriate σ and π overlap integrals.

Similarly, the contribution form the $3p$ orbitals of iron becomes

$$q'_l(3p - i\gamma) = \frac{8e}{5} \langle r^{-3}\rangle_{3p} \left(\sum_{i\gamma=1\sigma,2\sigma,3\sigma} \left| \sum_{tN_tL_t} d^*_{ti\gamma N_tL_t0} M^{n_tL_t0}_{3p}(t)\right|^2 - \sum_{i\gamma=1\pi,2\pi} \left|_{tN_tL_t} d^*_{ti\gamma N_tL_t1} M^{n_tL_t1}_{3p}(t)\right|^2 \right). \tag{17}$$

The expressions for $q_l(2p - i\gamma)$ can be obtained from the above expression for $q_l(3p - i\gamma)$ by replacing everywhere $3p$ by $2p$. The local contributions arising from $2s$ and $3s$ orbitals of iron, viz., $q_l(3s-i\gamma)$ and $q_l(2s-i\gamma)$ vanish.

The nonlocal part of EFG arising from the interaction between the $3d$ orbitals of iron and $i\sigma$ molecular orbitals of OH$^-$ may be written

$$q'_{nl}(3d-i\sigma)=-\frac{8e}{\sqrt{5}}\sum_{i\gamma=1\sigma,2\sigma,3\sigma}\left(\sum_{t'N_{t'}L_{t'}} M_{3d}^{N_tL_t0}(t)d_{t'i\gamma N_{t'}L_{t'}0}\right)$$

$$+\sum_{tN_tL_t} d_{ti\gamma N_tL_t0}\left(\frac{1}{2}\langle u_{3d}^0|r^{-3}|\alpha_0(N_tL_t0|a_tr)\rangle+\frac{\sqrt{5}}{7}\langle u_{3d}^0|r^{-3}|\alpha_2(N_tL_t0|a_tr)\rangle\right.$$

$$\left.+\frac{3}{7}\langle u_{3d}^0|r^{-3}|\alpha_4(N_tL_t0|a_tr)\rangle\right). \tag{18}$$

Similarly, the contribution from $i\pi$ orbitals of OH$^-$ reads,

$$q'_{nl}(3d-i\pi)=-\frac{8e}{7}\sum_{i\gamma=1\pi,2\pi}\left(\sum_{t'N_{t'}L_{t'}} d_{t'i\gamma N_{t'}L_{t'}1}M_{3d}^{N_{t'}L_{t'}1}(t')\right)\sum_{tN_tL_t} d_{ti\gamma N_tL_t0}[\langle u_{3d}^0|r^{-3}|\alpha_2(N_tL_t1|a_tr)\rangle$$

$$+\sqrt{6}\langle u_{3d}^0|r^{-3}|\alpha_4(N_tL_t1|a_tr)\rangle]. \tag{19}$$

The "nonlocal part" of the EFG resulting from the interaction between the $3p$ orbitals of iron and $i\sigma$ molecular orbitals of OH$^-$ is

$$q'_{nl}(3p-i\sigma)=-\frac{16e}{5}\sum_{i\gamma=1\sigma,2\sigma,3\sigma}\left(\sum_{t'N_{t'}L_{t'}} d_{t'i\gamma N_{t'}L_{t'}0}M^{n_{t'}L_{t'}0}(t')\right)\sum_{tN_tL_t} d_{ti\gamma N_tL_t0}\left[\langle u_{3p}^0|r^{-3}|\alpha_1(N_tL_t0|a_tr)\right.$$

$$\left.+\left(\frac{3\sqrt{3}}{2\sqrt{7}}\right)\langle u_{3p}^0|r^{-3}|\alpha_3(N_tL_t0|a_tr)\rangle\right]. \tag{20}$$

Analogously, the $3p$-$i\pi$ contribution is expressed as

$$q'_{nl}(3p-i\pi)=-\frac{18e}{5}\sum_{i\gamma=1\pi,2\pi}\left(\sum_{t'N_{t'}L_{t'}} d_{t'i\gamma N_{t'}L_{t'}1}M_{3p}^{n_{t'}L_{t'}1}(t')\right)$$

$$\times\sum_{tN_tL_t} d_{ti\gamma N_tL_t1}\left[-\langle u_{3p}^0|r^{-3}|\alpha_1(N_tL_t1|a_tr)\rangle+\left(\frac{3\sqrt{2}}{\sqrt{7}}\right)\langle u_{3p}^0|r^{-3}|\alpha_3(N_tL_t1|a_tr)\rangle\right]. \tag{21}$$

The "nonlocal" interaction of the $3s$ orbitals of iron with the $i\sigma$ and $i\pi$ molecular orbitals of OH$^-$ yields,

$$q'_{nl}(3s-i\sigma)=-\frac{8e}{\sqrt{5}}\sum_{i\gamma=1\sigma,2\sigma,3\sigma}\left(\sum_{t'N_{t'}L_{t'}} d_{t'i\gamma N_{t'}L_{t'}0}M_{3s}^{N_{t'}L_{t'}0}\right)\sum_{tN_tL_t} d_{ti\gamma N_tL_t0}\langle u_{3s}^0|r^{-3}|\alpha_2(N_tL_t0|a_tr\rangle, \tag{22}$$

$$q_{nl}(3s-i\pi)=0. \tag{23}$$

To obtain $q'_{nl}(2s-i\sigma)$ and $q'_{nl}(2s-i\pi)$one replaces $3s$ by $2s$ throughout the above expressions.

The expressions for q'_d and q'_{db}, are more difficult to deal with because they contain both the two-center and three-center integrals depending on whether t and t' represent the same or different locations. Since the "distant" parts of EFG are enhanced by the Sternheimer factor $(1-\gamma_\infty)$, these integrals are to be calculated accurately. The q'_d component which depends on the interaction of the $3d$ orbitals of iron with the molecular orbitals of OH$^-$ may be simplified to

$$q'_d(3d-i\gamma)=\left(\frac{2}{\sqrt{5}}\right)e\sum_{i\gamma tN_tL_tM_t} d^*_{i\gamma tN_tL_tM_t}\sum_{i'\gamma't'N_{t'}L_{t'}} d_{i'\gamma't'N_{t'}L_{t'}M_t}M_{3d}^{N_tL_tM_t}(t)M_{3d}^{N_{t'}L_{t'}M_t}(t)$$

$$\times\sum_{\tau N_\tau L_\tau M_\tau}\sum_{\tau'N_{\tau'}L_{\tau'}} d^*_{i\gamma\tau N_\tau L_\tau M_\tau}d_{i'\gamma'\tau'N_{\tau'}L_{\tau'}M_{\tau'}}\langle\chi_{\tau N_\tau L_\tau M_\tau}|Y_2^0/r^3|\chi_{\tau'N_{\tau'}L_{\tau'}M_{\tau'}}\rangle \tag{24}$$

Similar expressions for $q_d'(3p-i\gamma)$, $q_d'(3s-i\gamma)$, $q_d'(2p-i\gamma)$, and $q_d'(2s-i\gamma)$ are also obtained on simplification from Eq. (13). All these expressions involve, in general, the three-center integrals $\langle \chi_{\tau N_\tau L_\tau M_\tau} | Y_2^0 | r^3 | \chi_{\tau' N_{\tau'} L_{\tau'} M_{\tau'}} \rangle$ which, for the general Slater-type orbitals, read

$$\left\langle R_1^{N_1-1} e^{-\eta_1 R_1} Y_{L_1}^{M_1}(\Theta_1,\Phi_1) \right| \frac{Y_2^0(\theta,\phi)}{r^3} \left| R_2^{N_2-1} e^{-\eta_2 R_2} Y_{L_2}^{M_2}(\Theta_2,\Phi_2) \right\rangle$$

$$\begin{aligned} = \delta_{M_1,M_2} \Bigg\{ \sum_{l_1} \sum_{l_2=|2-l_1|}^{2+l_1} F_{2,l_2}^{0,M_2}(l_1) \sum_{k_1'=0}^{l_1+N_1} \sum_{k_2'=0}^{l_2+N_2} \left(\frac{1}{a_1}\right)^{k_1'-l_1} \left(\frac{1}{a_2}\right)^{k_2'-l_2} \Big[& A_{k_1'}^{(1)} A_{k_2'}^{(2)} a_1^{k_1'-l_1+k_2'-l_2-2} E_{n'} | 0,\infty;(\eta_1+\eta_2)a_1 | \\ & + A_{k_1'}^{(1)} B_{k_2'}^{(2)} a_2^{n'+1} E_{n'}[0,1;(\eta_1-\eta_2)a_2] + B_{k_1'}^{(1)} A_{k_2'}^{(2)} a_1^{n'+1} E_{n'}[0,1;(\eta_2-\eta_1)a_1] \\ & + B_{k_1'}^{(1)} B_{k_2'}^{(2)} a_1^{n'+1} E_{n'}[0,1;(-\eta_1-\eta_2)a_1] + D_{k_1'}^{(1)} A_{k_2'}^{(2)} a_1^{n'+1} E_{n'}[1,\infty;(\eta_1+\eta_2)a_1] \\ & + D_{k_1'}^{(1)} B_{k_2'}^{(2)} a_1^{n'+1} E_{n'}\left[1,\frac{a_2}{a_1};a_1(\eta_1-\eta_2)\right] + (A_{k_1'}^{(1)}+D_{k_1'}^{(1)}) D_{k_2'}^{(2)} a_2^{n'+1} E_{n'}[1,\infty;a_2(\eta_1+\eta_2)] \Big] \Bigg\} , \end{aligned} \tag{25}$$

where the coordinates r, θ, ϕ are measured with respect to the origin located at the iron nucleus; the coordinates $R_1\Theta_1\Phi_1$ and R_2, Θ_2, Φ_2 are measured with respect to the locations $(0,0,a_1)$ and $(0,0,a_2)$, respectively. The symbols A_k, B_k, C_k, and D_k are defined by Eq. (29) of Ref. 50. $A_k^{(1)}$ is obtained from A_k by substituting $a_1N_1\eta_1 l_1 L_1 M_1$ for $aN\eta lLM$ in the expression for A_k, whereas $A_k^{(2)}$ by substituting $a_2N_2\eta_2 l_2 L_2 M$. Analogous meanings stand for the other parameters $B_k^{(1)}$, $B_k^{(2)}$, $C_k^{(1)}$, $C_k^{(2)}$, $D_k^{(1)}$, and $D_k^{(2)}$. In Eq. (25), n' is defined by

$$n' = k_1' - l_1 + k_2' - l_2 - 3 ,$$

and, in general,

$$E_n[a,b;\eta] = \int_a^b r^n e^{-\eta r} dr , \tag{26}$$

where n is any (negative, positive, or zero) integers. The molecular orbitals $\Phi_{\mathrm{OH}^-,i\gamma}$ contain the basis functions

$$\begin{aligned} \chi_{t,N_tL_tM_t} &\equiv (1s)_t = (Z_1^3/\pi)^{1/2} e^{-Z_1R} \\ &\equiv (2s)_t = (2S)_t' - C(1S)_t \\ &\equiv (2p_0)_t = (\tfrac{4}{3} Z_2^5)^{1/2} R e^{-Z_2R} Y_1^0(\Theta,\Phi) \\ &\equiv (2p_{\pm 1})_t = (\tfrac{4}{3} Z_2^5)^{1/2} R e^{-Z_2R} Y_1^{\pm 1}(\Theta,\Phi) , \end{aligned} \tag{27}$$

where

$$(2s)_t' = (Z_2^5/3\pi)^{1/2} R e^{-Z_2R} , \tag{28}$$

and $Z_1 = 7.70$ and $Z_2 = 2.275$ for oxygen. For hydrogen $\chi_{t,N_tL_tM_t} \equiv (1s)_t = 1/\sqrt{\pi}\, e^{-R}$. In Eq. (27), C is determined so as to orthogonalize the $(2s)$ function with the $(1s)$ function.

Though the expression (25) is useful for both the two- and three-center integrals, the two-center integrals in the present case can be obtained in closed and elegant forms following an alternate route. Explicitly, one expands the operator, instead of the wave functions, in the integrand as explained in Ref. 48 and, on simplification, obtains the desired results. The closed forms for the integrals are important and, therefore, expressed as follows for convenience:

$$\begin{aligned} \sqrt{4\pi} \left\langle (1s)_t \right| \frac{Y_2^0}{r^3} \left| (1s)_t \right\rangle &= \frac{\sqrt{5}}{a_t^3} [1 - \exp(-2Z_1a_t) h_2(2Z_1a_t)] , \\ \sqrt{4\pi} \left\langle (1s)_t \right| \frac{Y_2^0}{r^3} \left| (2s)_t' \right\rangle & \end{aligned} \tag{29}$$

$$= \frac{8\sqrt{15}}{a_t^3} \frac{(Z_1^3 Z_2^5)^{1/2}}{(Z_1+Z_2)^4} \times \{1 - \exp[-(Z_1+Z_2)a_t] h_3[(Z_1+Z_2)a_t]\} , \tag{30}$$

$$\sqrt{4\pi} \left\langle (1s)_t \right| \frac{Y_2^0}{r^3} | (2p_0)_t \rangle$$

$$= \frac{96\sqrt{5}}{a_t^4} \frac{(Z_1^3 Z_2^5)^{1/2}}{(Z_1+Z_2)^5} \times \{1 - \exp[-a_t(Z_1+Z_2)] h_4[(Z_1+Z_2)a_t]\} , \tag{31}$$

$$\sqrt{4\pi} \langle (2s)_t' | \frac{Y_2^0}{r^3} | (2s)_t' \rangle$$

$$= \frac{\sqrt{5}}{a_t^3} [1 - \exp(-2Z_2a_t) h_4(2Z_2a_t)] , \tag{32}$$

$$\sqrt{4\pi} \langle (2s)_t' | \frac{Y_2^0}{r^3} | (2p_0)_t \rangle$$

$$= \frac{5\sqrt{15}}{2Z_2} \frac{1}{a_t^4} [1 - \exp(-2Z_2a_t) h_5(2Z_2a_t)] , \tag{33}$$

$$\begin{aligned} \sqrt{4\pi} \left\langle (2p_0)_t \right| \frac{Y_2^0}{r^3} \left| (2p_0)_t \right\rangle = \frac{4}{3} Z_2^5 \Bigg(& \frac{3}{4} \frac{\sqrt{5}}{a_t^3 Z_2^5} [1 - \exp(-2Z_2a_t) h_4(2Z_2a_t)] \\ & + \frac{27\sqrt{5}}{2} \frac{1}{a_t^5 Z_2^7} [1 - \exp(-2Z_2a_t) h_6(2Z_2a_t)] + \frac{\sqrt{5}}{10} \frac{1}{Z_2^2} \exp(-2Z_2a_t)(1 + 2Z_2a_t) \Bigg) , \end{aligned} \tag{34}$$

$$\sqrt{4\pi}\left\langle(2p_\pm)\left|\frac{Y_2^0}{r^3}\right|(2p_\pm)\right\rangle = \frac{4}{3}Z_2^{\frac{5}{2}}\left(\frac{3}{4}\,\frac{\sqrt{5}}{a_t^3 Z_2^{\frac{5}{2}}}\left[1-\exp(-2Z_2a_t)h_4(2Z_2a_t)\right] - \frac{27\sqrt{5}}{4}\,\frac{1}{a_t^5Z_2^7}\left[1-\exp(2Z_2a_t)h_6(2Z_2a_t)\right] - \frac{\sqrt{5}}{20}\,\frac{1}{Z_2^2}\left[\exp(-2Z_2a_t)(1+2Z_2a_t)\right]\right), \tag{35}$$

where we have defined,

$$h_n(\alpha)=1+\alpha+\alpha^2/2!+\cdots+\alpha^n/n!\ , \tag{36}$$

and a_t is assumed to be the distance between the two centers. The expression for q'_{db} giving the contribution from the OH$^-$ can be obtained from Eq. (14) in a similar manner. One needs again the expressions for the two- and three-center integrals $\langle\chi_{tN_tL_tM_t}|Y_2^0/r^3|\chi_{t'N_{t'}L_{t'}M_{t'}}\rangle$ which have already been treated above. The above expressions have been employed in Sec. III to evaluate the EFG's and hence the hyperfine splitting at the iron nucleus in HiOH.

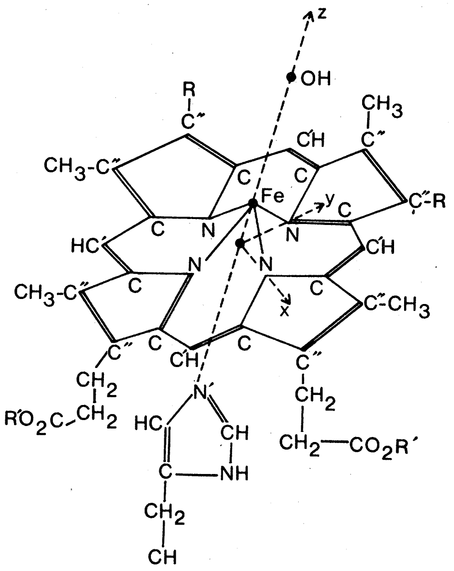

FIG. 1. Depicts part of hemoglobin hydroxide surrounding the iron ion. Four nitrogens (N) are located in the porphyrin plane. The nitrogen (N′) of the histidine lie at the fifth ligand site, whereas the OH$^-$ complex at the sixth ligand site. The carbon atoms C, C′, and C″ are the first, second, and the third nearest carbon atoms, respectively, to the iron. The axes system used for the calculations is also shown.

III. CALCULATIONS, RESULTS, AND COMPARISON WITH EXPERIMENTS-FERRIHEMOGLOBIN HYDROXIDE

Hemoglobins are large complex molecules with molecular weights of 10^4–10^5 consisting of four polypeptide chains, which are identical in pairs, and of four heme groups. Their atomic structure parameters have been determined by Kendrew and co-workers[19-21] and by Perutz and co-workers[17,18] from x-ray scattering experiments by a rather enormously complicated process. The resolution attained in the x-ray analysis is 5.5 Å which gives only approximate locations of the atoms surrounding the heme iron. For quantitative purposes, however, one needs much better accuracy. To the end, we follow an alternate route which is based on the structural information of similar compounds containing heme groups; it is justifiable, partly, because the heme structure is known to be rigid.

Accordingly, for HiOH the distance of the four nitrogen atoms of the porphyrin plane may be assigned as 2.03 Å measured from the porphyrin center as determined by x-ray diffraction studies of ferric porphyrins by Fleischer, Miller, and Webb.[55] As for the coordinates of the carbon atoms in the heme we use the values obtained by Hoard *et al.*[56] from the x-ray data with slight modifications so that the atoms lie in the porphyrin plane retaining the bond lengths as suggested by Zerner *et al.*[27] The distance between the iron and OH$^-$ is taken as 1.842 Å as evidenced by the structure of methoxyferrous mesoporphyrin IX dimethyl ester. The O-H bond length is set equal to the average of the corresponding bond lengths in various other systems,[57] and comes out close to the value 1.8103 Å assumed by Rosenfeld[58] for the molecular orbital calculations of OH$^-$. The position of the nitrogen *N′* of the histidine is obtained from the information on the covalent radii. As for the distance of the iron atom from the center of the heme plane we take 0.455 Å appropriate to the high spin ferric system. In Fig. 1 we depict the relative positions of the atoms immediately surrounding the iron. The coordinates of the atoms are listed in Table I.

Various explicit expressions [such as Eqs. (16)–(24)] useful for the estimation of the EFG's have been described in Sec. II. These expressions require many overlap two and three-center matrix elements. For their evaluation one may follow, as mentioned in Sec. II, the method of expansion of a function from one center onto the other center using the analytical expressions derived in Ref. 50. We obtained analytical closed forms of some of the two-center matrix elements [Eqs. (29)–(35)]

TABLE I. List of the Coordinates of various atoms in A^0 units. The origin of the axes system is assumed to be at the center of the porphyrin plane (see Fig. 1).

Atom	x	y	z
Fe^{3+}	0.000	0.000	0.455
N	2.030	0.000	0.000
N'	0.000	0.000	−2.100
C	1.098	2.839	0.000
C'	2.444	2.444	0.000
C''	4.217	0.681	0.000
O	0.000	0.000	2.297
H	0.000	0.000	3.249

and the general analytical expression [Eq. (25)] of the three-center matrix elements in Sec. II. For the overlap matrix elements we have adopted the general and closed expression derived in Ref. 50.

For our calculations we have made use of the HF self-consistent wave functions[59] for Fe^{3+}, N, and C and the molecular orbitals of OH^- as calculated by Rosenfeld[58] in the self-consistent-field molecular-orbital approximation employing a minimum basis set of Slater-type atomic orbitals [Eq. (27)]. The coefficients $d_{t,i\gamma,NLM}$ of the linear combination of the atomic orbitals [see Eq. (3)] and the occupation number $\epsilon_{i\gamma}$ of the molecular orbitals for OH^- are listed in Table II for easy reference.

The calculated multicenter matrix elements considering all the orbitals of various atoms are numerous and, therefore, not listed here. However, some of the two- and three-center matrix elements which are important for the "distant" bonding and antibonding components of the EFG's, are tabulated in Table III. As emphasized earlier, since these integrals effectively get exemplified by the Sternheimer antishielding factor, we require to calculate them accurately. Special attention is to be paid for the evaluation of the three-center integrals [Eq. (25)], where one needs the generalized exponential integrals [Eq. (26)] which have been calculated by modification of a recent method.[60]

In order to give information of the relative magnitudes of various integrals we mention that the overlap integral between the $3d$ orbitals of Fe and $2p_\sigma$ orbital of the nitrogen of the porphyrin plane comes out to be 0.0621 a.u. The overlap of $3d$ orbital of Fe with the $(2p_\sigma)$ oxygen basis function is also of the same order of magnitude. The similar overlap with the nitrogen of the histidine at the fifth ligand site is, however, smaller by a factor 2. The overlaps of the $3d$ Fe orbitals with the distant atoms such as the first, second, and the third nearest carbon atoms are much smaller in magnitude as expected. The nonlocal integral $\langle u^0_{n'L'}\,|1/r^3|\,\alpha_0(nLM)\rangle$ between the $n'L'$ orbital of iron and nLM orbital of a neighboring atom is found to be always larger than the corresponding overlap integral by a factor of 2–3.5 depending on the neighboring atom and the quantum numbers $n'L'$ and nLM. The distant two-center integrals [see Eqs. (13) and (14)] for neighboring atoms are about 0.036 or lower in magnitude.

Knowing the values of the required integrals one next calculates the "local," etc., parts of EFG arising from various orbitals and various neighbors. For the shielding factors we use $1-R = 0.68$,[61] and $1-\gamma_\infty = 10.14$.[62] The various components of the EFG arising from the complex OH^-, the four nitrogens (N) of the porphyrin, the nitrogen (N′) of histidine, the eight first-neighbor carbons (C), the four second-neighbor carbons (C′) and the eight third-neighbor carbons (C″) are tabulated separately in Table IV.

The perusal of Table IV reveals that the most dominant contribution comes from the nitrogens (N) of the prophyrin. The contribution from the

TABLE II. Tabulation of the molecular-orbital coefficients $d_{ti\gamma,N_t,L_t,M_t}$, the occupation number $\epsilon_{i\gamma}$, and the Slater exponents for the molecular orbitals $i\gamma$ of OH^- as given by Rosenfeld (Ref. 58) with the basis orbitals $\chi_{t,N_tL_tM_t}$ [see Eq. (3)] used in the present calculations.

$\chi_{t,N_tL_tM_t}$	(1σ)	(2σ)	(3σ)	$(1\pi^+)$	$(1\pi^-)$	Slater exponents
$1s$(O)	1.00017	−0.03253	−0.03845	···	···	7.700
$2s$(O)	0.01725	0.79366	−0.65715	···	···	2.275
$2p_\sigma$(O)	0.00305	0.07282	0.52289	···	···	2.275
$1s$(H)	−0.00469	0.31196	0.71785	···	···	1.000
$2p_{\pi+}$ (O)	···	···	···	1.00000	···	2.275
$2p_{\pi-}$ (O)	···	···	···	···	1.00000	2.275
$\epsilon_{i\gamma}$	2	2	2	2	2	···

TABLE III. Calculated values in $1/\sqrt{4\pi}$ a.u. of the "distant" two- and three-center matrix elements useful for obtaining EFG from OH^- ligand. The subscripts O and H on the orbitals stand for the oxygen and hydrogen, respectively.

Matrix elements	$1/\sqrt{4\pi}$ a.u.
$\langle (1s)_O \| Y_2^0/r^3 \| (1s)_O \rangle$	5.3026×10^{-2}
$\langle (2s)'_O \| Y_2^0/r^3 \| (2s)'_O \rangle$	5.0112×10^{-2}
$\langle (2p_\sigma)_O \| Y_2^0/r^3 \| (2p_\sigma)_O \rangle$	6.8160×10^{-2}
$\langle (1s)_O \| Y_2^0/r^3 \| (2s'_O) \rangle$	2.0×10^{-7}
$\langle (1s)_O \| Y_2^0/r^3 \| (2p_\sigma)_O \rangle$	2.4701×10^{-3}
$\langle (2s)'_O \| Y_2^0/r^3 \| (2p_\sigma)_O \rangle$	2.8374×10^{-2}
$\langle (2p_{+1})_O \| Y_2^0/r^3 \| (2p_{+1})_O \rangle$	4.5425×10^{-2}
$\langle (2s)_H \| Y_2^0/r^3 \| (1s)_H \rangle$	1.5160×10^{-2}
$\langle (2s)'_O \| Y_2^0/r^3 \| (1s)_H \rangle$	9.2335×10^{-4}
$\langle (2p_\sigma)_O \| Y_2^0/r^3 \| (1s)_H \rangle$	5.6300×10^{-5}
$\langle (1s)_O \| Y_2^0/r^3 \| (1s)_H \rangle$	3.5759×10^{-5}

nitrogen (N′) at the fifth ligand site is an order of magnitude smaller and opposite in sign. The reason for the larger magnitude lies, partly, in the fact that there are four nitrogens in the porphyrin plane thereby enhancing the results by a factor of 4 and partly, in that they are closer to the iron. The contribution from OH^- is amazingly small because the local part almost cancels the distant part and because the nonlocal part is very small. This exhibits the importance of accurate numerical evaluations particularly of the distant part which involves both the two- and three-center matrix elements. The contribution from the first-neighbor carbon atoms is also large because there are eight such atoms. However, it is smaller than the N contribution. The EFG's from the second- and third-neighboring carbon atoms (C′ and C″) are decreasingly much smaller. This emphasizes that the contributions from the atoms further away from Fe can be safely neglected. The net EFG (see Table IV) comes out to be 25.59×10^{14} esu which, assuming the quadrupole moment $Q(^{57}\mathrm{Fe}) = (0.18 \pm 0.02)$[46]$b$, yields the hyperfine splitting $\Delta E = \frac{1}{2} |e| Qq = 1.44 \pm 0.16$ mm/sec in very good agreement with the experimental value 1.57 mm/sec observed by Lang and Marshall.

IV. DISCUSSION AND CONCLUSION

Utilizing the electron charge distribution described by the GMO's in Eq. (2) and the explicit expressions for the EFG's derived in Sec. II we estimated the nuclear hyperfine interaction in ferrihemoglobin hydroxide (HiOH) in Sec. III. Different contributions arising from the orbitals of iron and from surrounding atoms and the complex OH^- were shown in Table IV. On combining various contributions the hyperfine splitting was found to be 1.44 ± 0.16 mm/sec which compared excellently with the experimental value 1.57 mm/sec given by Lang and Marshall.

It is gratifying that the calculated result is very close to the observed splitting particularly when we have not used any parameters to fit with the experimental data. It should be remarked, however, that the sign of the splitting in HiOH has not been determined experimentally. Because of this uncertainty we explore the possibility of determining the sign indirectly as follows.

Eo animo we compare the calculated total EFG

TABLE IV. Calculated electric field gradient components (in 10^{14} esu) and the nuclear hyperfine splitting ΔE of Fe^{57m} in ferrihemoglobin hydroxide.

Field gradient components	Fe orbitals	OH^-	N	N′	C	C'	C''
q'_l	$3d$	−1.007	1.260	−0.141	0.181	0.026	0.004
	$3p$	−10.347	20.637	−1.817	3.659	0.535	0.100
	$2p$	−0.144	0.462	−0.037	0.087	0.012	0.002
q'_{nl}	$3d$	1.125	−1.884	0.179	−0.432	−0.066	−0.014
	$3p$	0.174	−0.320	0.026	−0.050	−0.007	−0.001
	$3s$	0.074	−0.192	0.014	−0.034	−0.005	−0.001
	$2p$	0.022	−0.076	0.006	−0.014	−0.002	0.000
	$2s$	0.006	−0.023	0.002	−0.004	−0.001	0.000
q'_d		−0.053	0.082	−0.004	0.005	0.001	0.000
q'_{db}		−12.107	9.095	−2.882	5.688	1.933	2.030
q'_n		12.739	−9.155	2.885	−4.935	−1.724	−1.877
q (Subtotal)		−0.994	13.730	−1.212	9.993	2.465	1.612
q (Total)				25.594			
ΔE (mm/sec)				1.44 ± 0.16			

TABLE V. Compilation of the separated and total electric field gradients (in 10^{14} esu) in hemin and hemoglobin hydroxide (HiOH) for various atomic and complex units. The experimental hyperfine splittings ΔE (in mm/sec) have also been listed. Since in hemin the fifth ligand site is empty, the relevant contribution from N' is absent. Also, the EFG from the third-nearest carbons (C'') in hemin is not available and , therefore, left unfilled; it is approximately equal to the corresponding value in HiOH as also evident from the calculated EFG's from the first- and second-nearest carbons (C and C') in both cases.

								ΔE	
q	OH^- or Cl^-	N	N′	C	C'	C''	Total	Calculated	Expt.
Hemin	−16.431	14.273	···	9.347	2.526	···	+9.715	+0.55	+0.78[a]
HiOH	−0.994	13.730	−1.212	9.993	2.465	1.612	+25.594	+1.44 ± 0.16	1.57[b]

[a] Reference 63.

[b] Reference 8.

and the EFG's separated for various surrounding atoms in HiOH with the corresponding values[41] for hemin in Table V; the experimental hyperfine splittings in these systems are also shown therein. From Table V one observes that the difference in the EFG values in the two cases is mainly due to the widely different contributions coming from the ligands (OH^- and Cl^-) at the sixth ligand sites; the contribution from N′ in HiOH is small and the other contributions in the two systems are almost equal. Also, the experimental splitting[63] in hemin has been established to be positive. Further, there is a close agreement, in sign as well as in magnitude, between the calculated and the experimental splittings in hemin. Since the method of calculations and the mechanisms contributing to the EFG's in hemin (without polarizations) are the same as adopted here, the closeness in magnitude of the theoretical and experimental values of the splitting in HiOH could be exploited to deduce the sign of the splitting in HiOH. It is clear from Table V that the experimental value of the splitting should be positive (consistent with the present calculations) since one would demand, otherwise, in order to reverse the sign of the calculated value, the contribution to EFG from OH^- alone to be about -50×10^{14} esu—a large negative value—to override the net positive contribution from all the other atoms. Since such a high negative value is not possible from OH^- (or from any other ligand—as our experience shows) we are lead to predict the positive sign for the splitting in HiOH.

Now the good agreement between the calculated and the experimental results can be made evident. In the present calculations not only the valence but also the core states of the constituent atoms and all the molecular states of the complex OH^- have been included with the result that the dominant contributions are taken into account. Also, the relevant multicenter matrix elements and the "distant" contributions which get enhanced by the antishielding effect have been calculated correctly. Besides, all the expressions for the EFG's have been completely retained without further approximations and evaluated adequately.

In our calculations we have ignored the molecular-orbital nature of the histidine complex at the fifth ligand site. In fact, we have taken into account only the closest atom of the complex, viz., the nitrogen N′. This approximation does not seem to be serious since the contribution from N′ itself is almost negligible, being only 5% of the total contribution (see Table IV). Also, the molecular orbitals of the complex are not expected to change the final results severely because the atoms other than N′ in the complex are further away and produce negligible effects. However, slight changes in the results are likely to occur owing to the changes in the N′ orbitals themselves. Unfortunately, the molecular orbitals of the histidine complex are not yet available otherwise it would have been informative to adopt them here to check our conclusions.

We have also neglected the contributions to EFG arising from the atoms beyond the C″ atoms. Besides, the heme-heme interaction has not been included in the present treatment. Such effects are negligible since the EFG's decrease rapidly as inverse cube of the distance. Our rough estimates indicate that these would produce about 4% variation in the splitting.

The experimental value of the splitting in HiOH is considered to be high[7] for a ferric system but no explanation for it has yet been given. The present calculations, however shows that the combined contribution from the N, C, C′, and C″ atoms is positive and appreciately dominates the negative and small contributions from the OH^- and N′. Accordingly, one obtains a net high value of the splitting in HiOH.

To make the point further transparent we compare our results with the similar results previously calculated for hemin. In hemin the observed splitting is 0.78 mm/sec, only half of the observed splitting in HiOH. Calculationally, the Cl^- contribution to EFG in hemin is large and positive, which cancels a large part of the EFG from other atoms (see also Table V) whereby yielding a net positive but small results.

Some comments are due as regards the spin state of iron in HiOH. The assignment of spins from the Mössbauer spectra is not always unambigious and sometimes leads to difficulties. This is the situation in HiOH Mössbauer spectra as for as the spin state of iron is concerned. It is conceivable that the spin state of iron depends on the temperature of the system since the observed Mössbauer splitting changes from 1.57 mm/sec at 195 °K to 1.9 mm/sec at 77 °K. A similar situation seems to exist in Hartree's[64] susceptibility measurements which assume the iron to be in a mixed state of high and low spins ($S=\frac{5}{2}$ and $\frac{1}{2}$).

Our calculations reveal that at high temperatures HiOH should be a high spin system. This is inferred from the calculated result which is close to the experimental splitting at high temperature (195 °K) and which corresponds (i) to the room-temperature atomic-structure parameters and (ii) to the high spin system since the molecular orbitals constructed for calculations assume the high spin state of iron. This conclusion is also supported by Zerner *et al.*'s analysis of the optical spectra of OH^- porphin complex in which the iron has been determined to be in the high spin state.

Next we turn to the discussion of our results in relation to the observed splitting in various other ferric-hemoglobin compounds. The observed values of the hyperfine splittings are 0.60, 1.39, 2.00, and 2.30 (in mm/sec) for HiF, HiCN, HiH_2O, and HiN_3, respectively. Perusal of the calculated EFG's in hemin and hemoglobin hydroxide (see Table V) points out that, since the relative difference in the splitting in various compounds is (as discussed above) due mainly to the difference in the nature of the ligands at the sixth ligand site and since the experimental splitting in hemin is positive, the splitting in these compounds is likely to be positive. This owes to the fact that the negative contribution arises only from the ligand at the fifth and sixth ligand sites and is too weak to override the positive contribution of other atoms. This is an important deduction particularly, when the signs of the splittings are not known from experiments.

Comparing our results with the semiempirical estimates made by Weissbluth and Maling on model compounds of iron porphin complexes one finds that they obtained negative sign for the splitting in the hydroxide compound in contrast with our positive sign. Besides, they arrived at the conclusion that the ligand effect is relatively minor in importance which is against our result as is evident from our large "distant" contribution (designated as the ligand-effect contribution by Weissbluth and Maling).

In tracing the sources of the above disagreements Weissbluth and Maling employed semiempirical molecular orbitals of Zerner *et al.* which were obtained by fitting with the observed optical properties of porphin complexes. These molecular orbitals are good for describing only the spectroscopic results and are inadequate for explaining the hyperfine splittings. More importantly, the orbitals do not include the core orbitals (for example, the iron $3p$ orbitals) which are found to give dominant contributions. Further, they approximated the "distant" (or ligand-effect) contributions assuming that the ligands are effectively point charges though the values of the point charges were determined from the molecular orbital coefficients. In view of our calculations the last approximation seems to be severe since the ligand charges are, *de facto*, diffused and change the results drastically because of the large antishielding enhancements. Weissbluth and Maling also appear to have ignored completely the effect of the charge distribution of the nitrogen which binds the imidazole ring of histidine. Similar conclusions have also been derived by Bhide.[37]

En somme, we have estimated the hyperfine splitting of iron in hemoglobin hydroxide without resorting to semiempirical treatment or fitting with uncertain parameters. The results are stimulating and suggestive of performing improved calculations on different hemoglobin compounds incorporating better molecular orbitals and induced polarization effects.

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