Mössbauer Effect in Hemoglobin with Different Ligands

Abstract. *Recoil-free nuclear gamma-ray resonance adsorption was observed in the iron-57 of blood. The spectral parameters are dependent on the ligand bound to the iron atoms in hemoglobin. The results are interpreted in terms of isomeric shifts and quadrupole splittings.*

A Mössbauer effect has been observed in the red cells of blood (*1*). The nuclei absorbing the γ-ray in a recoil-free fashion are those of Fe^{57}. The experimental difficulties result mainly from the small natural isotopic abundance of Fe^{57}—2.2 percent (atomic)—and the large photoelectric absorption; however, a resonance effect of about 2 percent can be observed. The central configuration around the iron in the hemoglobin molecule is indicated in Fig. 1. The iron is located at the middle of the porphyrin ring formed by four nitrogen atoms. The globin molecule is attached to the iron in the 5-position and the 6-position is usually occupied by a ligand (*L* in Fig. 1). The purpose of this work was to investigate the change of the Mössbauer spectrum caused by different ligands. This should make possible an interpretation of the spectrum obtained from natural blood, and furthermore it should lead to information concerning the binding of the ligands to the central iron atom.

The absorbers were prepared by taking blood from a vein, washing it several times with saline solution, and allowing the red cells to sediment. Approximately 4 mc of radioactive Co^{57} diffused into metallic platinum and kept at room temperature was used as a source. The width of the resonance line was about twice the width of the natural line as determined from the absorption spectrum of metallic iron.

In Fig. 2*a* the spectrum obtained by using an absorber of rat red cells isotopically enriched to contain 3.5 percent Fe^{57} at the temperature of liquid helium is shown. The enrichment with Fe^{57} increases the resonance absorption. The absorption spectrum obtained by using human blood was found to be very similar (*1*). The absorber in the spectrum shown in Fig. 2*b* was freshly prepared crystalline rat oxyhemoglobin. A spectrum taken with oxygenated blood was the same. The absorbers in the spectra shown in Fig. 2, *c*–*e*, were obtained by exposing human blood to the atmospheres of CO_2, N_2, and CO, respectively. This was accomplished by bubbling the gases through blood samples at room temperature. The Mössbauer spectra Fig. 2, *b*–*e*, were taken with absorbers at liquid-nitrogen temperature.

The hyperfine interactions which effect the spectra can be interpreted by a two-term Hamiltonian (*2*)

$$H = E + P\left[I_z^2 - \frac{I}{3}(I+1)\right]$$

where E is the isomeric shift, P is the quadrupole coupling constant (we have assumed an axially symmetric electric-field gradient), I is the nuclear spin, and I_z is the projection of I along the axially symmetric electric-field gradient. The isomeric shift represents an electrostatic interaction between the nucleus and the *s*-electron density at the nucleus. The second term is due to the interaction of the nuclear quadrupole moment with the electric-field gradient at the nucleus. The numerical parameters obtained from the spectra in Fig. 2 are given in Table 1. The spectrum obtained by using an absorber of blood (Fig. 2*a*) consists of a quadrupole-split strong component which coincides in splitting and in shift with the spectrum obtained with crystalline O_2-hemoglobin and a quadrupole-split weak component which coincides in splitting and shift with the spectrum obtained with blood which was exposed to either N_2 or CO_2 atmospheres. The intensity ratio of the two-line sets (about 6 to 1) is roughly the probability of occupancy of the 6-position by an O_2 molecule. Since the blood was taken from a vein, this relative concentration of O_2-hemoglobin seems reasonable. The fact that similar spectra are obtained with blood exposed to CO_2 and N_2 atmospheres is evidence that the binding of CO_2 to form CO_2-hemoglobin is not effecting the central electronic configuration of the heme to an appreciable extent and is confirmation (*3*) that the CO_2 is not bound as a ligand in the 6-position but rather to some other part of the molecule. The spectrum of Fig. 2*b* on the one side and the spectra of Figs. 2*c* and

Table 1. Quadrupole splitting and isomeric shift for hemoglobin with different ligands.

Ligand	Quadrupole splitting (peak separation in mm/sec)	Isomeric shift (mm/sec)
	Blood	
O_2	2.3 ± 0.1	-0.05 ± 0.05
(H_2O, --)?	$2.3 \pm .1$	$+ .55 \pm .05$
	O_2-Hemoglobin (crystalline)	
O_2	$2.1 \pm .1$	$- .05 \pm .05$
	Blood (CO_2-atmosphere)	
(H_2O, --)?	$2.3 \pm .1$	$+ .55 \pm .05$
	Blood (N_2-atmosphere)	
(H_2O, --)?	$2.3 \pm .1$	$+ .55 \pm .05$
	Blood (CO-atmosphere)	
CO	$0.0 \pm .1$	$.0 \pm .05$

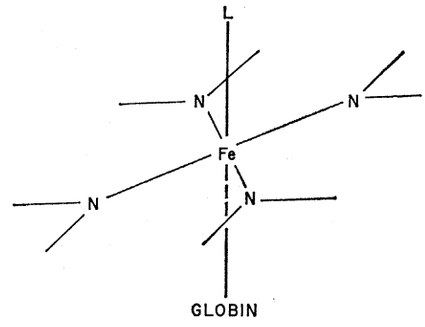

Fig. 1. Structure of hemoglobin immediately surrounding the iron atom.

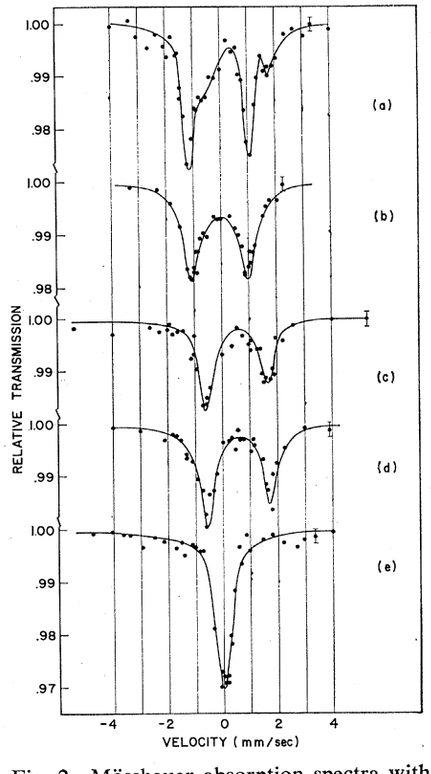

Fig. 2. Mössbauer absorption spectra with a source of Co^{57} diffused into Pt and kept at room temperature and absorbers of (*a*) rat red cells at 4°K and isotopically enriched with Fe^{57}, (*b*) crystalline rat oxyhemoglobin at 77°K, (*c*) human CO_2-hemoglobin (in a CO_2 atmosphere) at 77°K, (*d*) human hemoglobin (in a N_2 atmosphere) at 77°K, and (*e*) human CO-hemoglobin (in a CO atmosphere) at 77°K. We have used the standard notation that source approaching absorber is positive velocity.

2*d* on the other can be regarded as cases where either the 6-position is completely occupied by O_2 molecules or is not occupied by O_2 molecules. The question whether the 6-position is empty or is occupied by H_2O molecules (*4*) is still unanswered. A characteristic feature of the spectra in Figs. 2*c* and 2*d*, corresponding to the hemoglobin without an O_2 ligand, is the different intensity of the two lines. Similar behavior has been previously reported and has been attributed to either a preferential orientation of a polycrystalline source or absorber where the crystal *c*-axes and the direction of observation is not random (*5*) or to a directionally dependent Debye-Waller factor (*6*). In blood one does not have a preferential orientation. However, an asymmetry can be expected if the fundamental vibrational frequencies of the iron bound in the ring are directionally dependent. If we assume that the fundamental vibrational frequency is higher "in-plane" than "out-of-plane" and then use the known positive sign of the nuclear quadrupole moment for the 14.4 kev state (*7*) of Fe^{57}, we can conclude that the weaker line centered at + 1.7 mm/sec corresponds to the two absorption lines $+1/2 \rightarrow +3/2$, $-1/2 \rightarrow -3/2$ and that the stronger line centered at − 0.6 mm/sec corresponds to the four absorption lines $\pm 1/2 \rightarrow \pm 1/2$; and thus the sign of the electric-field gradient is positive for the spectra in Figs. 2*c* and 2*d*. In summary, the quadrupole splitting, and thus the magnitude of the electric-field gradient in the spectra of Fig. 2, *a–d*, is the same although there is a considerable difference in isomeric shift between the two basically different spectra.

The spectrum of Fig. 2*e* was taken with blood which was exposed to a CO atmosphere. The isomeric shift observed in this case is similar to O_2-hemoglobin; however, no quadrupole splitting was observed indicating the absence of a sizable electric-field gradient. The CO-hemoglobin absorption spectrum demonstrates the ease with which a change of the hemoglobin ligand can be detected and points out the fact that recoil-free nuclear resonance absorption could conceivably become an interesting biological tool.

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References and Notes

1. U. Gonser, R. W. Grant, J. Kregzde, *Appl. Phys. Letters* **3**, 189 (1963); *ibid.* **4**, 23 (1964).
2. P. H. Barrett, R. W. Grant, M. Kaplan, D. A. Keller, D. A. Shirley, *J. Chem. Phys.* **39**, 1035 (1963).
3. F. J. W. Roughton in *Haemoglobin*, F. J. W. Roughton and J. C. Kendrew, Eds. (Butterworth, London, 1949).
4. P. George and R. L. J. Lyster, *Conference on Hemoglobin*, (Natl. Acad. of Sciences–Natl. Research Council, Washington, D.C., 1958), p. 33.
5. A. J. F. Boyle, D. St. P. Bunbury, C. Edwards, *Proc. Phys. Soc. London* **79**, 416 (1962).
6. V. I. Goldanskii, E. F. Makarov, V. V. Khrapov, *Appl. Phys. Letters* **3**, 344 (1963).
7. R. W. Sternheimer, *Phys. Rev.* **130**, 1423 (1963).
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Toxic Residues in Soil 9 Years after Treatment with Aldrin and Heptachlor

Abstract. *In silt loam soil heavily infested with European wireworm, a single treatment with aldrin or heptachlor prevented reinfestation for 9 years, even when the soil was under continuous cultivation. Toxic residues were determined by bioassay with* Drosophila melanogaster *Meig. By gas-liquid chromatographic analysis, the residues were found to be mainly dieldrin and heptachlor epoxide.*

In 1953 a long-term experiment was set up on the control of the introduced European wireworm, *Agriotes obscurus* (L.), at Agassiz, British Columbia. The experiment included seven chemical treatments and untreated controls, replicated three times. Each plot was 9 by 18 m. The materials were applied as dusts or sprays to the surface and immediately worked into the soil to a depth of 15 cm by plowing and disking. No further treatments were made. Crops were grown as follows: 1953, potatoes; 1954, oats, peas, and vetch; 1955, oats, peas, and clover; 1956 to 1958, clover; 1959 and 1960, corn; 1961, peas. The wireworm population, determined by sifting untreated soil every year for 7 years, averaged 130 per square meter.

Aldrin and heptachlor at 5.6 kg toxicant per hectare were the most effective treatments. Immediate protection was given to potatoes and complete control was obtained by the 2nd year. No reinfestation occurred. Reinfestation did occur within 5 years in plots treated with aldrin at 2.8 kg, heptachlor at 3.4 kg, and DDT at 16.8 kg per hectare.

In January 1962, 20 laboratory-reared, 7-month-old larvae of *A. obscurus* were put in soil from the plots treated with aldrin dust, aldrin emulsion, and heptachlor dust, at 5.6 kg toxicant per hectare and from the untreated control. The wireworms were immobilized and stopped feeding soon after being placed in the treated soil but remained healthy looking for periods up to 6 weeks. After that time they were unable to burrow into the soil when they were left on the surface and soon became desiccated. The wireworms in the untreated soil burrowed immediately and survived.

The soil treated with aldrin and heptachlor was analyzed further to determine the toxic residue remaining after 9 years of cropping. Thirty 15-cm cores, 10 cm in diameter, were taken, ten from each plot, and were mixed by tumbling and screening. *Drosophila melanogaster* Meig. was the test insect used for bioassay. The method was based on that of Edwards *et al.* (*1*), with modifications that we had developed. Four-gram samples of air-dried soil were weighed into 22-ml bottles, wetted with ¾ ml of a boiled mixture of one part of apple juice to nine parts distilled water. Four or five dosages were used in each run and each dosage was replicated eight times. Untreated soil was included in each assay to correct for natural mortality. Twenty-five flies, from 1 to 3 days old, were aspirated into each jar and left for 24 hours in a climate chamber at 26°C under constant light. Dead and moribund flies were counted.

Since aldrin and heptachlor are con-

Table 1. Recoveries of toxic residues from silt loam soil 9 years after treatment with aldrin or heptachlor, applied at 5.6 kg/hectare in 1953.

Pesticide (2.5 ppm)	Pesticide residues found in 1962 (ppm)		
		GLC*	Bio-assay†
Aldrin dust	Aldrin	0.005	0.230
	Dieldrin	.098	
Aldrin emulsion	Aldrin	.006	.175
	Dieldrin	.153	
Heptachlor dust	Heptachlor	.009	.317
	Heptachlor epoxide	.169	

* Wilkens Hi-FI gas chromatograph with electron capture detector at a potential of 20 volts. Injector temperature 210°C, column 0.318 cm × 0.6 m aluminum packed with 5 percent Dow Silicone II on Chromosorb W60–80 mesh, at a temperature of 137°C for the heptachlor fraction, and 153°C for the other fractions. Inlet pressure 0.7 kg/cm², carrier gas nitrogen. Output sensitivity 10 times; attenuation 2 times; 5-μl injections used throughout. † Against dieldrin and heptachlor expoxide standards.