

SHORT COMMUNICATIONS

MÖSSBAUER EFFECT IN FERRITIN*

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Ferritin, the iron storage protein, is made up of a roughly spherical apo-protein shell enclosing a micelle of composition $(\text{FeOOH})_8(\text{FeO} \cdot \text{OPO}_3\text{H}_2)$ (Granick and Hahn 1944; Farrant 1954). Electron microscopy (Farrant 1954; van Bruggen, Wiebenga, and Gruber 1960) showed that the micelle is approximately 55 Å in diameter.

Recent X-ray studies by Fischbach and Anderegg (1965) of ferritin fractions separated according to density (and hence according to iron content) by the ultracentrifuge showed that, while the micelles of "full" ferritin (i.e. that containing the maximum amount of iron) are roughly spherical, the micelles in the lighter fractions have a different shape, becoming elongated so as to bridge the hollow centre of the molecule.

Chemical analysis shows that unfractionated ferritin contains approximately 20% by weight of iron.

Schoffa (1965) measured the magnetic susceptibility between 4.2 and 300°K and found that it attained a maximum at $20(\pm 3)^\circ\text{K}$. He attributed this to the onset of antiferromagnetic ordering. From measurements of susceptibility and the Mössbauer effect, Blaise, Chappert, and Giradet (1965) concluded that the iron-containing core exhibited superparamagnetic properties. Their Mössbauer measurements established that spectra characteristic of magnetic ordering appeared at temperatures below 30°K. Our results are in general agreement with those of Blaise, Chappert, and Giradet, but differ in detail as discussed below.

The experiments were performed on horse-spleen ferritin prepared by the method of Granick (1942). Susceptibility measurements made at low fields (5000 G) and between 90 and 295°K are in agreement with previous results. Both the susceptibility and the Mössbauer results indicated that repeated cycling between 4.2 and 295°K did not alter the magnetic state of the iron, even though the protein may be denatured in the process.

For the Mössbauer experiments, a source of ^{57}Co in copper was used. The absorber was in the form of a pressed disk of ferritin of thickness 0.5 mm. Spectra were recorded with source and absorber at the same temperature using a 400-channel pulse height analyser in time mode operation (Wertheim 1964).

Results at fixed temperatures within the range 4.2–295°K are shown in Figures 1 and 2. The spectra at 77°K and above show two well-resolved lines, caused by quadrupole interaction at the iron nuclei. The quadrupole splitting of 0.37 ± 0.02 mm/sec and the isomer shift (with respect to stainless steel) of 0.47 ± 0.05 mm/sec indicate that the iron is in an Fe^{3+} state (Duncan and Golding 1965). These values are in agreement with those of Blaise, Chappert, and Giradet (1965). At 4.2°K the

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spectrum exhibits a magnetic splitting corresponding to a field of 493 ± 10 kG acting on the iron nuclei. We note here that almost identical values of isomer shift, quadrupole splitting, and internal magnetic field have been reported for various forms of FeOOH (Rossiter and Hodgson 1965).

The spectra recorded from 20 to 45°K show a gradual increase in the intensity of the central doublet characteristic of ferritin at 77°K and a corresponding decrease in the intensity of the broadened six-line spectrum. These results may be interpreted, as Blaise, Chappert, and Giradet (1965) have done, on the assumption that the fine particles of the core are superparamagnetic. Particles of similar dimensions and chemical composition exhibit superparamagnetic behaviour down to low temperatures (see, for example, Creer 1962).

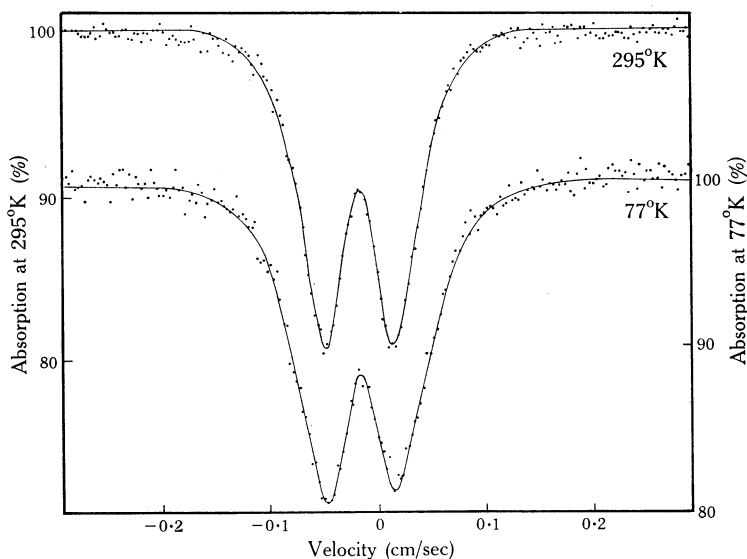


Fig. 1.—Mössbauer spectra of ferritin at $T = 295$ and 77°K .

The temperature dependence of the ferritin spectra is similar to that observed for superparamagnetic particles of $\alpha\text{Fe}_2\text{O}_3$ by Kundig *et al.* (1966) and is characteristic of a superparamagnetic system with a range in particle size and/or shape. This is not surprising in view of the X-ray results of Fischbach and Anderegg (1965) referred to earlier. If the iron ions were characterized by a definite spin-lattice relaxation time, the temperature dependence of the Mössbauer spectra would be quite different. Furthermore, the magnetic susceptibility results of Blaise, Chappert, and Giradet (1965) show that the behaviour of the Mössbauer spectrum must be due to superparamagnetism rather than to long spin-lattice relaxation times of the iron ions in the ferritin molecule.

Néel (1962) has shown that for sufficiently small antiferromagnetic particles (100 Å diameter or less) the direction of antiferromagnetism will not be fixed with respect to the lattice when the anisotropy energy is less than kT , where k is the Boltzmann constant and T the absolute temperature. This fluctuation of the direction

of antiferromagnetism will be characterized by a relaxation time

$$\tau = \tau_0 \exp(KV/kT),$$

where K is the anisotropy energy per unit volume, V is the volume of the particle, and τ_0 is a frequency factor of the order of \hbar/KV . Results characteristic of the ordered or disordered state will be observed when the time of measurement is less than or greater than the relaxation time.

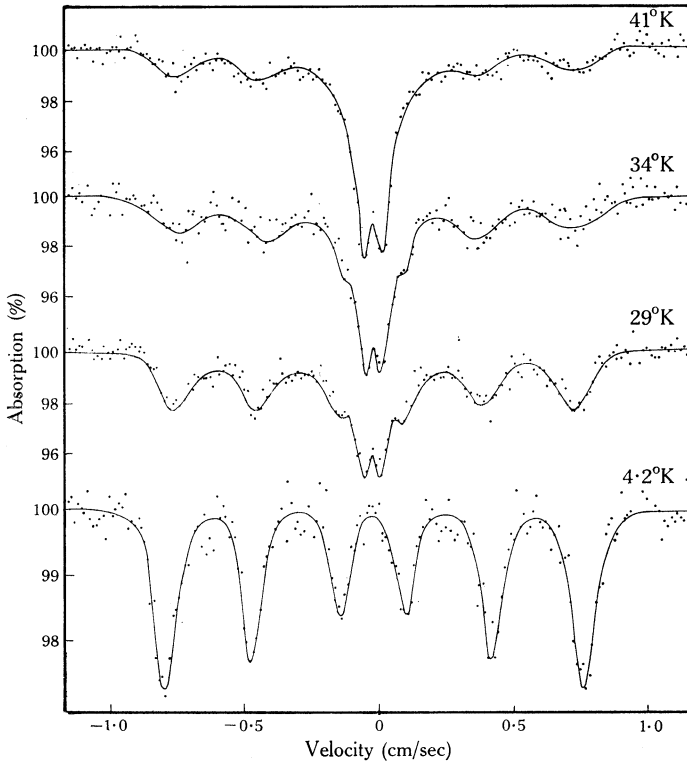


Fig. 2.—Mössbauer spectra of ferritin at $T = 4.2, 29, 34$, and 41°K . The absorber used for the 4.2°K spectrum was thinner than that used at the higher temperatures.

For susceptibility experiments, the measurement time is of the order of seconds. For Mössbauer experiments, magnetic ordering will be observed if the Larmor frequency of the nuclear spin around the magnetic field is much larger than $1/\tau$. For a field of 500 kG acting on an ^{57}Fe nucleus in the excited state the Larmor frequency is $4 \times 10^7 \text{ sec}^{-1}$. Using a value of $\tau_0 = 10^{-12} \text{ sec}$ (Néel 1962) and the susceptibility ordering temperature of 20°K (Schoffa 1965), an order of magnitude calculation predicts that ordering, as determined by the Mössbauer measurements, would be observed below 45°K . Our experimental results are in agreement with this prediction.

We conclude that ordering as observed by the Mössbauer effect occurs at significantly higher temperatures in our ferritin samples than it does in the ferritin of Blaise, Chappert, and Giradet (1965). This difference could be due to a different

size and shape distribution of the micelles in our ferritin, and it would therefore be of interest to carry out experiments on the components of ferritin as separated in an ultracentrifuge.

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