

FIG. 3. Optical absorption spectra of imidazole photohemichrome vs pressure.

and  $\beta$  bands. The effect of pressure on the absorption spectrum of imidazole protohemichrome is shown in Fig. 3. All the bands shift to lower energy, and the  $\alpha$  and  $\beta$  bands fade relative to the Soret peak. The Soret peak shifts by about 1 kK in the pressure range 20–140 kbar. The shift of the Soret peak and the fading of the  $\alpha$  and  $\beta$  bands with increasing pressure make the shifts of the latter peaks difficult to measure. An approximate shift for these bands is about 1–1.5 kK. The Soret peak also loses intensity with pressure, from 20 to 140 kbar by a factor of about 5 to 10. Ferric compounds usually have ligand to metal charge transfer bands, but the existence of such a band in imidazole protohemichrome is uncertain.

As shown in Fig. 1, at 1 atm hemin and hematin have their Soret peaks at approximately 25 kK and their  $\alpha$  and  $\beta$  bands at about 18.3 and 19.5 kK, respectively. In both hemin and hematin the Soret peak consists of two bands. The more intense band is at about 25 kK, and the weaker band is at higher energy. In addition, there is a new transition at lower energy, which has been assigned as a ligand to metal charge transfer. In hemin this peak lies at approximately 15.3 kK, and in

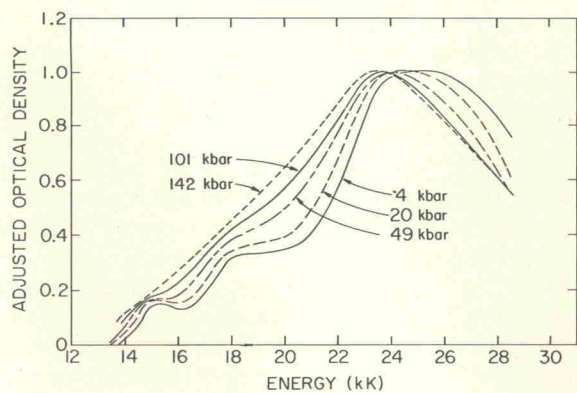


FIG. 4. Optical absorption spectra of hemin vs pressure.

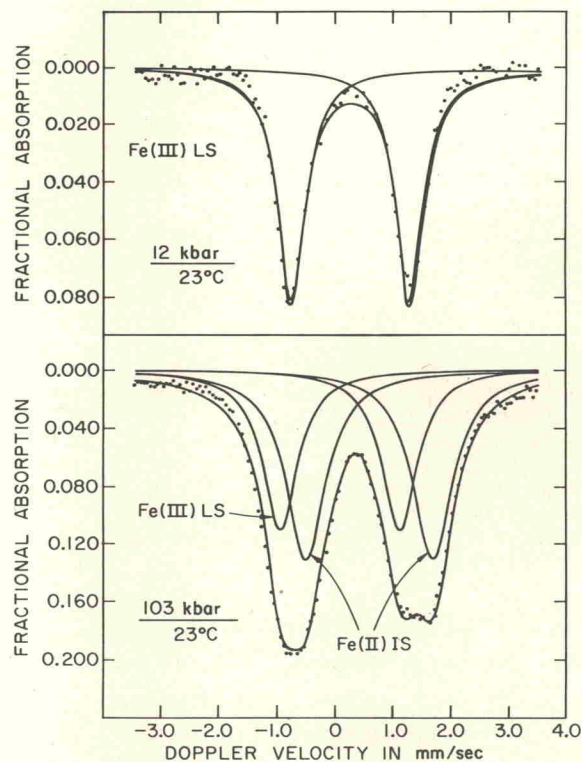


FIG. 5. Mössbauer spectra of imidazole protohemichrome at 23°C for 12 and 103 kbar.

hemin it lies at about 15.5 kK. The effect of pressure on the optical absorption of hemin is shown in Fig. 4. The pressure effects are similar to the previous two compounds in that the Soret peak maximum shifts to lower energy by approximately 1.2 kK, and the  $\alpha$  and  $\beta$  bands shift to lower energy and fade relative to the Soret band. The Soret band at higher energy appears to fade with increasing pressure. The Soret peak in hemin lost a factor of about 5 to 10 in intensity in the pressure range 20–140 kbar. The charge transfer peak shifts to lower energy and loses intensity rapidly with increasing

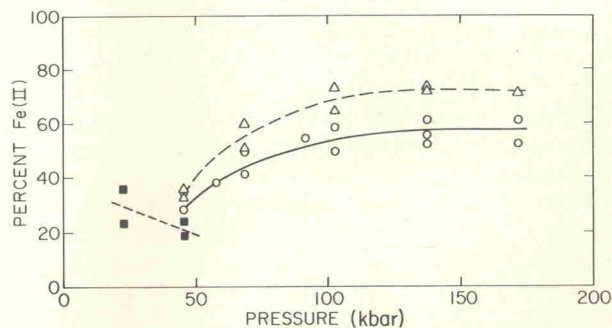


FIG. 6. Conversion of ferric to ferrous iron vs pressure for imidazole protohemichrome. O, Fe(II) [I.S.] 23°C;  $\Delta$ , Fe(II) [I.S.] 110°C;  $\blacksquare$ , Fe(II) [L.S.] 110°C.

TABLE I. Mössbauer parameters for imidazole protohemichrome.

Pressure (kbar)	Isomer shift (mm/sec) <sup>a</sup>							
	4	25	45	70	100	135	170	
Fe(III)[L.S.] <sup>b</sup>	0.17	0.15	0.09	0.03	0.0	-0.02	-0.03	(23°C)
Fe(II)[I.S.]	...	...	0.52	0.52	0.52	0.53	0.53	(23°C)
Fe(III)[L.S.]	...	0.17	0.14	0.06	0.01	-0.04	-0.06	(110°C)
Fe(II)[I.S.]	...	...	0.51	0.53	0.53	0.52	0.51	(110°C)
Fe(II)[L.S.]	...	0.30	0.30	...	...	...	...	(110°C)
	Quadrupole splitting (mm/sec)							
Fe(III)[L.S.]	2.07	2.06	2.05	2.04	2.03	2.01	2.00	(23°C)
Fe(II)[I.S.]	...	...	2.10	2.11	2.13	2.15	2.17	(23°C)
Fe(III)[L.S.]	...	2.06	2.10	2.08	2.07	2.05	2.04	(110°C)
Fe(II)[I.S.]	...	...	2.18	2.19	2.21	2.23	2.25	(110°C)
Fe(II)[L.S.]	...	1.22	1.22	...	...	...	...	(110°C)

<sup>a</sup> Relative to iron metal.<sup>b</sup> [L.S.], low spin; [I.S.], intermediate spin; [H.S.], high spin.

pressure. The optical absorption of hematin as a function of pressure is similar to hemin, and therefore is not presented.

### MÖSSBAUER RESULTS

The spin state of iron in both ferric and ferrous hemes has been a subject of considerable theoretical interest in recent years. Almost exclusively, the theoretical work has been done on ferric compounds. Harris<sup>12</sup> has shown that in ferric compounds, high spin, low spin, and intermediate spin complexes are possible if the symmetry of the molecule is lower than octahedral. In addition, if spin-orbit coupling is included in the calculation, a mixed spin state is possible. Zerner<sup>13</sup> has calculated that an intermediate spin ferrous state is possible if the iron is kept in the plane of the porphyrin. There is no experimental evidence of a true intermediate spin ferric or ferrous heme, although the magnetic moments of heme proteins indicate the possibility of mixed spin states. Ehrenberg<sup>14</sup> has shown that a mixed spin state is most probable for ferric iron in some heme proteins. As will be discussed below, a ferrous spin state resembling known intermediate spin ferrous compounds has been observed at high pressure. However, a mixed spin ferrous state, as opposed to a true intermediate spin state is entirely possible. In fact, wherever there is indicated "intermediate spin" in the following discussion, one should read the parenthetical expression (or mixed spin).

The room temperature Mössbauer spectra of imidazole protohemichrome at 12 and 103 kbar is shown in Fig. 5. At high pressure the spectrum indicates the presence of an iron state different from the original low spin ferric iron. The high pressure state is labeled intermediate spin ferrous iron. Most ferric compounds studied to date show a pressure induced reduction to ferrous iron, so it is reasonable that the high pressure

state observed in imidazole protohemichrome is the ferrous state. In most cases the ferrous iron produced at high pressure has the same spin state as the ferric iron, but pressure can cause a change in spin state, as discussed below. The percent conversion to ferrous iron is shown in Fig. 6. At 110°C the conversion is higher, and also starts at a lower pressure. Thus, at 110°C and 23 kbar, there is approximately 30% ferrous iron present, as shown in Fig. 7(a). Note that the ferrous iron, because of the values of its Mössbauer parameters, is labeled low spin ferrous. By 46 kbar there is increased conversion, but in addition, there are two types of ferrous iron present. The new ferrous iron is labeled intermediate spin. By 103 kbar all the ferrous iron present is in this state as shown in Fig. 7(b).

At 1 atm the quadrupole splitting of the ferric iron is 2.08 mm/sec, and the isomer shift is 0.17 mm/sec. These values are in agreement with the values obtained by Epstein *et al.*<sup>15</sup> of 2.17 mm/sec for quadrupole splitting and 0.15 mm/sec for isomer shift. The effect of pressure on the isomer shift and quadrupole splitting of the ferric iron and both spin states of ferrous iron is given in Table I. Epstein<sup>15</sup> measured a low spin pyridine hemochrome and found at 25°C an isomer shift of 0.40 mm/sec and a quadrupole splitting of 1.18 mm/sec. He did not measure the imidazole protohemochrome at room temperature, but at liquid nitrogen temperature he found an isomer shift of 0.43 mm/sec and a quadrupole splitting of 0.95 mm/sec. Using Epstein's data for compounds at both temperatures, a range of 0.35-0.43 mm/sec isomer shift, and 0.95-1.0 mm/sec quadrupole splitting would be reasonable values for the room temperature parameter of (low spin) imidazole protohemochrome. At 110°C and 25 kbar, the measured isomer shift and quadrupole splitting of the ferrous iron are 0.30 and 1.22 mm/sec, respectively. These values are sufficiently close to the expected values above to