Physical Investigations on Colloidal Iron-Dextran Complexes

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Three iron-dextran complexes used as parenteral hematinic agents were studied by physical techniques. Electron microscopy showed that Imferon contained very small particles, approximately spherical and 3 mμ in diameter, whereas Imposil and FPL 52001 contained larger, spindle-shaped particles. Shadow casting revealed the presence of an electron-transparent sheath, probably dextran, around the electron-dense, iron-containing core of the particles. Calculated particle sizes from diffusion studies were in close agreement with the electron microscope dimensions of the core without its dextran sheath.

Other studies were carried out on the complexes and on α-, β-, and γ-FeOOH for comparison. Correlation of interplanar spacings from electron and X-ray diffraction measurements indicated the presence of β-FeOOH in the complexes. IR spectroscopy in the region 700–1100 cm^{-1} suggested that β-FeOOH was more likely to be present than α-FeOOH but dextran absorptions prevented firm assignments. Mössbauer spectroscopy gave two-line spectra for the complexes at 300°K but differences in the spectra at 77°K were interpreted in terms of particle sizes and superparamagnetic-antiferromagnetic transitions.

INTRODUCTION

Aqueous solutions of iron-dextran complexes, or more correctly of FeOOH–dextran complexes, are important parenteral hematinic agents (1) but few studies on the structures and physical properties of the complexes have so far been reported, perhaps because of the difficulties involved in examining polydisperse colloidal particles. The particle size and molecular weight of the clinical product Imferon (2) have been briefly discussed (3) and valency investigations on the same material have also been reported (4). The low molecular weight dextran derivative which is the protective colloid in Imferon and in Imposil, a veterinary hematinic, has been the subject of a recent paper (5).

The adsorption of dextran on ferric oxide sol particles has been studied by Fujita and Terato (6) and their results have led them to suggest that adsorption occurs through the terminal carbonyl-containing glucose unit in the dextran chain. Müller (7) has reviewed both the complexing behavior of various forms of FeOOH with a range of carbohydrates and the application of certain physical techniques for characterizing the complexes. Further reference is made to this review in the discussion.

This paper reports certain physical investigations carried out on the established hematinics Imferon and Imposil and on the experimental product FPL 52001. The object of this study was to characterize them as fully as possible by the physical techniques available, with the ultimate aim of correlating their properties and structures with their biological behavior.

MATERIALS AND METHODS

The iron-dextran complexes used are the products of neutralization of ferric chloride in the presence of dextran derivatives: Imferon (batch 1586/5) is an aqueous solution of an FeOOH–complex with alkali-modified dextran; it contains 5% (w/v) iron. Imposil (batch 2186/20/100) is a similar colloidal solution containing 10% (w/v) iron. Preparations of iron-dextran complexes have been described (17).

FPL 52001 is an experimental colloidal solution prepared in a similar manner, containing 20% (w/v) iron, and in which the dextran involved has been previously treated with cyanide and subsequently hydrolyzed (18).

All these complexes may be dried and reconstituted without apparent change. The FeOOH was separated from the carbohydrate in FPL 52001 by boiling with $Al_2(SO_4)_3$ and neutralizing, followed by filtration of the solid. The standard sample of α-FeOOH was prepared by hydrolysis of ferric nitrate (19) and that of β-FeOOH by hydrolysis of ferric chloride (20). The sample of γ-FeOOH was obtained by oxidation of dichlorotetrapyridinoiron(II) (21).

Electron microscopy was carried out at the Shirley Institute, Manchester, England. For direct microscopy, samples were diluted with water, a drop was placed on a glass plate and a carbon-coated grid was floated on top with its carbon film uppermost. The preparation was then allowed to dry.

Shadow-casting was carried out with platinum/iridium at an angle of 15° to the surface. Samples for electron diffraction were prepared by allowing a drop to dry on a carbon grid. Measurements were made with a minimum beam intensity to avoid melting the sample.

Diffusion measurements were also performed at the Shirley Institute using a cell of the type described by Stokes (22) and thermostated at 25°C.

Mössbauer spectra were measured on a constant acceleration type spectrometer with gas proportional counter and ^{57}Co source by Dr. S. J. Lyle at University of Kent at Canterbury.

Infrared spectra were measured in Nujol mulls on a Perkin-Elmer 237 spectrophotometer.

RESULTS AND DISCUSSION

It has been shown by electron microscopy that Imferon contains electron-dense particles approximately spherical in shape (8). The same technique in our hands has now revealed that all three complexes Imferon, Imposil, and FPL 52001 contain electron-dense particles and that the sizes and shapes of the particles are characteristic for each complex. In addition, much larger particles have been revealed after the samples have been shadow-cast with platinum–iridium. Electron photomicrographs demonstrating these points are reproduced in Figs. 1–6.

Small spherocolloidal particles can be distinguished in Imferon even though there is a tendency for the particles to aggregate in large masses during the preparation of the sample for electron microscopy in which the sample of solution is allowed to dry on the carbon surface. The particulate nature of the complex is more clearly seen in the shadow-cast material (Fig. 4). The unshadowed particles are approximately 3 mμ in diameter, in agreement with the dimensions of 2–3 mμ found by Muir and Goldberg (8) and of 3–4 mμ for a fractionated sample of Imferon examined in later studies (3). However these measured dimensions are in contradiction to those of 3–4 μ quoted by Hall and Ricketts (9) and of 3 μ recently referred to by Henderson and Hillman (10), who give no details on the origin of this value.

Small spherical particles have been described in other iron-dextran preparations (7, 11).

The electron photomicrographs of Imposil show short rod-like structures some of which are apparently linear aggregates of spherical particles. The overall particle size is greater than that of Imferon, and this is even more obvious in the shadow-cast example (Fig. 5), where lozenge-shaped structures can be seen.

The FPL 52001 particles are largest and have a spindle shape, with a tendency for the particles to align in clusters. The particle shape is strongly reminiscent of the cigar-shaped crystals of β-FeOOH noted by Mackay (12) and described in detail by Watson *et al.* (13) and Gallagher (14). The shadow-cast sample gives a clear impression of the individual particles (Fig. 6).

The average dimensions of the particles

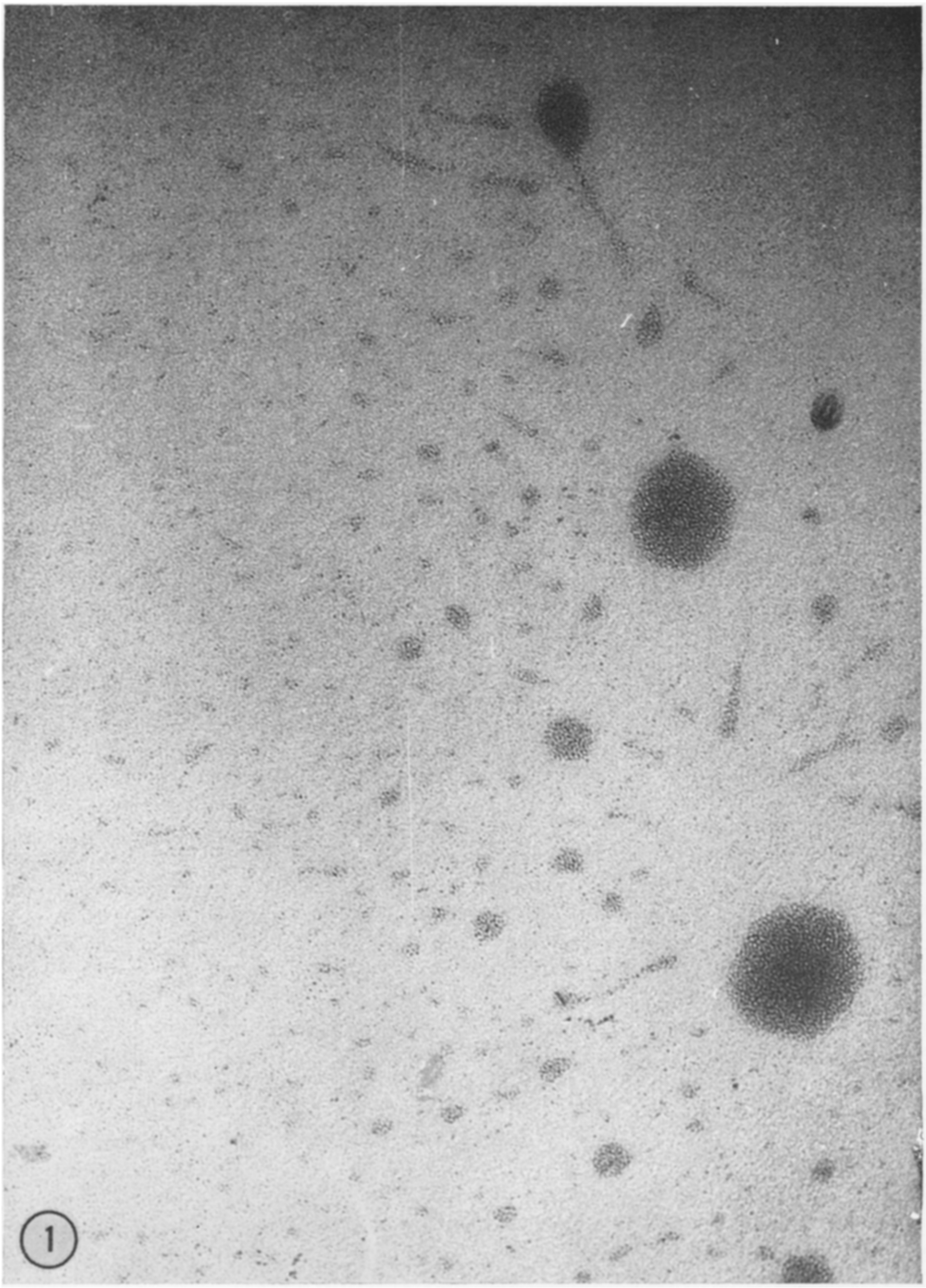

FIG. 1. Imferon × 100,000.

are listed in Table I. Random observations on other samples of these complexes indicate that these dimensions are typical.

The large differences in particle size (8–12 mμ in the largest dimension) between the shadowed and unshadowed material are evident from Table I and are much greater than can be attributed to the platinum/iridium layer which is only 0.7 mμ thick (personal communication from Mr. S. C.

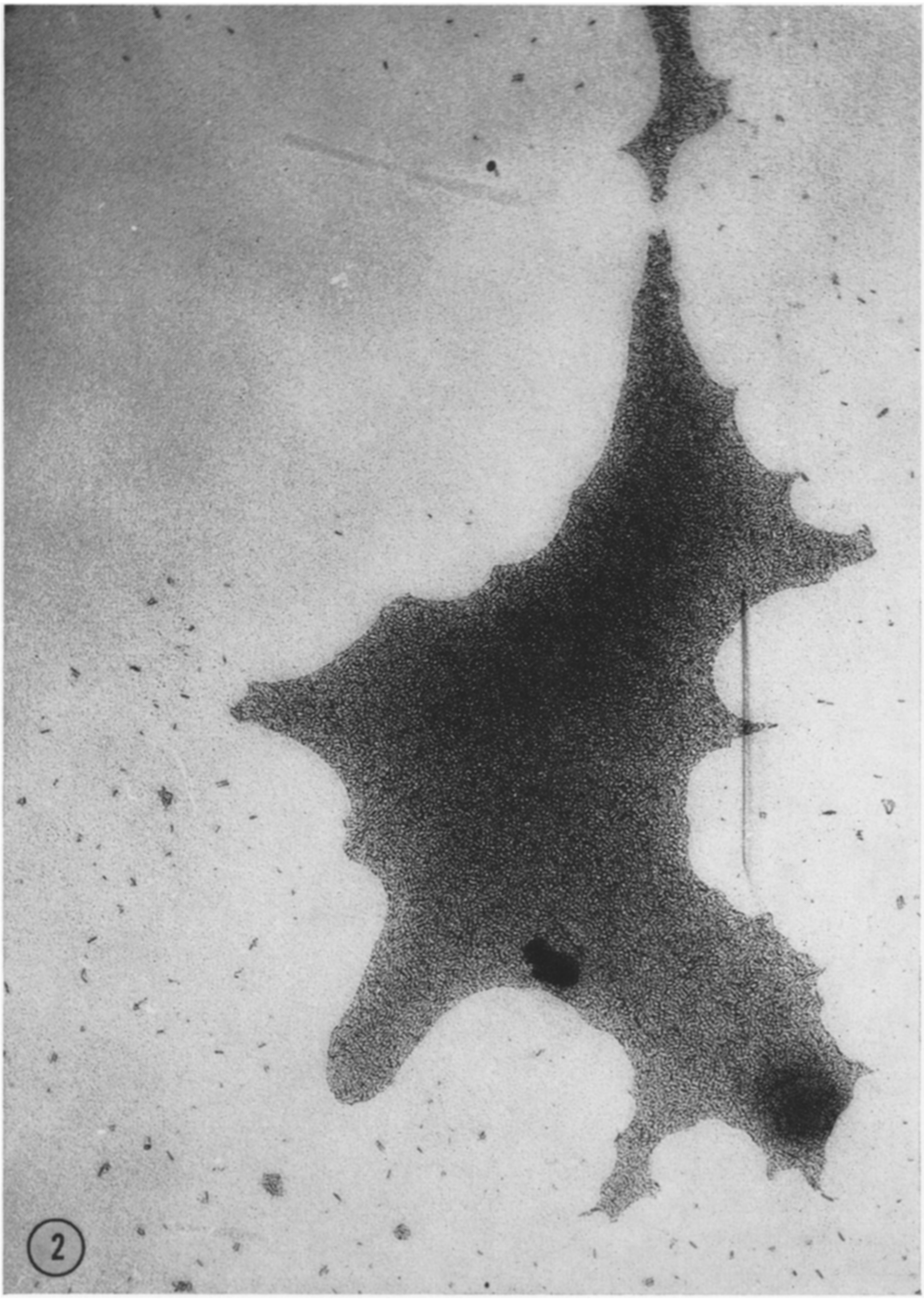

FIG. 2. Imposil × 100,000.

Simmens, Shirley Institute, Manchester). Clearly the particles have electron-dense nuclei surrounded by a sheath of electron-transparent material. Consideration of the chemical moieties involved in the complexes suggests that the nuclei contain FeOOH, the electron-dense component, and the electron-transparent sheath is the dextran, in

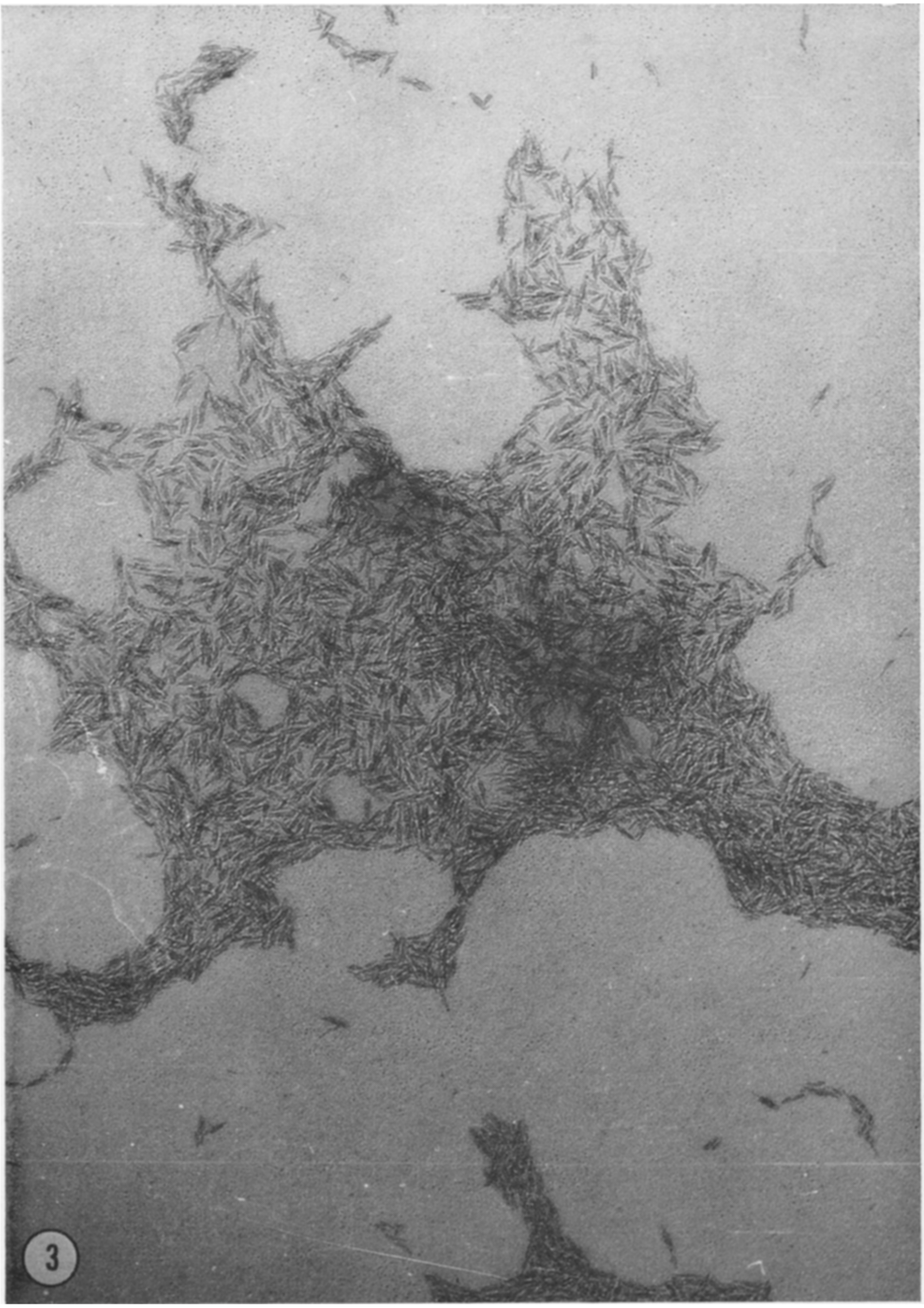

FIG. 3. FPL 52001 × 100,000.

accordance with its role as the protective colloid stabilizing the hydrophobic FeOOH particles.

To obtain independent evidence for the accuracy of these values, the rates of diffusion of Imferon and FPL 52001 have been investigated. The value of the diffusion coefficient, D, in three experiments each

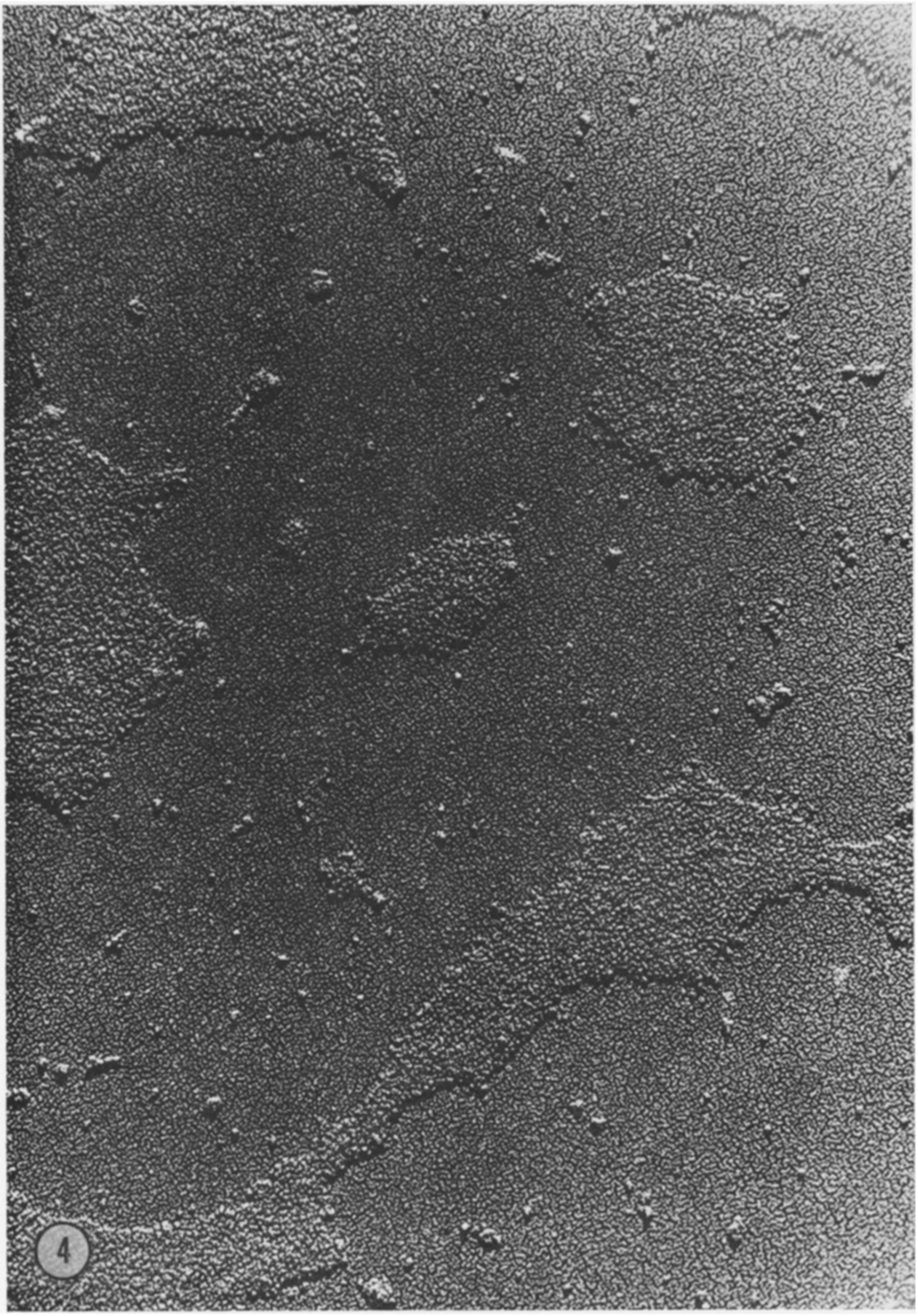

FIG. 4. Shadowed Imferon × 100,000.

extending over 1 week was $7.5 \pm 1.2 \times 10^{-11}$, $8.2 \pm 1.2 \times 10^{-11}$, and $8.4 \pm 0.8 \times 10^{-11}$ m^2 sec^{-1} for Imferon and the averaged result of two determinations on FPL 52001 was $3.4 \pm 1.5 \times 10^{-11}$ m^2 sec^{-1} over the same time period. Using the Stokes–Einstein equation [1] which applies to spherical particles,

FIG. 5. Shadowed Imposil × 100,000.

$$D = \frac{kT}{6\pi\eta_0 a} \qquad [1]$$

(k = Boltzmann constant, T = temp, η_0 = solvent viscosity and a = particle radius), the calculated radius was 27 Å, which is in agreement with the dimension of the "core" size as measured by direct electron microscopy. The calculation of the particle size of prolate spheroids necessitates use of the

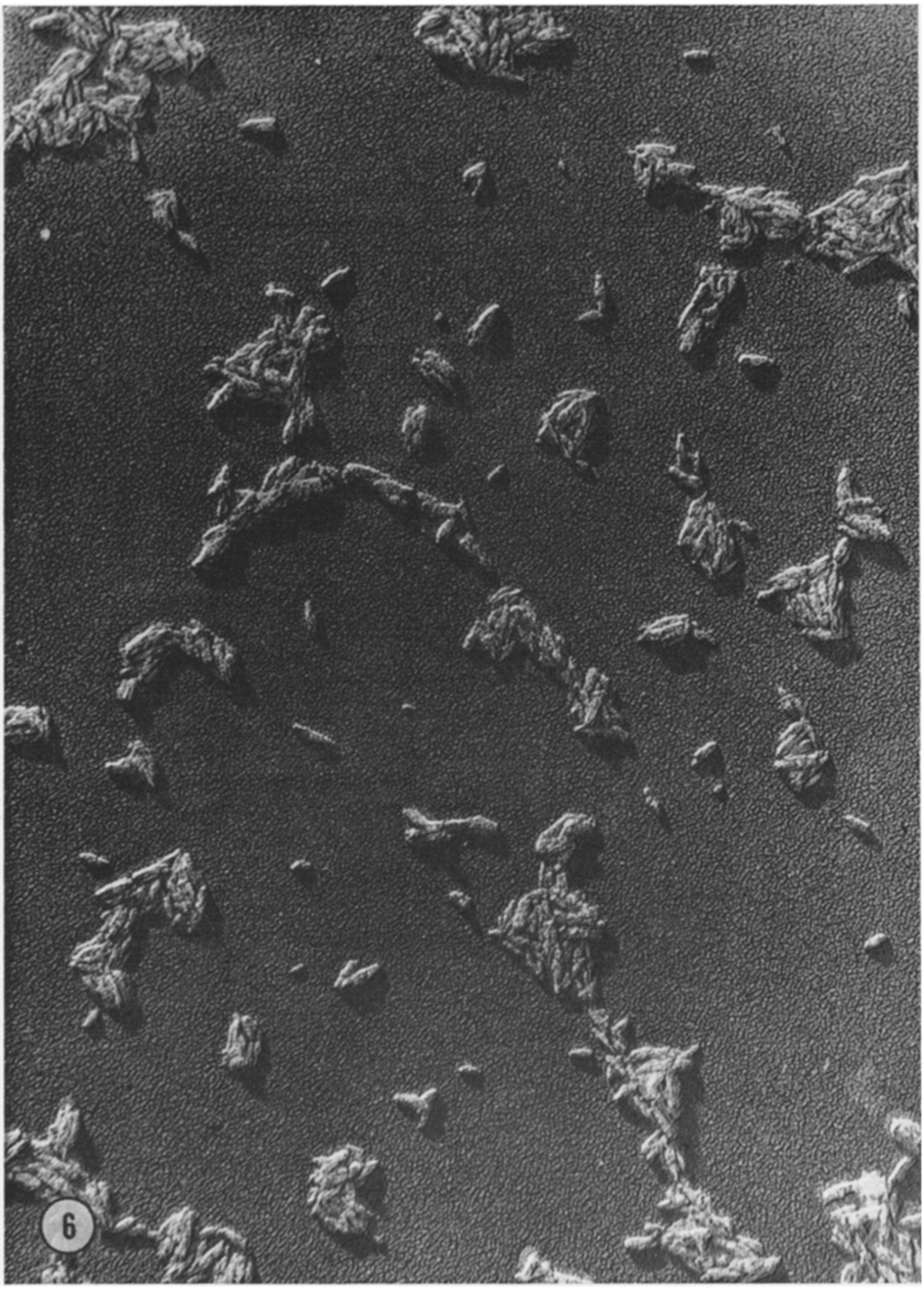

Fig. 6. Shadowed FPL 52001 × 100,000.

TABLE I

Particle Size Measurements (mμ) on Iron-Dextran Complexes

	Unshadowed	Shadowed	Largest unshadowed particle	Largest shadowed particle
Imferon (1586/5)[a]	3 × 3	11 × 7 (±2 × 2 rms)	4 × 4	23 × 11
Imposil (1286/20/100)[a]	11 × 3 (±5 × 0.5 rms)	21 × 12 (±4 × 2 rms)	23 × 4	35 × 11
FPL 52001 (50)[a]	31 × 7 (±8 × 2 rms)	43 × 15 (±15 × 6 rms)	46 × 8	72 × 23

[a] Batch number.

Gans equation (2) or, for long rods ($L{:}d > 10{:}1$), the Herzog-Kurdar equation (3).

$$D = \frac{kT}{3\pi\eta_0 L}\cdot\ln\frac{1+q}{(1-q)/2q}, \qquad [2]$$

$$D = \frac{kT}{3\pi\eta_0 L}\cdot\ln\frac{2L}{d}, \qquad [3]$$

where

$$q = \left[1 - \frac{d^2}{L^2}\right]^{1/2}.$$

Using the observed value of D, the $L \times d$ values calculated for FPL 52001 were:

1	2	3	4
58 × 2 mμ	40 × 5 mμ	29 × 8 mμ	24 × 10 mμ

Value 3, i.e., 29 × 8 mμ, fits well with the value of 31 × 7 mμ found by electron microscopic examination of the unshadowed material. This result and the corresponding agreement of the core size of Imferon with its overall size calculated from diffusion measurements suggest that in solution the particles behave as if they have a solid FeOOH core with a "collapsible" dextran sheath. This enables the particles to penetrate a membrane or barrier of pore size much smaller than might have been thought possible from measurements of the particle "uncollapsed" dimensions.

In his review, Müller (7) discussed the properties of the various forms of FeOOH and, as a result of his own experimental studies, came to the conclusion that only one subclass of β-FeOOH gave soluble complexes with carbohydrates, whereas none of the other forms did so. Therefore, it was decided to determine the nature of the FeOOH core of the three complexes as part of their general characterization and to see if their structures were in agreement with Müller's observation. Electron and X-ray diffraction experiments have been performed on the three samples and, for comparison, on pure samples of α-, β-, and γ-FeOOH. The results are shown in Table II.

The diffraction patterns of the three complexes fit most closely to those of β-FeOOH in agreement with Müller's findings. The best correspondence was obtained with FPL 52001 which contains the largest particles. Smaller particles have higher surface–volume ratios and consequently the effect of the associated dextran on the diffraction patterns would be greater in Imposil and Imferon.

Müller (7) also showed that the particular form of FeOOH could be identified by infrared spectroscopy. This was confirmed when spectra of the standard α-, β-, and γ-FeOOH samples were examined. The principle differences between the various forms lie in the region 700–1100 cm^{-1} where δ-OH vibrations occur. Figure 7 shows the spectra of the three standard forms and of Imferon, Imposil, and FPL 52001 for comparison.

In these α-, β- and γ-FeOOH are readily distinguishable but the spectra of the complexes are much more difficult to interpret because of the strong absorptions of the associated dextran components. Nevertheless, it is unlikely that α-FeOOH is present

TABLE II

INTERPLANAR SPACING BY ELECTRON AND X-RAY DIFFRACTION[a]

Imferon		Imposil		FPL 52001		α-FeOOH		β-FeOOH		γ-FeOOH	
ED	XRD	ED	XRD	ED	XRD	ED	XRD (15)	ED	XRD (12)	ED	XRD (16)
							4.94		7.40vs		6.30w
							4.20		5.25m		6.25vs
									3.70vw		
	3.30	3.28m	3.30	3.36s	3.30		3.36	3.32m	3.31vs		3.29vs
		2.65s				2.80ms	2.71		2.62m		
2.58s	2.54	2.35m	2.54	2.56vs	2.54	2.64ms	2.58	2.57s	2.54s		2.47ms
							2.40				
2.30m	2.28	2.22w	2.28	2.32s	2.28		2.26	2.27ms	2.34w	2.37m	2.36w
							2.19		2.29m		
2.07ms		2.04m		2.12w			2.08	2.08vw	2.09w		2.09w
							1.99		2.06w		
				1.95ms			1.91	1.95m	1.94ms	1.95vvs	1.93s
							1.83		1.85vw		1.85w
				1.77w			1.72	1.73vw	1.75m		1.73m
		1.70w							1.72vw		
				1.65m		1.60vs		1.63m	1.64vs	1.65vvw	1.70w
		1.55s					1.56				1.57m
1.52ms		1.50w		1.52vs		1.52vs	1.52	1.50s	1.52m	1.52vs	1.53vw
									1.50w		1.52m
									1.48w		
									1.46vw		
						1.39w	1.46	1.44w	1.44s	1.40m	1.45w
											1.43w
						1.34w	1.32	1.37w	1.37m		1.39vw
											1.37w
								1.30vw		1.27vw	1.26vw
								1.22vw		1.25s	1.22w
											1.20vw
								1.15m			1.19m
						1.03w		1.05wm			
						0.99wm				0.97m	
						0.92				0.88m	
						0.88wm				0.81m	

[a] v = very; w = weak; m = medium; s = strong.

in the complexes since they do not have strong absorptions at 790 and 890 cm^{-1}. The presence of α-FeOOH cannot be excluded, however, because the absorptions of this form coincide closely with those of the carbohydrate. The strongest indications are for the presence of β-FeOOH in the complexes since the absorptions at 680 and 840 cm^{-1} (marked with arrows in Fig. 7) correspond well with those of the pure sample.

Mössbauer spectroscopy has been frequently employed to provide information on the general environment of iron atoms in compounds. It was therefore applied to the complexes and to the standard forms of FeOOH for comparison in a further attempt to characterise the iron-containing core of the complexes. It was also hoped that it might give information on the iron atoms present on the surface of the core in contact with the dextran. In another experiment, a sample of FPL 52001 was treated to remove the carbohydrate and the residual FeOOH was then examined by Mössbauer spectroscopy. Table III gives the observed isomer shifts and quadrupole splittings.

All three complexes showed the expected two-line spectrum at room temperature and were identical within experimental error. At 77°K Imposil and the sample of FeOOH isolated following degradation of FPL 52001 gave six-line spectra due to antiferromagnetic interaction between the iron atoms. Imferon only gave a quadrupole split two-line spectrum at 77°K and no spectrum could be obtained from FPL 52001. The data suggest that the FeOOH core of the complexes bears the closest similarity in spectral properties to β-FeOOH in agreement with the evidence already presented by diffraction studies and infrared spectroscopy.

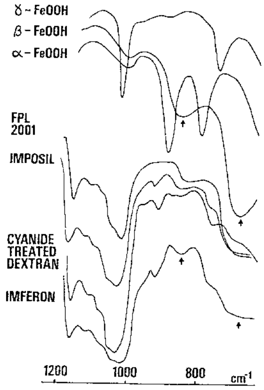

FIG. 7. Infrared spectra of α-, β-, and γ-FeOOH, iron-dextran complexes and cyanide-treated dextran as Nujol mulls between sodium chloride plates. (Cyanide-treated dextran was used in the preparation of FPL 52001 and its infrared spectrum in this region is typical of dextrans generally.)

The variation of behavior of the complexes at low temperature can be explained in terms of the different sizes of the particles. The smallest particles, found in Imferon, are below the critical size for observation of antiferromagnetism and, as a result, they show superparamagnetic behavior and only two lines in the spectrum. Imposil contains considerably larger particles, which are greater than the critical size for the observation of antiferromagnetism; and as a result a six-line spectrum was obtained. However, closer examination of the spectrum showed it to be a six-line spectrum superimposed on the remnants of a two-line spectrum. The asymmetry may be due to the effect of the iron atoms on the periphery of the FeOOH structures being in a different environment, as suggested above. However, the difference depends on whether the oxygen atoms around each iron atom are part of the FeOOH structure or part of the dextran attached to it and this difference is probably small. In addition, relatively few iron atoms will be in this different environment. For

TABLE III

ISOMER SHIFTS AND QUADRUPOLE SPLITTINGS FOR IRON-DEXTRAN COMPLEXES AND α-, β-, AND γ-FeOOH

Absorber	Absorber temp (°K)	Isomer shift, δ (mm/sec)	Quadrupole split, Δ (mm/sec)
Imferon	295	0.36 ± 0.05	0.62 ± 0.05
	77	0.47 ± 0.05	0.67 ± 0.05
Imposil	295	0.38 ± 0.05	0.65 ± 0.05
	77	6-Line spectrum	
FPL 52001	295	0.37 ± 0.05	0.65 ± 0.05
	77	No spectrum obtainable	
FeOOH from FPL 52001	295	0.37 ± 0.05	0.65 ± 0.05
	77	6-Line spectrum	
α-FeOOH	295	0.53 ± 0.06	6 lines
	77	0.78 ± 0.06	6 lines
β-FeOOH	295	0.43 ± 0.06	0.62 ± 0.06
	110	0.45 ± 0.06	6 lines
γ-FeOOH	295	0.48 ± 0.06	0.54 ± 0.06
	77	0.62 ± 0.06	0.62 ± 0.06

both reasons the overall effect on the spectrum is likely to be very small and the effect described was somewhat larger than would be expected on this basis. A superparamagnetic-antiferromagnetic transition is a more likely explanation for the phenomenon.

FPL 52001, which did not give a low-temperature spectrum, consists mainly of large particles; however, the electron photomicrograph (Fig. 3) reveals the presence of a small number of very small particles. It is suggested that these cause a superparamagnetic-antiferromagnetic transition giving rise to extreme line-broadening which prevents any spectrum being observed.

SUMMARY

The iron-dextran complexes examined in this study, Imferon, Imposil, and FPL 52001, are readily distinguishable by electron microscopy. Thus, Imferon possesses small, roughly spherical particles approximately 3 mμ in diameter, while Imposil and FPL 52001 contain larger spindle-shaped particles similar in shape to crystals of β-FeOOH. Shadow-casting demonstrated that the electron-dense core is surrounded by an electron-transparent sheath which is assumed to be dextran. Diffusion studies on Imferon and FPL 52001 led to calculated dimensions of the particles which agreed closely with those from electron microscopy measurements of the core without its dextran sheath. This possibly indicates that the dextran streamlines as the particle passes through pores.

Information on the nature of the core was obtained by physical studies: Electron and X-ray diffraction measurements support characterization of the iron-containg component as β-FeOOH but correlation of the interplanar spacings is too limited to be definitive. Infrared spectroscopy also suggests that the β-form is present and that the presence of α-FeOOH is unlikely, but the strong absorptions of the dextran make assignments tentative. Finally, Mössbauer spectroscopy gave little additional information on the structure of the core; spectrum differences between 77° and 300°K were rationalized in terms of particle size effects and superparamagnetic-antiferromagnetic transitions. All the physical studies reported, favor the presence of β-FeOOH in the complexes in support of Müller's observations (7).

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