# THE CELLULAR TRANSFORMATION OF INJECTED COLLOIDAL IRON COMPLEXES INTO FERRITIN AND HEMOSIDERIN IN EXPERIMENTAL ANIMALS

## A Study with the Aid of Electron Microscopy*

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Although there have been numerous studies on the fate of injected iron compounds in experimental animals (1–8), the transformation of the iron compounds within individual cells has not yet been traced at the molecular level. Recent work in which electron microscopy was combined with other techniques (9–12) has provided information on the molecular structure and the disposition of hemosiderin in several types of cells under varying circumstances, on the relationship of hemosiderin to ferritin, and on the possible role of specialized mitochondria ("siderosomes") in hemosiderosis. In the work now to be presented it has proved possible to distinguish intracellular deposits of iron compounds, given parenterally, from ferritin and from indigenous hemosiderin, and to gain insight into the transformation of the injected iron compounds into ferritin and hemosiderin. The findings bear on several aspects of iron metabolism, especially on the utilization of colloidal iron preparations within macrophages and endothelial cells.

### *General Plan of Experiments*

The general plan of the experiments was, *first*, to trace the sequence of changes in the fine structure of intracellular deposits of injected iron compounds in several types of cells in mice and rats at various intervals following intraperitoneal injection; *second*, to compare the physico-chemical nature of the intracellular material with that of the iron compounds prior to their injection; *thirdly*, to confirm the actual presence of ferritin, about or in relation to deposits

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of injected iron compounds; *finally*, to study the association of the injected compounds with cytoplasmic organelles.

### *Definition of "Hemosiderin"*

In earlier studies (11, 12) it was demonstrated that ferritin is often a component of typical hemosiderin granules, and that *some* hemosiderin granules have the essential attributes of crystalline ferritin while others are much less homogeneous. Furthermore, it was determined that crystalline ferritin gives histochemical reactions for ferric iron in the same way as hemosiderin granules. For these reasons the term *hemosiderin* will be used in this paper according to the following definition:

*Hemosiderin is a substance, occurring in the form of intra- or extracellular deposits that (1) are visible in the light microscope as brown granules, (2) contain trivalent iron as demonstrated by histochemical tests such as Perls' test (18); and (3) often contain variable amounts of ferritin as revealed by electron microscopy.*

### *Material and Methods*

*Animals.*—Mature, female mice of the D2BC strain were used. These animals were kept on diets of Purina chow and were given water *ad libitum*.

*Iron Compounds.*—Three iron compounds were used for injections and studied in the electron microscope.

(*a*) Iron-dextran (imferon), obtained from Lakeside Laboratories, Inc., Milwaukee, and manufactured by Benger Laboratories, Ltd. Holmes Chapel, Cheshire, Great Britain. This preparation is a complex of ferric hydroxide with partially depolymerized dextran (obtained from cultures of *Leuconostoc mesenteroides*). Its preparation is specified in United States Patent No. 2,820,740. In aqueous media this preparation forms a colloidal dispersion which is stable over a wide pH range and does not precipitate out in blood or serum. The ferric hydroxide (more properly it should be called hydrous ferric oxide), is physically bound to the dextran rather than chemically linked with it. As supplied by Lakeside Laboratories, the material has been sterilized by heating and contains the equivalent of 50 mg. of elemental iron per ml. As measured in our laboratory, the pH of the dispersions was 5.0.

(*b*) Saccharated iron oxide (ferric oxide), N.F. VII, obtained from Amend Drug and Chemical Company, Inc., New York. This material contained the equivalent of approximately 3.0 gm. of elemental iron per 100 gm. of dry solid, and yielded colloidal dispersions in water. 17.5 gm. was dispersed in 100 ml. of aqueous 0.9 per cent NaCl solution, the final concentration of iron being equivalent to approximately 5 mg./ml. The pH of these dispersions was 8.6. Prior to injection they were passed through Seitz filters. Unlike some other iron-sucrose preparations (6), this dispersion did not produce precipitates of hydrous ferric oxide when mixed with serum. As has been pointed out by others (4, 40), different preparations of saccharated iron oxide ("iron-sucrose") can vary considerably in their colloidal stability.

(*c*) A hydrous gel of ferric oxide (*i.e.* so-called ferric hydroxide) was prepared as follows:—

To 100 ml. of a 10 per cent solution of $FeCl_3$ a few drops of concentrated $NH_4OH$ were added to produce a permanent, reddish-brown precipitate. Several drops of concentrated HCl were then added to dissolve the precipitate. The solution was then put into cellophane bags and dialyzed against distilled water. This resulted in the formation of a brown gel which was very weakly soluble in water, and was used for study in the electron microscope

and for injection into animals. Sterilization was not employed in order that the physicochemical state of the gel might be preserved. The final pH of the gel was 2.9.

Hereafter the following abbreviations will be used occasionally for these three preparations:

ID[1]

SIO[2]

HIO[3]

*Injections.*—Imferon and saccharated iron oxide were injected intraperitoneally into mice in doses and at intervals to be detailed in the section on the experiments. The hydrous gel of ferric oxide was injected subcutaneously into mice in one divided dose, 1 ml. being injected into each flank.

In general, the injections were non-lethal although several mice died within 24 hours after intraperitoneal administration of 1 cc. of iron-dextran dispersion.

*Preparation of Tissues.*—To obtain tissue for study, animals were anesthetized with ether, and organs or blocks excised prior to death. Blocks not exceeding 1 mm. in greatest dimension were fixed in Palade's fixative containing 5 per cent sucrose or in Caulfield's fixative (15, 16). The blocks were generally fixed during 1 to 1½ hours, dehydrated in graded concentrations of ethyl alcohol, and embedded either in *n*-butyl methacrylate or in araldite (17). Sections were cut with Porter-Blum microtomes. For electron microscopy, sections (about 200 A in thickness) were mounted on carbon-coated copper specimen grids. For light microscopy, thick sections (0.5 to 1.0 microns) were used. These were then soaked in xylol to dissolve most of the methacrylate. They were stained with basic fuchsin, and a modification of Perls' stain for ferric iron was used to demonstrate the ferric iron compounds (18). Extraction of iron by means of HCl was used as a confirmatory test when deemed desirable (18).

Tissues were also fixed in 10 per cent neutral formalin, dehydrated, embedded in paraffin, and sectioned with a Spencer microtome. Some sections were stained with hematoxylin and eosin, some with the periodic acid–Schiff stain, and some with basic fuchsin and Perls' stain for iron as already mentioned.

*Electron Microscopy.*—

(*a*) *Transmission electron microscopy:* Electron microscopy was done with an RCA electron microscope, model EMU-3b. The objective and projector apertures described in another report (19) were used, and the instrument was operated with a beam potential of 100 kv. Magnifications were calibrated with a diffraction grating replica and by means of films of ferritin on carbon substrates.

(*b*) *Electron diffraction:* This was generally done with the beam at 100 kv., and after removal of the projector pole piece. But on occasion "selected area diffraction" with the projector pole piece in place was also done. Films of iron-dextran, saccharated iron oxide and hydrous ferric oxide were dried on carbon-coated specimen grids after such preliminary treatment as will be indicated in the appropriate sections below. Hydrous ferric oxide was also heated on specimen grids to produce anhydrous ferric oxide suitable for diffraction. The powder patterns obtained were evaluated using conventional crystallographic methods (20, 21). But intensities of reflections were estimated only by inspection of patterns.

*Shadow-Casting.*—Several types of specimens, each to be described, were doubly shadowed

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[1] ID, iron-dextran (imferon).

[2] SIO, saccharated iron oxide.

[3] HIO, Hydrous gel of ferric oxide (gel of $Fe_2O_3 \cdot 3H_2O$).

from opposite directions with chromium or palladium according to the method of Kahler and Lloyd (22) as applied to ferritin by Farrant (23).

*Isolation of Ferritin.*—Using the method of Granick (24), ferritin was crystallized from pooled livers and spleens of mice that had been given several injections of iron-dextran during periods of 3 weeks. The crystals were characterized by means of procedures described by others (20, 25), and were studied in the electron microscope as previously reported by the author (9–12). Solutions of such crystals were also studied as previously reported (11, 23).

## EXPERIMENTS AND RESULTS

### *Structure and Identification of the Iron Compounds by Means of Electron Microscopy. Comparison with Ferritin*

According to specifications (United States Patent No. 2,820,740) iron-dextran is a complex of ferric hydroxide with partially depolymerized dextran, the latter being derived from the dextran synthesized by *Leuconostoc mesenteroides*. In aqueous solution the complex apparently forms a colloidal, substantially non-ionic dispersion. When the material is dried, however, the ferric hydroxide must undergo dehydration, at least to the monohydrate of ferric oxide ($Fe_2O_3 \cdot H_2O$), and eventually to anhydrous $Fe_2O_3$. The latter compound can be readily prepared by heating $Fe_2O_3 \cdot 3H_2O$ (so-called Fe $(OH)_3$. The properties of saccharated iron oxide are similar in these respects to those of iron-dextran although the aqueous colloidal dispersions of SIO are less stable than those of ID.

As was shown by Weiser and Milligan (26), hydrous gels of ferric oxide can give sharp electron diffraction powder patterns if they have been aged or heated. Dehydrated ferric oxide gives the sharp pattern of natural crystalline hematite ($\alpha$-$Fe_2O_3$). As determined by Weiser and Milligan (26), and confirmed by the author, fresh hydrous ferric oxide [$Fe_2O_3 \cdot 3H_2O$, also called $Fe(OH)_3$], produces only a few weak and rather diffuse reflections in powder patterns. However, the author also found that upon prolonged examination in the electron microscope the patterns became progressively sharper, presumably owing to continuing dehydration of the material, and to heating by the electron beam. This observation was utilized in the study of ID and SIO. Preliminary heating of specimens containing either of these compounds, in a porcelain dish, at 100°C. and for 3 to 5 minutes, produced sufficient change to indicate spacings of $\alpha$-iron oxide in electron diffraction patterns (Figs. 1, 2). As shown, there was agreement of spacings ($d$) and of the relative intensities of arcs calculated to be at 2.87, 2.55, 2.33, 2.18, and 1.69 A. Prolonged exposure of ID or SIO to the electron beam had similar effects. Thus it was revealed that the dense particles seen in electron micrographs of these compounds, as described below, do in fact represent the loci of hydrous ferric oxide.

Another aspect of the identification of the hydrous forms of iron oxide relates to the size of the so called primary crystals. As is well known, crystals or crystallites may be present in specimens examined by x-ray or electron diffraction methods, but the size may be too small to give rise to distinct, well defined patterns (20). In this

sense such material can be considered amorphous. According to Weiser and Milligan (26) this is true of fresh hydrous ferric oxide.

To study the structure of iron-dextran in conventional transmission electron micrographs, drops of the dispersions previously described were dried on carbon-coated specimen grids, and the grids were then examined in the electron microscope. As shown in Fig. 4, images recorded at a resolution of about 10 A reveal that the iron-dextran contains innumerable particles that are relatively dense to electrons, as well as short, filamentous structures of similar density. It may now be briefly recalled that the iron hydroxide micelles of individual ferritin molecules have a different structure in electron micrographs, since they appear as characteristic quadruplets of spherical dense particles (11, 12, 23, 27), each of which measures about 30 A in diameter (Farrant: 27 A; Richter: 30 A). Each quadruplet as a whole thus has a diameter of about 60 A (Farrant: 55 A; Richter: 60 A). These quadruplets form the cores of ferritin molecules, the protein, apoferritin, being located about them. Depending upon the orientation of the ferritin molecules in the specimen with respect to the beam of incident electrons, one may see profiles of four, three, or two particles in the electron micrographs (Fig. 3). But crystallographic considerations make it likely that in the natural (*i.e.* undenatured, undehydrated) state of ferritin the four dense particles have a tetrahedral configuration. The reasons for the belief that these dense particles represent the iron hydroxide micelles of ferritin have been summarized by Farrant (23) and by the author (11). A simple calculation based on the molecular weight of ferritin (approximately 600,000), the iron content of ferritin (23 per cent), and the atomic weight of iron (55.8), leads to the result that there are approximately 2400 iron atoms in each ferritin molecule. Thus, each of the four particles (30 A in diameter) in a quadruplet contains approximately 2400, or 600 atoms of iron. And as Michaelis and Granick have indicated, these atoms are situated in FeOOH chains that form so called micelles (24, 28). In the ferritin molecule a great many atoms of iron are packed into a comparatively small space, and this fact accounts for the opaqueness of the particles seen when ferritin is examined in the electron microscope.

As is shown in Figs. 3 and 4, the dense particles of iron-dextran differ from those of ferritin in size and in configuration. In the iron-dextran the size of the particles varies more, some particles having diameters of about 20 A, while others have diameters of 70 A. There is a random distribution of these particles with respect to each other, and quadruplets are absent. Furthermore, specimens of iron-dextran that were doubly shadowed with chromium did not display the features that characterize shadowed ferritin molecules, *viz.* rings of evaporated metal surrounding the central iron micelles with a space between the former and the latter (*cf.* Fig. 17).

When samples of saccharated iron oxide were examined in the electron microscope in a comparable way, the images obtained were essentially similar to those

obtained when iron-dextran was used (Fig. 5). The possibility that the dense particles seen in images ID and SIO represent merely organic material (*i.e.* dextran, sucrose, or their derivatives), or carbon produced by the interaction of the electron beam and organic material, seemed remote. To clarify this point, samples of hydrous ferric oxide, prepared as already described, were "dried" (in air at 25°C.) on carbon-coated specimen grids. Electron micrographs and diffraction patterns were then obtained with this material. As shown in Fig. 23, these specimens displayed opaque particles and filaments similar to those found in iron-dextran and in saccharated iron oxide. A comparison of electron diffraction patterns indicated the presence of hydrous ferric oxide. Considered together, the findings make it clear that the opaque particles and filaments present in electron micrographs of ID and SIO represent loci of ferric hydroxide (hydrous ferric oxide) in these compounds.

### *The Intracellular Disposition of Iron-Dextran*

To study the intracellular disposition of iron-dextran, 14 female D2BC mice were given a single intraperitoneal injection of 1 ml. of iron-dextran (equivalent to 50 mg. of elemental iron). Two animals were sacrificed after 1 hour, 4 hours, 18 hours, 42 hours, 6 days, 2 weeks, and 3 weeks, respectively. Sections of spleen, liver, and kidney were studied with light and electron microscopes. The principal findings after the various intervals will now be considered in sequence.

*One hour following injection* many macrophages and sinusoidal endothelial cells in spleen and liver contained cytoplasmic aggregates of opaque particles and filaments identical with those present in samples of iron-dextran as described in the preceding section (Fig. 7). At this time the aggregates were not enclosed or surrounded by cytoplasmic membranes and were not situated in organelles such as the "siderosomes" (11). Some of these aggregates measured 1 to 3 microns in cross-section. But many particles and filaments were scattered through the cytoplasmic matrix. Sections of splenic tissue from five *untreated control animals* did not reveal such particles or filaments, but did reveal—as one might expect—scanty accumulations of iron micelles of ferritin in the cytoplasm of macrophages, as well as aggregates of such micelles. In the macrophages and endothelial cells of the two animals that had received ID, ferritin iron micelles were also present, but their number did not seem to be increased in comparison to the controls. From these observations, it was evident that one could not tell whether any ferritin had been formed within the 1st hour following injection. But the presence of the iron-dextran particles was unmistakable in pictures taken at sufficiently high resolution. Prussian blue tests were done on "thick" sections that had been matched with the thin sections used for electron microscopy. In each case the iron-positive, blue cytoplasmic granules were represented by aggregates of dense particles in the corresponding electron micrographs. It became clear, however, that, as expected, histochemical tests for ferric iron are equally positive when applied to synthetic compounds such as iron-dextran, and when applied to endogenous hemosiderin or to crystals of ferritin (11, 12, 29). So it was not possible to distinguish by means of the histochemical tests between injected iron-dextran and endogenous hemosiderin; and this was true when acid extraction of iron, as recommended by

Gomori (30) and by Lillie (18), was used. Other tests, such as the Tirmann-Schmelzer reaction (18), also failed in this respect.

*In specimens obtained 4 hours after injection* there were several noteworthy features that had not been observed in specimens taken 1 hour following injection. Quite a few cytoplasmic iron-dextran aggregates in splenic macrophages and in various endothelial cells were now enclosed by single membranes. Such cytoplasmic regions might represent phagocytic vesicles (Figs. 6 and 8). Also, in specimens obtained 4 hours after injection, there was vastly more ID in the cytoplasm of splenic macrophages and of endothelial cells than was present in specimens taken only 1 hour after injection. Furthermore, many ferritin iron micelles were situated in the cytoplasm of cells containing ID, although only a small number of these micelles was in close proximity to large aggregates of ID (Fig. 9).

*Eighteen hours following injection* iron micelles of molecular ferritin were more frequent in the vicinity of ID aggregates. Iron-dextran aggregates were abundantly present in macrophages and vascular endothelial cells in all samples of tissue examined. As seen in cross-sections, many of them had dimensions as large as 2 microns. But there were also many ID aggregates with much smaller dimensions, and many of these were enclosed by membranes. Cytoplasmic bodies demarcated by single membranes and containing ID particles as well as ferritin iron micelles were also present (Figs. 10 and 11). There were few scattered ID particles and filaments in the cytoplasmic matrix in the *vicinity* of aggregates, and still fewer in more remote cytoplasmic regions.

*Forty-eight hours following injection* ferritin iron micelles were present in moderate number in the vicinity of most ID aggregates in the cytoplasm of macrophages and vascular endothelial cells. Although one often finds ferritin in such cells taken from normal, untreated animals, its presence in the vicinity of ID aggregates seemed more than coincidental in the light of other findings. For now both ferritin iron micelles and the ID particles were often situated in discrete cytoplasmic bodies enclosed by single membranes, and often within cells that apparently were devoid of ferritin in other cytoplasmic regions (*cf.* Figs. 10 and 11).

*Six days following injection* there were vast numbers of ferritin iron micelles in the cytoplasm of macrophages and endothelial cells. These micelles were extremely numerous in the immediate vicinity of ID aggregates, especially in organelles enclosed by membranes. As shown in Fig. 12, the ID particles were clearly distinguishable from the ferritin iron micelles, and often there were sharp zones of transition between ID aggregates and the surrounding ferritin molecules. But it was also evident that at this stage ferritin was abundant throughout the cytoplasmic matrix. Observations on the disposition of iron-dextran in vascular endothelial cells of renal capillaries (tubular or glomerular) were essentially similar (Fig. 14).

The findings on sections of tissue taken *2 and 3 weeks after injection* were also essentially similar. At this time the majority of dense cytoplasmic particles in macrophages and endothelial cells appeared as ferritin iron micelles; and now there were many aggregates of such micelles that were indistinguishable in electron micrographs from the indigenous type of hemosiderin granules as previously described by the author (11, 12). It may be recalled here that the previous work indicated that indigenous hemosiderin contains variable amounts of ferritin, some hemosiderin granules

being crystals of ferritin while others are less homogeneous, but often contain ferritin. Furthermore, very little is known about the nature of organic material other than apoferritin that seems to be present in many hemosiderin granules.

It was also noteworthy that 2 to 3 weeks following injection cytoplasmic bodies with cristae and peripheral double membranes, and containing ferritin iron micelles were frequently seen in parenchymal liver cells (Fig. 13). In earlier reports the name "siderosomes" was applied to such organelles, which were believed to be derived from mitochondria (11). The presence of aggregates of ferritin in mitochondria has also been noted by Bessis and Breton-Gorius (13, 14).

In parenchymal liver cells and in cells of proximal convoluted tubules of mice that had received a single injection of ID, aggregates of particles of the injected ID were scarce even after 3 weeks, and when present, were inconspicuous. Such aggregates did not differ from those in macrophages and endothelial cells, while the presence and distribution of ferritin particles was also comparable in all types of cells. But 2 and 3 weeks after the injections had been given, siderosomes were encountered more frequently in the parenchymal liver cells than in the other cell types.

These last mentioned observations may indicate that parenchymatous cells, such as those of the liver, do not ingest colloidal particles of the size of iron-dextran complexes as actively as do macrophages and endothelial cells. On the other hand, the vascular endothelial barrier may to some extent impede the passage of colloidal iron-dextran from the lumina of capillaries to the surfaces of parenchymatous cells. The significance of the siderosomes (specialized mitochondria) in the parenchymatous cells of animals given iron-dextran is not apparent. As will be pointed out in the Discussion they may bring about the oxidation of ionic ferrous iron to ferric hydroxide. If some of the ferric iron, contained in ID, were reduced by constituents of cells, blood, or tissue fluids, the ferrous iron thus formed would have to be oxidized before incorporation into ferritin iron micelles. But, for reasons given in the Discussion, such a mechanism does not seem to be the primary one in the transformation of ID ferric hydroxide to ferritin ferric hydroxide.

*Further Identification of Ferritin in Cells of Animals Treated with Iron-Dextran*

Although in the previous work of Farrant (23) and of the author (11, 12) it was shown that in electron micrographs the appearance of the iron hydroxide micelles of purified ferritin is characteristic, it was not shown that such micelles were regularly associated with the protein component of ferritin, the apoferritin. In the previous work, methods of chemical fractionation and of shadow-casting were combined with electron microscopy to identify ferritin obtained from tissues. There was a high degree of correlation between the presence of "quadruplets" in large number in sections of splenic macrophages, and the quantity of ferritin that could be extracted from the spleen and identified chemically. The same was true when differential centrifugation was substituted for chemical extraction (11, 12, 29). In the work on which the present report is

based, it seemed important for reasons set forth in the Discussion to explore the possibility that ferritin iron micelles in the vicinity of ID aggregates might be "naked" iron hydroxide micelles; *i.e.*, not enveloped by apoferritin. To demonstrate the presence of entire ferritin molecules *in situ* within cells, sections from which the methacrylate embedding medium had been dissolved with xylol (24 hours) were doubly shadowed with chromium. Prior to removal of the methacrylate these sections had been mounted on carbon-coated specimen grids. Fig. 17 shows the result obtained with a representative section of a splenic macrophage. The tissue had been taken from a mouse 6 days after a single intraperitoneal injection of ID. In places indicated by arrows in Fig. 17, there are rings of evaporated metal with diameters of about 105 A around quadruplets (doublets or triplets), and there is a small space between the rings and the quadruplets (doublets or triplets). This is the characteristic image of isolated ferritin molecules, first demonstrated by Farrant and since confirmed (23, 27). Thus, one may assume that the ferritin iron micelles in the unshadowed sections signify the presence of *entire* ferritin molecules.

### *Decomposition of Iron-Dextran during Incubation with Tissue in Vitro*

A different type of experiment was done to provide confirmation for the inference that cell components can bring about a separation of ferric hydroxide (hydrous ferric oxide) from the iron-dextran complex. Such a separation is necessary if ferric hydroxide contained in iron-dextran is to precipitate out within cells and to form large aggregates; for colloidal iron-dextran is quite stable over a wide pH range (31). The closely packed aggregates of particles and "filaments" seen in electron micrographs are visible under the light microscope as brown granules (*i.e.* "precipitates") in fixed and in living cells.

Since the component of the brown granules which is opaque to electrons represents ferric hydroxide (hydrous ferric oxide), it became necessary to account for the precipitation of ferric hydroxide from iron-dextran *in vivo*.

Preliminary tests in which ID was mixed with normal rat or rabbit serum indicated that when the pH was artificially varied over a wide range (*e.g.* between 3 and 9) precipitation of ferric hydroxide did not take place. It seemed unlikely, therefore, that the rather small changes in pH that may occur in living cells could produce the precipitation of ferric hydroxide from iron-dextran. On the other hand, it appeared likely that the partially depolymerized dextran of the ID preparation may be metabolized by cells sufficiently to abolish the colloidal stability of the ID preparation. To test this inference the following experiment was done twice.

A D2BC mouse was exsanguinated. The entire liver was minced with scalpels and the mince suspended in 6 ml. of a 1:1 dilution of iron-dextran dispersion. This suspension was put into a small flask which was plugged with cotton and was kept in a water bath at 37°C. After 6 hours some of the liquid in the flask was withdrawn and observed under a light micro-

scope. It was found that small, brown granules that had not been present at the outset were now present in the liquid. Furthermore, upon addition of a few drops of distilled water (pH 7.2) a flocculent brown precipitate formed immediately. This precipitate was quite insoluble in water, gave a strong Prussian blue reaction, and was soluble in dilute hydrochloric acid, precipitating again upon addition of alkali. Examination of the precipitate in the electron microscope revealed closely packed particles and filaments similar to those found in ID, SIO, and HIO. After prolonged dehydration *in vacuo* on carbon-coated specimen grids, the material gave the electron diffraction pattern of $\alpha$-ferric oxide (Figs. 15 and 16). When this experiment was repeated using pooled, minced spleens instead of liver, the results were similar; but incubation of ID in mouse serum or blood did not result in the formation of precipitates.

The results of these experiments indicate that mouse tissues can bring about —in a way as yet to be determined—the precipitation of ferric hydroxide from iron-dextran. Since conditions under which this took place *in vitro* were comparatively mild, it is reasonable to assume that similar events take place within cells *in vivo*.

### *The Disposition of Saccharated Iron Oxide within Cells in Vivo*

Experiments with SIO were similar in essentials to those carried out with ID. In a representative experiment each of 12 D2BC mice was given a single intraperitoneal injection of 1 ml. of the SIO dispersion described before. Two animals were then sacrificed 1, 6, 12, 14, 21, and 28 days later.

In all instances the results were similar to those obtained with iron-dextran. The particulate or filamentous component of SIO was evident in splenic macrophages and in vascular endothelial cells examined 24 hours after injection (Fig. 18); and after 6 days the particles in dense cytoplasmic aggregates still had the same appearance though ferritin "quadruplets" were often found in the vicinity of the aggregates (Fig. 20). 12, 14, and 21 days following injection there were many ferritin iron micelles in splenic macrophages and sinusoidal endothelial cells, and in vascular endothelial cells in the kidneys. Ferritin was thus present about and occasionally within dense aggregates of SIO, but particles that were clearly not ferritin iron micelles were still abundant 21 days after injection of SIO. Again some aggregates of SIO particles, and of ferritin iron micelles were bounded by membranes (Fig. 19). Siderosomes, *i.e.* cytoplasmic bodies with cristae and peripheral double membranes that contained ferritin iron micelles, were also encountered in splenic macrophages. Twenty-eight days after injection relatively few SIO particles or filaments were seen in the macrophages and various endothelial cells examined, but ferritin was present in massive quantity (Figs. 21 and 22). At this time parenchymal liver cells, and cells in proximal convoluted tubules also contained much ferritin, as judged from the number of visible iron micelles, and in these cells siderosomes were plentiful at this time.

In another experiment 6 mice were given three intraperitoneal injections of SIO dispersion, 1 ml. being injected on the 1st, 6th, and 11th day of the experiment, respectively. The

mice were sacrificed 30 days after the first injection. Electron micrographs of macrophages, sinusoidal endothelial cells, and endothelial cells lining capillaries disclosed no features other than those already described.

### *Sequential Events Following the Subcutaneous Injection of Hydrous Ferric Oxide*

Two groups of four female D2BC mice were given single injections of hydrous ferric oxide subcutaneously. Injections of 1 ml. were given into each flank. Two animals were sacrificed after 24 hours, 5 days, 7 days, and 16 days, respectively. The soft, brown lumps that had developed in the subcutaneous tissues in the flanks of these animals were excised, dissected, and prepared for electron and light microscopy. In the gross, they consisted largely of brown precipitate about the site of injection in the subcutaneous tissues.

As expected, preliminary study with the light microscope revealed abundant, granular, brown, extra- and intracellular deposits. These deposits gave the Prussian blue reaction, and in their intracellular form were contained principally in macrophages. 24 hours and 5 days following injection most of the deposits were extracellular. In specimens obtained 7 and 16 days following injection, a much larger proportion of the deposits was contained in macrophages though much extracellular iron-positive material remained even after these intervals.

Electron micrographs revealed intracellular dispositions of the injected material and of ferritin that were in many ways analogous to those observed in splenic macrophages of animals that had been given ID or SIO intraperitoneally. As shown in Figs. 23 and 24, much injected hydrous ferric oxide was clearly identifiable even 16 days following injection. Much of the material was extracellular, however. While it was true that 24 hours after injection most of the deposits in macrophages contained no ferritin iron micelles, some of the latter were present nevertheless as scattered cytoplasmic particles. It was also evident that in tissues taken 5, 7, and 16 days after injection of HIO, much of the HIO in the cytoplasm of macrophages was enclosed by single membranes (Fig. 24). But the specimens taken 5, 7, and 16 days following injection also contained much ferritin. It was noteworthy that most of the ferritin was intracellular, being present mostly in macrophages, and that 16 days after injection the *extracellular* iron-positive deposits were those of injected hydrous ferric oxide. Moreover, 16 days after injection there was more ferritin in macrophages than 5 or 7 days after injection. However, after all three intervals much intra-cytoplasmic HIO still remained. Siderosomes (*e.g.* mitochondria containing ferritin iron micelles) were encountered very rarely in any of the macrophages, and never as early as 24 hours after injection.

Hence it seemed clear, that even 16 days after injection most of the ferritin in the specimens was intracellular. For comparison, further observations were made on sections prepared from the spleens of the animals that had been given HIO subcutaneously. Briefly, it was found by means of light microscopy that 24 hours following subcutaneous injection of HIO the two spleens examined contained only slightly more iron-positive deposits than did spleens from untreated control animals. Nearly all of this iron-positive material was contained in macrophages. Electron microscopy failed to disclose particles and filaments characteristic of the injected HIO. The particles present in the splenic macrophages had the typical structure of ferritin and hemosiderin iron micelles. The spleens taken 5, 7 and 16 days following injection

contained far more deposits that gave a Prussion blue reaction than did the spleens from control mice. Again, these deposits were almost wholly confined to the cytoplasm of macrophages though some were present in sinusoidal endothelial cells; and again, HIO particles were not found. In some macrophages ferritin iron micelles were present in large cytoplasmic aggregates, and occasionally in mitochondria. Moreover, ferritin iron micelles were found in the vascular spaces of splenic sinusoids, and in intercellular spaces in the splenic "cords," usually close to macrophages containing deposits. The most significant aspect of these observations is that injected HIO, while abundantly present in the subcutaneous tissue near the site of injection after 16 days, was not found in the spleen. On the other hand, there seemed to be a considerable increase in the amount of splenic ferritin (and hemosiderin granules containing ferritin).

## DISCUSSION

The findings demonstrate that the ferric hydroxide in the iron compounds used for injection differs in physical state from the ferric hydroxide that is present in ferritin or in endogenous hemosiderin. By virtue of this difference one can trace the transformation of the injected iron compounds with the electron microscope. It is not necessary to consider here the problem of what can be inferred from electron micrographs about the internal structure of various forms of hydrous ferric oxide (*i.e.* so called ferric hydroxide). It seems clear enough that the images of the iron compounds used for injection, and of ferritin, as recorded in electron micrographs, depend upon the state of the material examined during exposure to the electron beam (*cf.* reference 32). The images obtained are therefore not only related to, but are dependent on, the physicochemical nature of the individual compounds prior to electron microscopy. To deal with the extent or degree to which the real molecular structure of the iron compounds is represented in the electron micrographs, is outside the scope of this report, but it seems evident that analogous problems arise when results are obtained with the aid of research tools other than the electron microscope. It is noteworthy that with the chemical and histochemical methods generally used for the demonstration of trivalent iron (*e.g.* of iron in ferric hydroxide) one cannot discriminate between physical states of ferric hydroxide. Indeed, the possibility that a change in the physical state of ferric hydroxide might be involved in the formation of ferritin from injected ferric iron compounds has been neglected in many of the studies bearing on this point. Yet, more than a decade ago the work of Michaelis, Coryell, and Granick (33) on the paramagnetic susceptibility of iron in ferritin and hemosiderin clearly indicated a unique physical state of the iron.

It is clear that the observations which are the basis of the present report must be considered in the light of pertinent knowledge gained without the use of electron microscopy. There is abundant evidence that the injection of various iron compounds stimulates the production of ferritin, thus raising the level

of ferritin in several organs (11, 34–37). For example, Fineberg and Greenberg (37, 38) have shown by means of radioactive tracers that in guinea pigs ferritin is synthesized rapidly in the liver following the parenteral administration of trivalent iron. These investigators concluded that the administration of the iron compound accelerated the synthesis of apoferritin. One hour following injection of ferric ammonium citrate there was an appreciable increase of ferritin in the liver, and 4 hours after injection the increase was quite considerable. It is of special interest that Bielig and Bayer have reported that apoferritin combines readily with ferric hydroxide *in vitro* (39). In their experiments ferric hydroxide was prepared *in vitro* in the presence of apoferritin. The iron in the ferritin that was thus produced had the characteristic paramagnetic susceptibility, equivalent to three unpaired electrons. These results are consistent with earlier work of Granick (24) in which it was shown that ferric hydroxide micelles can be readily removed from ferritin by means of reducing agents without denaturing the protein moiety, apoferritin. Thus, the ferric hydroxide and apoferritin that constitute ferritin are bound together but loosely. The paramagnetic susceptibility of the iron in crystalline ferritin and in hemosiderin is the same, and is equivalent to three unpaired electrons per atom of iron, a fact that is in harmony with the author's findings on the relationship between ferritin and hemosiderin (9–12). So far as is now known, none of the other iron compounds that have been found in living matter have this property. Since ferritin iron hydroxide micelles are commonly present in endogenous hemosiderin, and often abundantly so (12), the utilization of ID or SIO in the formation of ferritin and hemosiderin may be considered together.

In thinking about the possible meaning of the evidence presented in the present report, five observations must be taken into account. These are:—

1. The presence of aggregates (precipitates) of ferric hydroxide (hydrous ferric oxide) particles derived from the injected preparations within cells such as macrophages and endothelial cells.

2. The presence of membranes—usually single—around some but not all of the aggregates of injected material.

3. The abundance, after varying time intervals, of ferritin in close proximity to aggregates of injected material; and the presence, in the cytoplasm, of bodies that have membranous borders and contain injected particles and ferritin.

4. The rapid rise in the ferritin content of organs or tissues containing injected iron compounds.

5. The paucity of siderosomes (or mitochondria containing ferritin iron micelles) in cells that are laden with iron-dextran or saccharated iron oxide.

How, then, does ferric hydroxide, present in iron-dextran and in saccharated iron oxide, become part of ferritin? Although any answer to this question can at present be only tentative, and incomplete, a possible mechanism will now be proposed.

The colloidal dispersions (ID or SIO) presumably reached the splenic macrophages and sinusoidal cells, as well as endothelial cells in various other locations, through the blood stream following penetration of the peritoneal surfaces. Presumably also, the colloidal dispersions crossed the mesothelial and endothelial barriers as do various macromolecules, including ferritin. It should be re-emphasized that neither the ID nor the SIO that were used gave rise to precipitates when mixed with blood or serum. Although some precipitation in the peritoneal cavity might have taken place, the wide dissemination of both materials in different organs within a few hours after injection clearly indicated entrance into the blood stream. Twenty-four hours after injection the peritoneal surfaces were generally brown but quite smooth, indicating entrance of the iron compounds into the mesothelial lining cells. Because of their colloidal state, the ID and SIO may have entered various types of cells through pinocytic vesicles. Once in the cytoplasm, much of the colloidal ferric hydroxide in ID or SIO precipitated out as a result of cell action on the stabilizing dextran or sucrose. The next step could then have been the union of ferric hydroxide with apoferritin to form ferritin in the cytoplasm, a union similar to the one that can be produced *in vitro*.

Some "free" apoferritin might be present in the cytoplasm prior to entry of ID or SIO, but, as found by Fineberg and Greenberg (38), much apoferritin may be synthesized *de novo* owing to the stimulating effect of the injected iron. According to a widely accepted hypothesis, proteins may be synthesized in close proximity to the endoplasmic reticulum (ergastoplasm). Since apoferritin is a protein, one might suppose that it is synthesized in the vicinity of the endoplasmic reticulum. But its synthesis in relation to other cell components is far from precluded, especially since many cell proteins, such as enzymes that are located in mitochondria, have no apparent relationship to the endoplasmic reticulum. In any case, the ferric hydroxide provided by ID and SIO must be remodeled into the characteristic ferric hydroxide micelles of ferritin at some stage in the sequence of events. Since the work of Bielig and Bayer (39) indicates that such a process can take place *in vitro*, it is possible that apoferritin molecules provide templates upon which preformed ferric hydroxide can be ordered into the typical micelles. It seems likely that the formation of ferritin—whatever its precise mechanism may be—is limited by the amount of apoferritin available in the cell. Hence, the persistence of ID or SIO within cells for many days may reflect the limited synthesis of apoferritin. It is clear, however, that the process whereby iron hydroxide is united with apoferritin need not involve mitochondria but can take place in any region of the cell where the two compounds come in contact under chemically suitable conditions. By contrast, if iron were present in the *ferrous* state, an oxidative process would be required to bring it to the *ferric* state. Thus, oxidative enzymes of mitochondria might be required for the formation of ferritin when divalent iron is supplied (*e.g.* iron liberated during the intracellular breakdown of hemoglobin, or the ferrous iron in some compounds used in therapy). Under such circumstances specialized mitochondria (siderosomes) might play an essential part in the formation of ferritin (11).

The presence of membranes about some aggregates of injected material need not have a special, functional significance. These membranes may be derived from vesicles formed during imbibition or ingestion of colloidally dispersed ID or SIO. Or, are they formed from cytoplasmic organelles, possibly even mitochondria? Do they delimit cytoplasmic regions that are specialized in function? Obviously, much more evidence must be gained before such questions can be answered.

In the light of the foregoing discussion the observations on material from mice that had been given subcutaneous injections of hydrous ferric oxide (HIO) may now be briefly considered. One can infer that HIO is no more soluble *in vivo* than it is *in vitro*. Precipitates form when such material is added to serum or to various aqueous solutions, especially when the pH is close to neutral (5, 29). In the experiments here reported, gross and microscopic findings left no doubt that the injected HIO remained sharply localized in the form of brown precipitates. Furthermore, the observations made with the electron microscope have indicated that the HIO was phagocytized before being utilized for the synthesis of ferritin, *i.e.* that the formation of ferritin was dependent upon intracellular processes. Among such processes the elaboration of apoferritin must have the leading part for it is obvious that in the absence of apoferritin there can be no ferritin. The lack of HIO particles or aggregates in cells of the spleen even 16 days after subcutaneous injection may have been due to insolubility of the HIO. Nevertheless it appears likely that the increase of ferritin in the spleen following subcutaneous injection of HIO was related to the presence of the subcutaneous deposits. Consideration of this finding suggests two possible explanations. Firstly, ferritin formed in macrophages at the site of injection was released into the blood stream, and reached the spleen as well as other organs in increased concentration; it was then stored by macrophages and other cells in the spleen and elsewhere. Also, macrophages containing ferritin might have wandered from the site of injection and reached the spleen *via* the blood stream. Secondly, it is possible that reduction of some of the iron in the HIO deposits took place, and that as a result ferrous iron was liberated in ionic (*i.e.* "soluble") form. Such iron can pass readily to various organs by the blood stream, and can be utilized in the oxidative synthesis of ferritin iron micelles in splenic macrophages and in other cells—a process that occurs in normal iron metabolism.

## SUMMARY

As revealed by electron microscopy and electron diffraction, the physical state of ferric hydroxide micelles contained in iron-dextran, saccharated iron oxide, and hydrous ferric oxide ("ferric hydroxide") differs notably from the state of the ferric hydroxide in ferritin or hemosiderin. By virtue of this difference one can trace the intracellular transformation of colloidal iron, administered parenterally, into ferritin and hemosiderin.

One hour after intraperitoneal injection of iron-dextran or saccharated iron oxide into mice, characteristic deposits were present in splenic macrophages, in sinusoidal endothelial cells of spleen and liver, and in vascular endothelial cells of various renal capillaries. Four hours after injection, small numbers of ferritin molecules were identifiable about intracellular aggregates of injected iron compounds; and by the 6th day, ferritin was abundant in close proximity to deposits of injected iron compounds. The latter were frequently situated in cytoplasmic vesicles delimited by single membranes. These vesicles were most frequently found in tissue obtained during the first 6 days after injection; and in certain of the vesicles ferritin molecules surrounded closely packed aggregates of injected material. Much unchanged ferric hydroxide was still present in macrophages and vascular endothelial cells 3 to 4 weeks after injection.

While electron microscopy left no doubt about the identity of injected ferric hydroxide on the one hand, and of ferritin or hemosiderin on the other, histochemical tests for iron failed in this respect.

Precipitation of ferric hydroxide (hydrous ferric oxide) from stabilized colloidal dispersions of iron-dextran was brought about *in vitro* by incubation with minced mouse tissue (*e.g.* liver), but not by incubation with mouse serum or blood.

Subcutaneous injections of hydrous gel of ferric oxide into mice initially produced localized extracellular precipitates. Most of the material was still extracellular 16 days after injection, though part of it was phagocytized by macrophages near the site of injection; but apparently none reached the spleen in unaltered form. Five days after injection and thereafter, much ferritin was present in macrophages about the site of injection and in the spleen.

The findings show that iron preparations widely used in therapy can be identified within cells, and that their intracellular disposition and fate can be followed at the molecular level. Considered in the light of previous work, they indicate that the characteristic structure of the ferric hydroxide micelles in molecules of ferritin is specific, and develops during the union of apoferritin with ferric hydroxide. Apparently this union does not depend upon specific cell components.

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## EXPLANATION OF PLATES

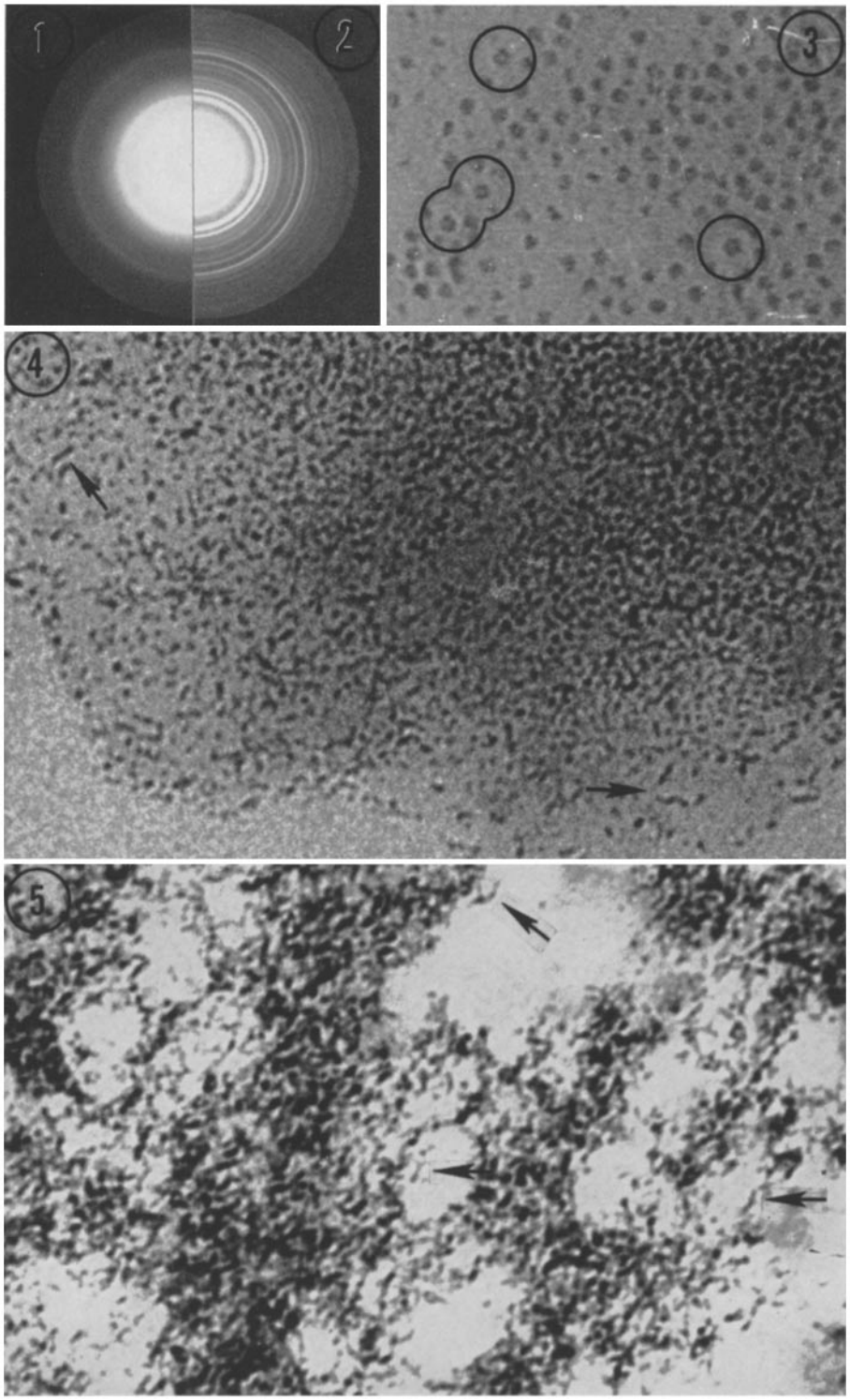
## PLATE 18

FIG. 1 and 2. Fig. 1 is an electron diffraction pattern obtained from dry iron-dextran that had been heated in a porcelain dish at 100°C. for 3 minutes. Fig. 2 is a similar pattern obtained from $\alpha$-$Fe_2O_3$. The spacings ($d$), that are identical in both patterns, were calculated to be at 2.87, 2.55, 2.33, 2.18, 1.84, and 1.69 A, respectively. Note also that the relative intensities of the arcs are of the same order.

FIG. 3. Electron micrograph showing ferric hydroxide micelles of mouse ferritin molecules. Note the "quadruplets" demarcated by circles. They show four particles, each with a diameter of 30 A, separated by a central space of 10 to 15 A. Other profiles (*e.g.* triplets, doublets) are also represented, and these presumably are oblique or lateral projections of "quadruplets". Imperfectly formed micelles may also be present [*cf.* Farrant (23)]. The specimen was prepared by dissolving a few crystals of mouse ferritin in water, and drying droplets of the solutions on carbon-coated specimen grids. The mouse ferritin was crystallized from pooled hemosiderotic mouse livers according to the method of Granick (24). × 366,000.

FIG. 4. Electron micrograph of iron-dextran from solution dried on a carbon-coated specimen grid. Note the many small particles with diameters between 15 and 40 A, and the filamentous profiles indicated by arrows. These particles and filaments represent the hydrous ferric oxide ("ferric hydroxide") contained in iron-dextran (see text). Compare with Figs. 5 and 23. × 256,000.

FIG. 5. Electron micrograph of saccharated iron oxide showing particles and filaments (arrows) similar to those found in iron-dextran. × 256,000.

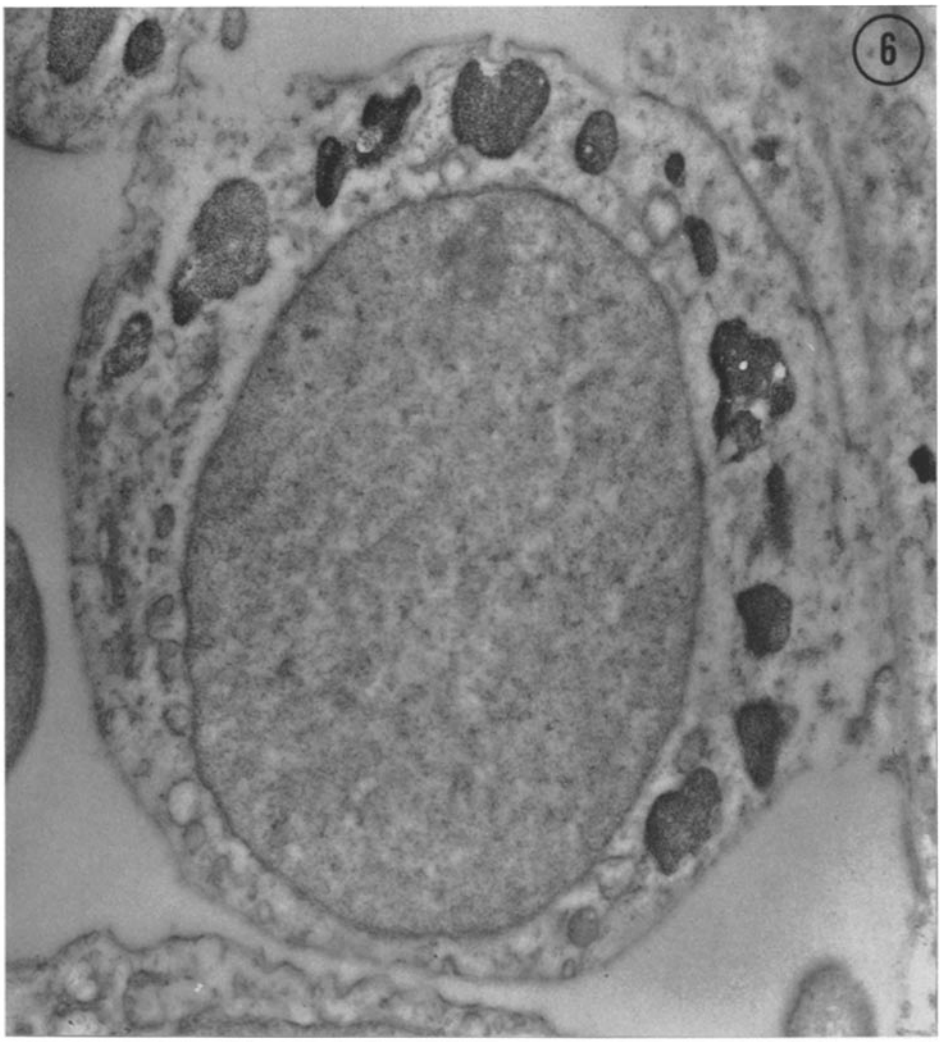


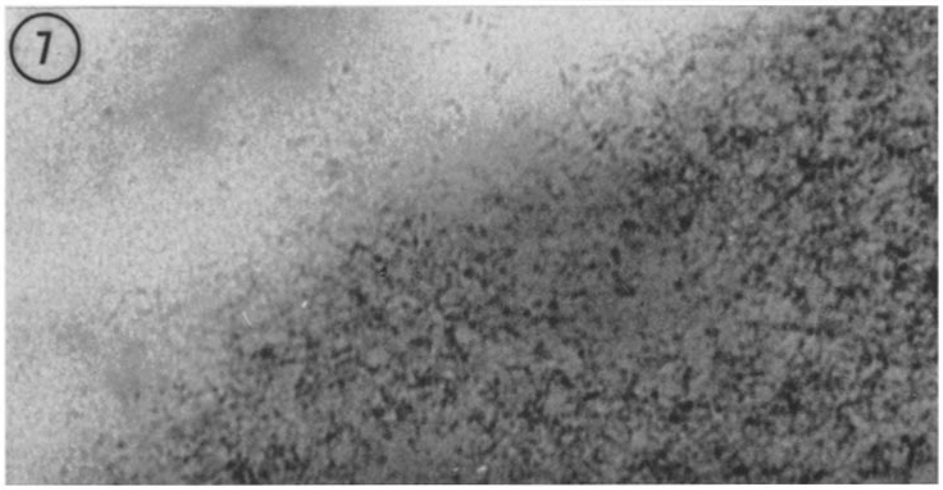

(Richter: Transformation of colloidal iron into ferritin)

PLATE 19

FIG. 6. Macrophage in a section from spleen of mouse given an intraperitoneal injection of iron-dextran 4 hours previously. Note the sharply outlined aggregates of dense particles in the cytoplasm. × 33,000.

FIG. 7. Part of cytoplasm of macrophage in spleen of a mouse that had been given an intraperitoneal injection of iron-dextran 1 hour before being killed. Note the small particles and filaments and compare with Fig. 4. × 225,000.



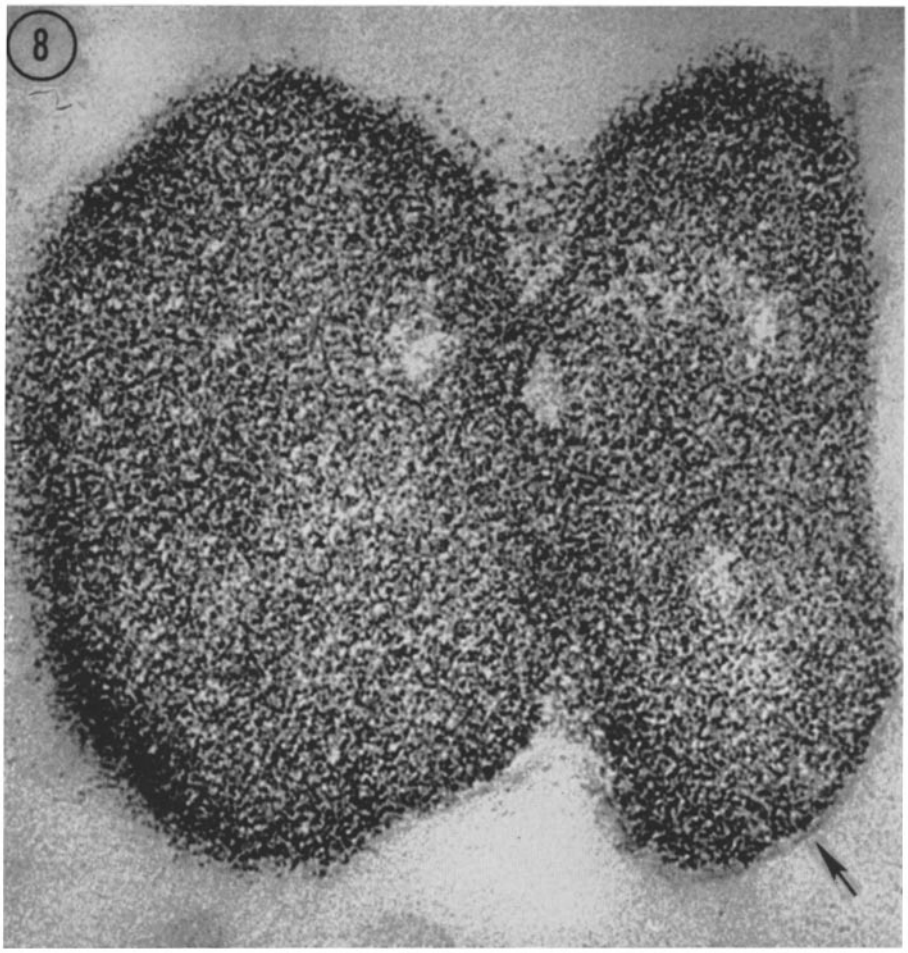



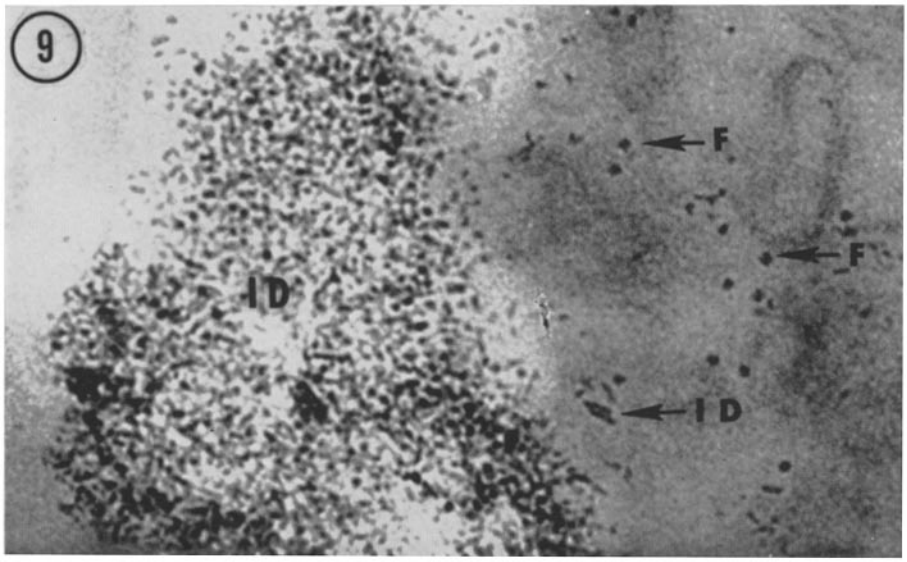

(Richter: Transformation of colloidal iron into ferritin)

PLATE 20

FIG. 8. Same specimen as in Fig. 6. An aggregate of dense particles in a splenic macrophage is shown at higher magnification. Most of the particles have diameters between 15 and 40 A, and apparently there are no ferritin iron micelles. Note the single membrane near the arrow. × 200,000.

FIG. 9. Part of cytoplasm of macrophage in spleen of mouse that had received an intraperitoneal injection of iron-dextran 4 hours before being killed. Particles and filaments (ID) representing "ferric hydroxide" contained in iron-dextran can be distinguished from ferric hydroxide micelles of ferritin molecules (*F*) which are not fully resolved. Note that there are only a few scattered ferritin micelles at this stage. × 256,000.

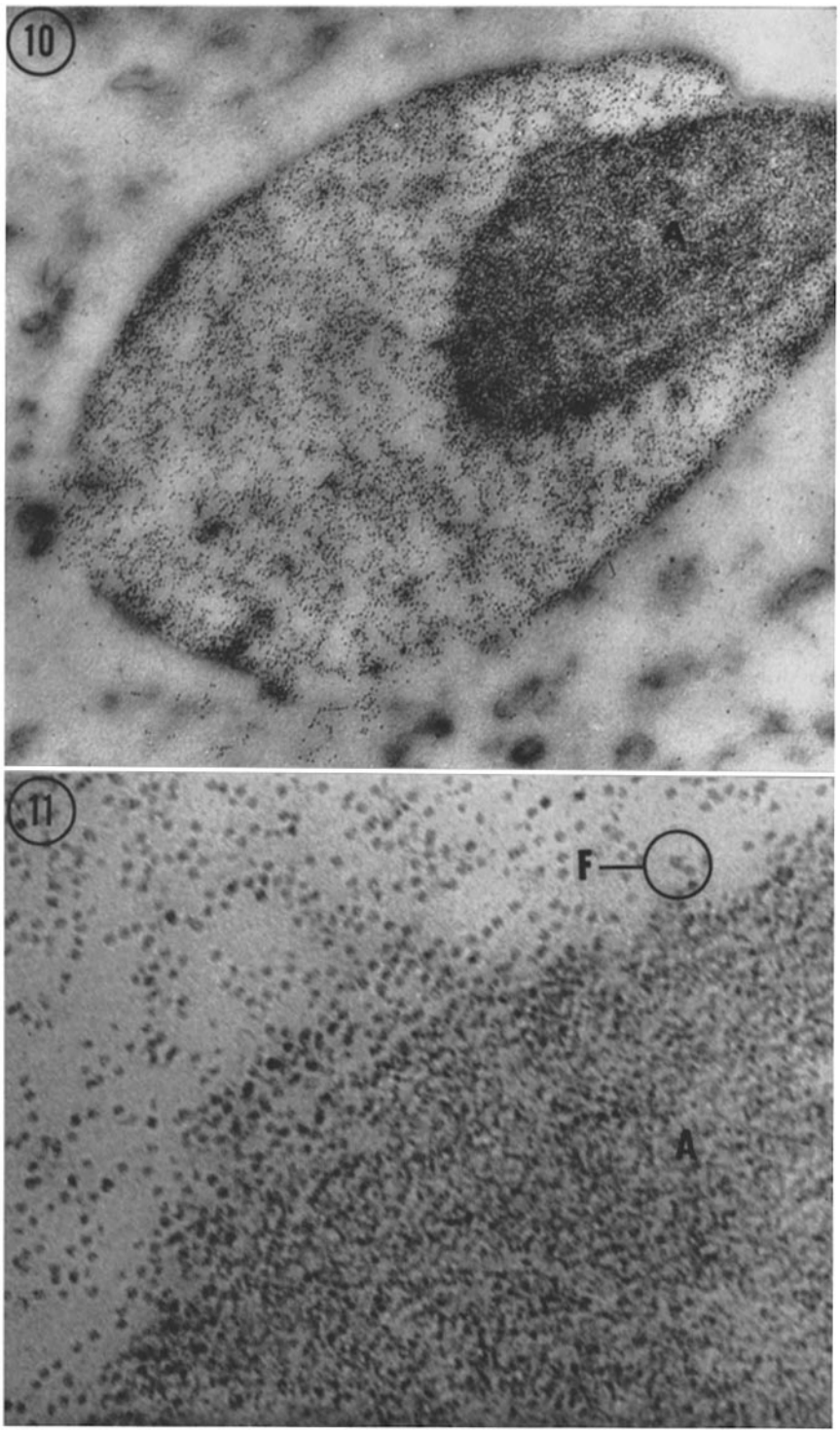



(Richter: Transformation of colloidal iron into ferritin)

PLATE 21

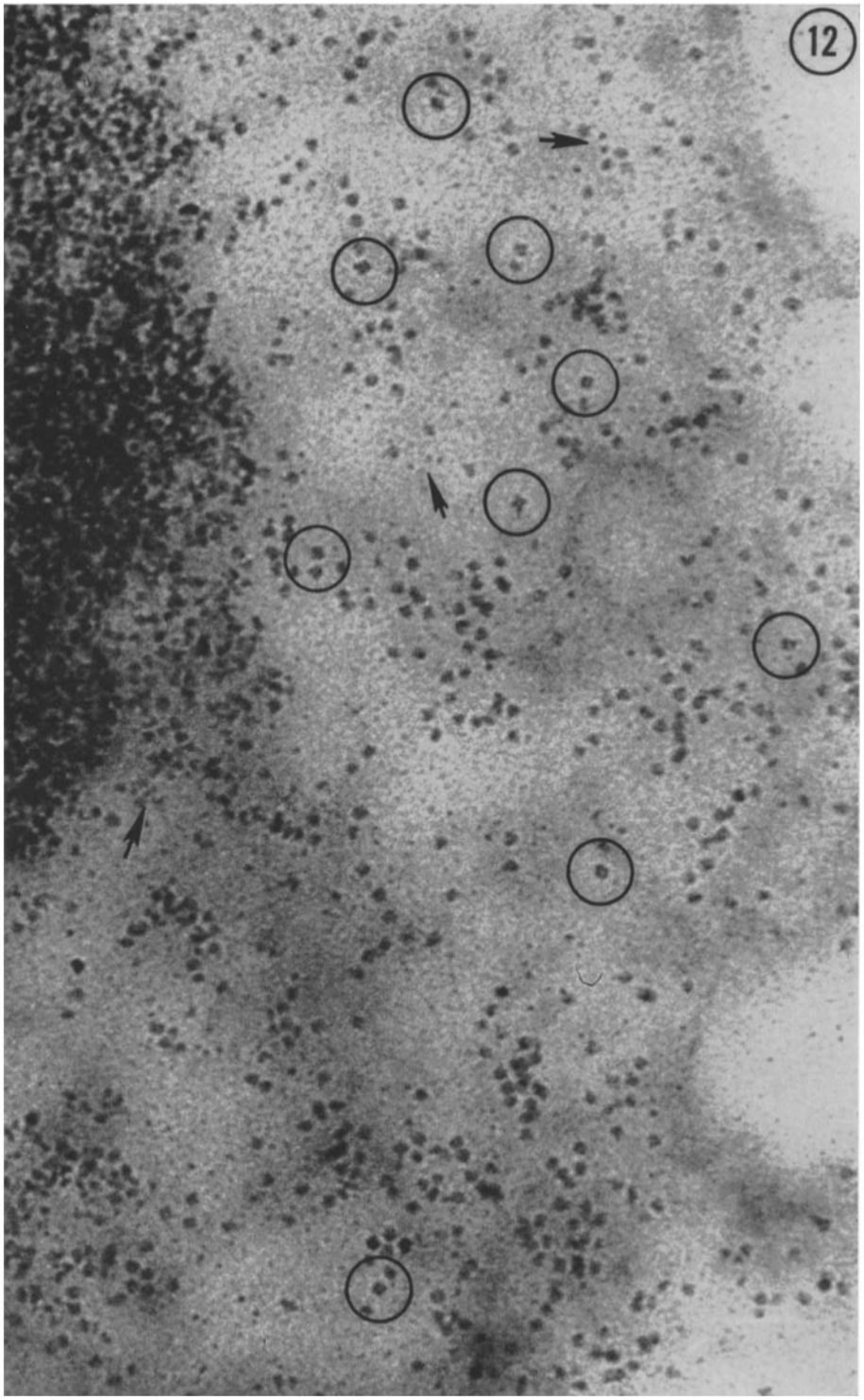
FIGS. 10 and 11. Cytoplasmic body in macrophage of mouse spleen 18 hours after intraperitoneal injection of iron-dextran. This body is bounded by a single membrane (Fig. 10) and it contains two kinds of particles (Fig. 11): (*a*) closely packed particles, about 15 to 40 A in diameter, that are situated in an aggregate (*A*) which is rather sharply demarcated, and (*b*) scattered particles that surround the aggregate (*A*) and most of which have the structure of iron micelles of ferritin molecules. Although there is some astigmatism in the image, characteristic subunits are visible in many of these micelles; *e.g.* at (*F*). Fig. 10, × 66,600; Fig. 11, × 225,000.




(Richter: Transformation of colloidal iron into ferritin)

PLATE 22

FIG. 12. Part of cytoplasm of macrophage in mouse spleen 6 days after intraperitoneal injection of iron-dextran. At the left is a closely packed aggregate of dense particles. Scattered in the cytoplasm about the aggregate are many ferritin molecules, as indicated by the presence of quadruplets (circles). But there are also innumerable, smaller, single particles (arrows). Some of these have diameters between 10 and 15 A. × 315,000.

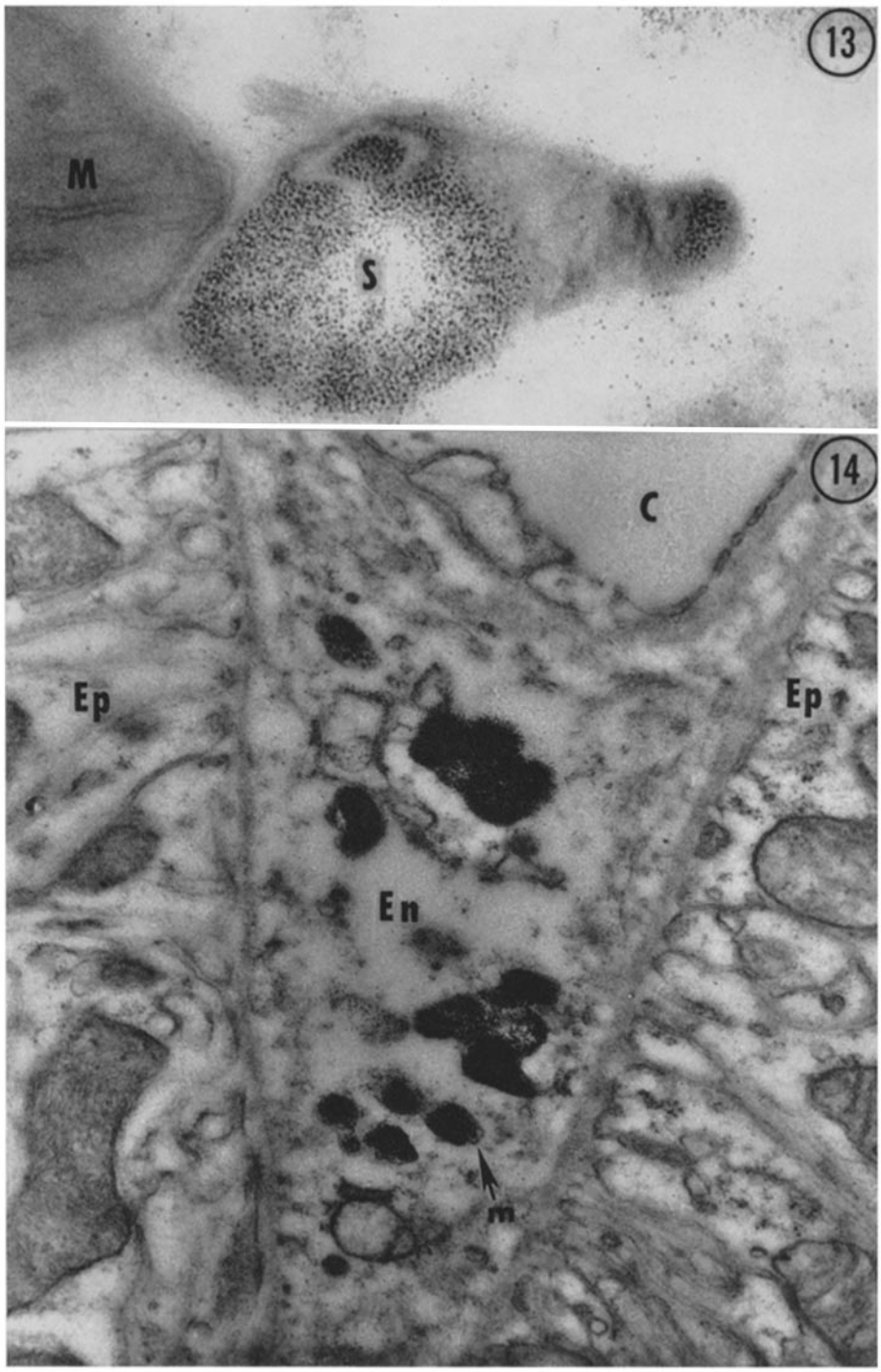



(Richter: Transformation of colloidal iron into ferritin)

PLATE 23

FIG. 13. Siderosome (*S*) in cytoplasm of parenchymal liver cell 3 weeks after intraperitoneal injection of iron-dextran. Note the internal membranes. Most of the dense particles have diameters of about 60 A and therefore probably represent ferritin iron micelles. Subunits are not visible at this resolution. At left is part of a mitochondrion (*M*). × 83,000.

FIG. 14. Section of mouse kidney 18 hours after intraperitoneal injection of iron-dextran. At the right and left of the image are parts of two epithelial cells (*Ep*) of proximal convoluted tubules. Between these is part of an endothelial cell (*En*) lining a capillary (*C*). Note the dense aggregates some of which are surrounded by membranes (*m*). Many particles are scattered in the cytoplasm of the endothelial cell. Most of them have diameters of about 60 A, and probably represent ferritin iron micelles at relatively low magnification. × 50,000.



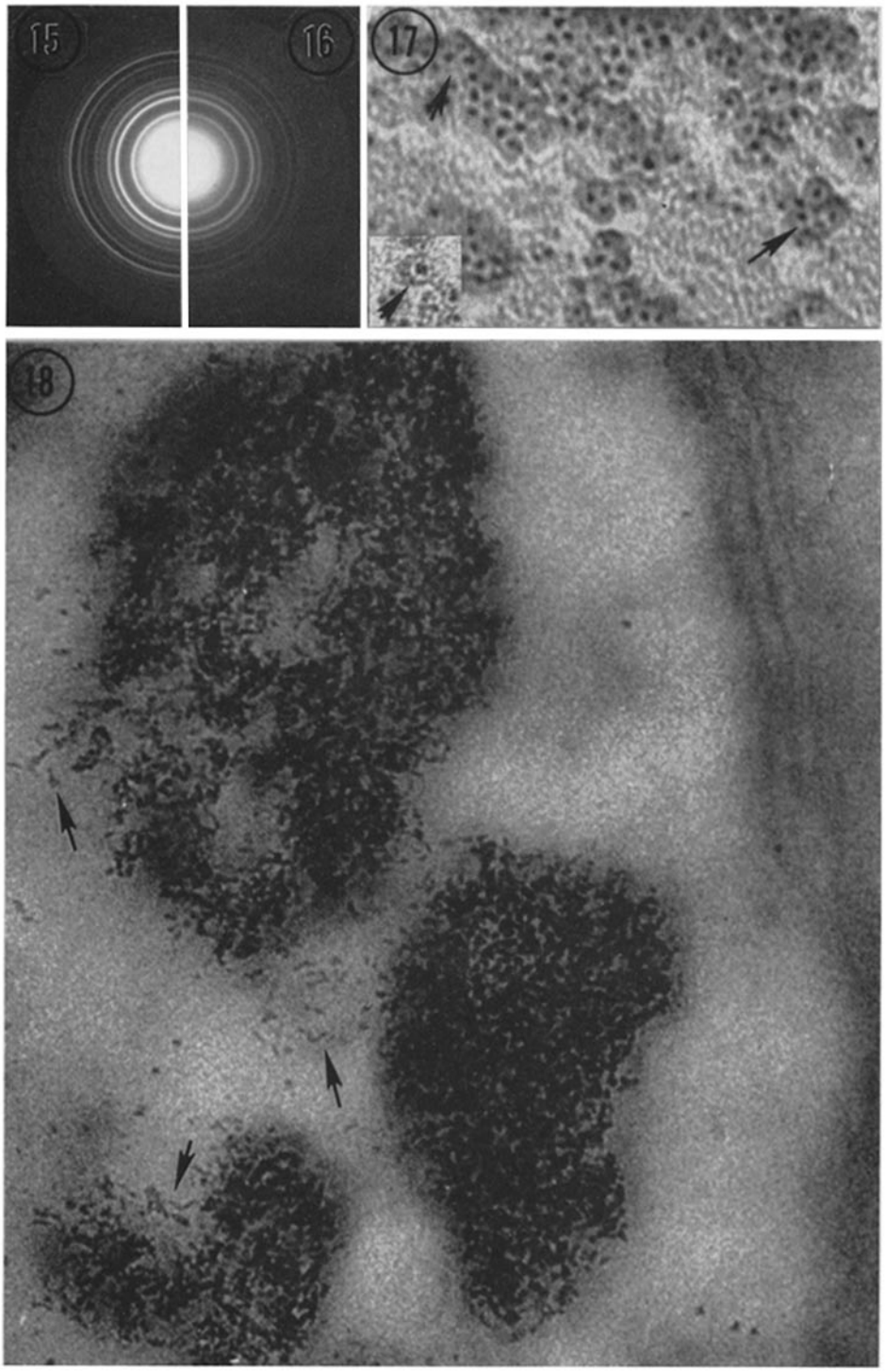

(Richter: Transformation of colloidal iron into ferritin)

PLATE 24

FIG. 15 and 16. Fig. 15 is an electron diffraction pattern of $\alpha$-$Fe_2O_3$ (compare with Fig. 2). Fig. 16 shows a similar diffraction pattern that was obtained on material derived from iron-dextran in the following experiment: Minced mouse liver was incubated in a solution of iron-dextran at 37°C. A fine brown precipitate was noted after 6 hours. Some of the liquid was decanted and a small volume of distilled water (pH 7.2) added to it. A coarse, flocculent, brown precipitate formed immediately. This precipitate was separated by means of centrifugation, and was dehydrated in a vacuum desiccator for 3 days. Comparison of Figs. 15 and 16 shows arcs in both patterns that are identical. The coincident spacings were calculated to be at 2.87, 2.82, 2.56, 2.32, 2.18, 1.97, 1.84, and 1.70 A.

FIG. 17. Doubly shadowed aggregates of ferritin molecules *in situ* in the cytoplasm of a splenic macrophage. Rings of evaporated chromium are situated at a distance around some ferritin iron micelles (arrows). This indicates the presence of apoferritin about the iron micelles (*cf.* Farrant (23)). Note also that there are rather uniform spaces between adjacent iron micelles in the aggregate at the upper left. Here and elsewhere only the periphery of the aggregate is outlined by evaporated chromium. The inset shows a characteristic "quadruplet" which is surrounded by a ring of chromium with a diameter of about 105 A. Compare with Fig. 3 which is a picture of unshadowed ferritin molecules. × 283,000.

FIG. 18. Saccharated iron oxide in macrophage of a mouse spleen 24 hours after intraperitoneal injection. The cytoplasmic aggregates are closely packed. Particles and filaments similar to those shown in Figs. 4, 5, and 23 are best seen near the periphery of each aggregate (arrows). × 200,000.

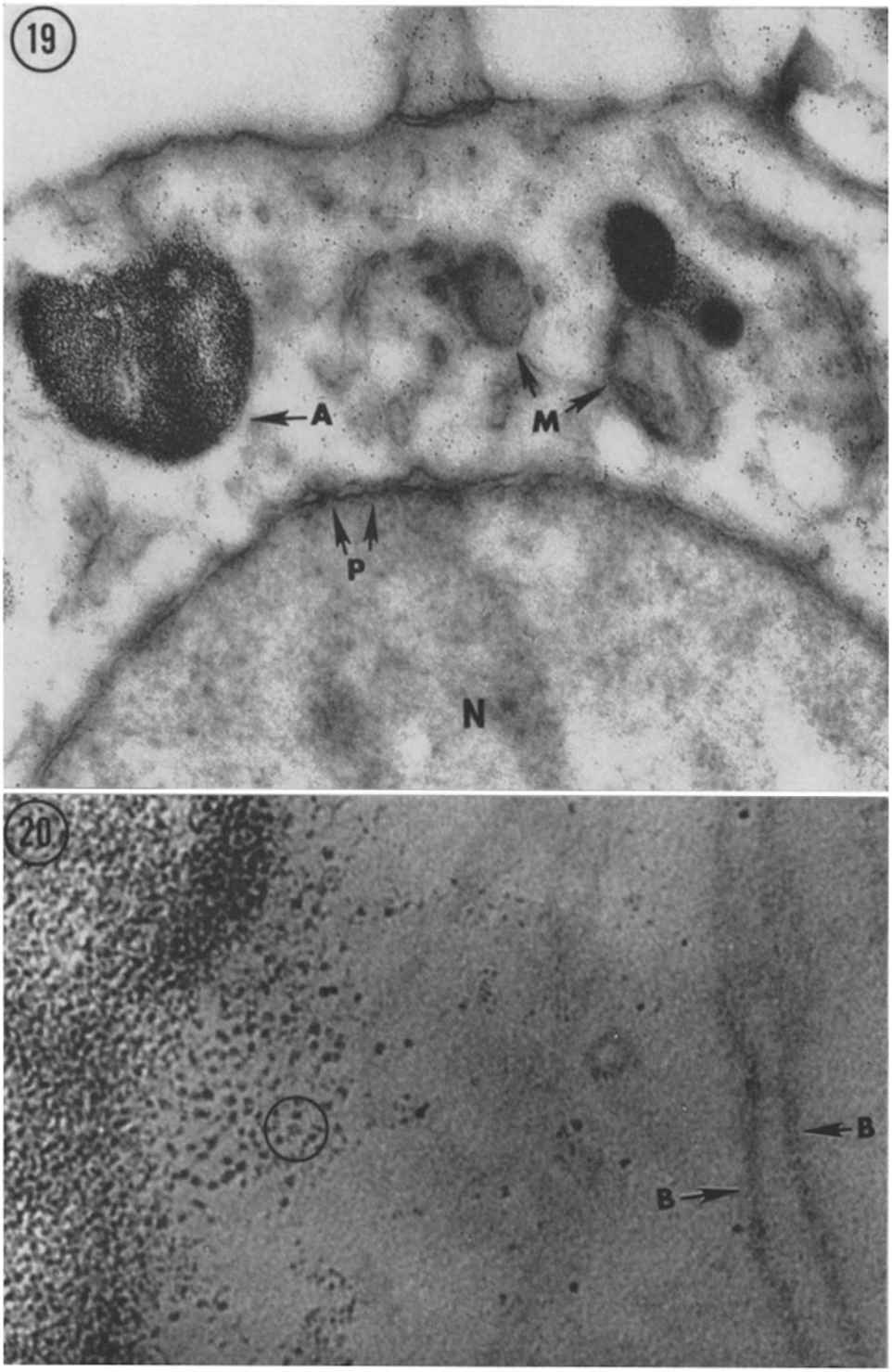



(Richter: Transformation of colloidal iron into ferritin)

## PLATE 25

FIG. 19. Part of macrophage in mouse spleen fourteen days after intraperitoneal injection of saccharated iron oxide. *N*, nucleus with pores (*P*); *A*, aggregate of dense particles with membranous border. The scattered dense particles have the dimensions of quadruplets of ferritin iron micelles (about 60 A), and some of them are present in mitochondria (*M*) and in the nucleus. × 66,600.

FIG. 20. Cytoplasm of sinusoidal endothelial cell in mouse spleen 6 days after intraperitoneal injection of saccharated iron oxide (SIO). The dense particles are mostly those of SIO. A ferritin "quadruplet" is indicated (circle) and several SIO filaments can be seen. The border of the cell and that of an adjacent one are also indicated (*B*). × 300,000.



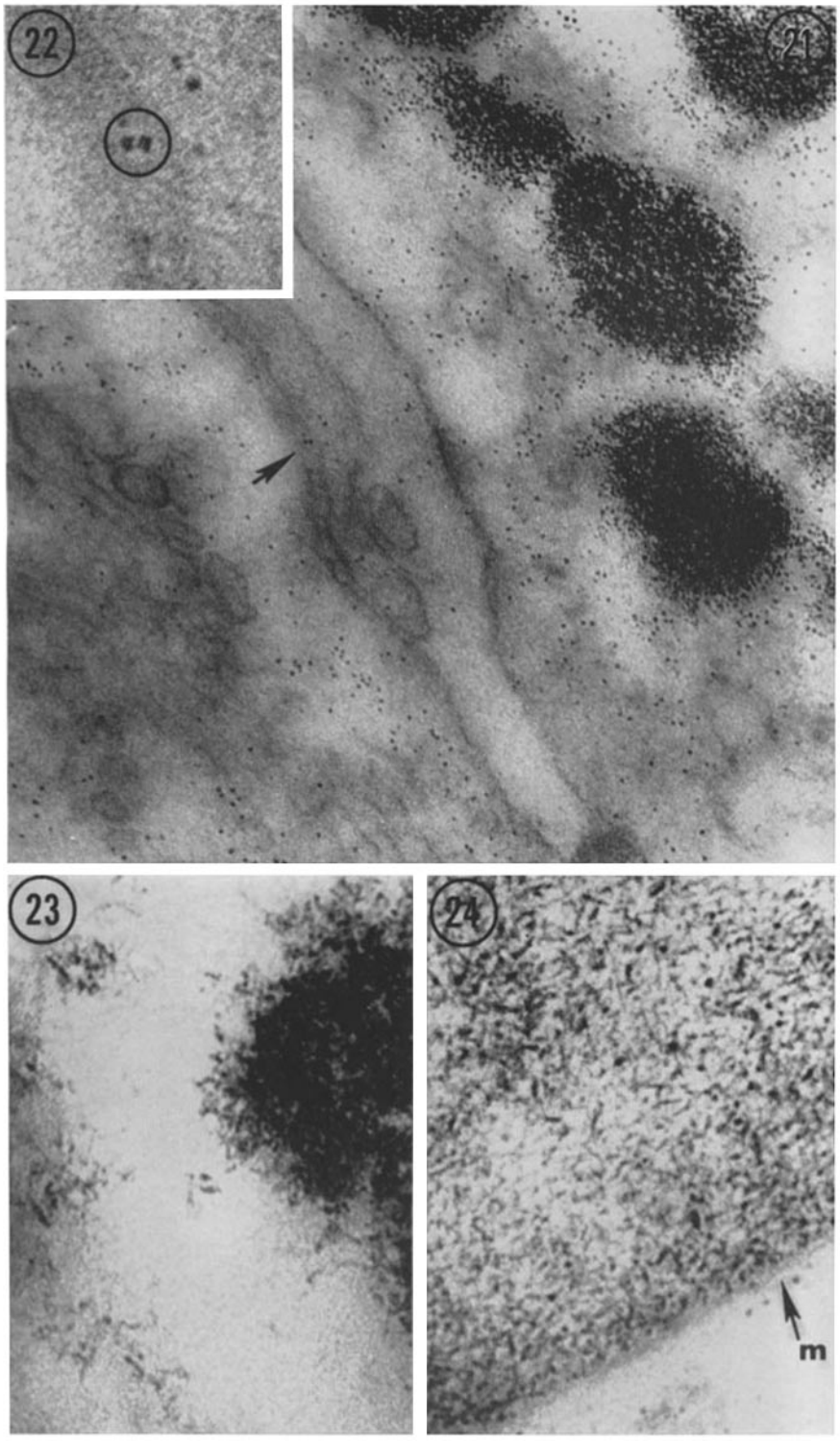

(Richter: Transformation of colloidal iron into ferritin)

PLATE 26

FIGS. 21 and 22. Parts of two sinusoidal endothelial cells of mouse spleen 28 days after intraperitoneal injection of saccharated iron oxide. Several aggregates of dense particles may be seen in the cytoplasm of the cell at the right, and there are innumerable scattered particles. In Fig. 22, the region indicated by an arrow in Fig. 21 has been enlarged to show the presence of ferritin. Similarly, most of the dense particles in Fig. 21 can be shown to represent ferritin iron micelles. Fig. 21, × 108,000; Fig. 22, × 300,000.

FIG. 23. Electron micrograph of hydrous ferric oxide, "dried" in air at 25°C. There are particles and filaments. The diameters of the particles range mostly between 15 and 40 A. Compare with Figs. 4 and 5. × 250,000.

FIG. 24. Part of cytoplasmic deposit in macrophage from subcutaneous tissue of mouse. The aggregate of particles and filaments is similar to those shown in Fig. 23, and is bordered by a membrane (*m*). The animal had been given a subcutaneous injection of hydrous gel of ferric oxide 5 days before the specimen was taken. × 250,000.




(Richter: Transformation of colloidal iron into ferritin)