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## Pathophysiological studies on ferric iron. Part 3. An electronmicroscopic studies on serum iron colloid and some iron preparates

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# Pathophysiological studies on ferric iron. Part 3. An electronmicroscopic studies on serum iron colloid and some iron preparates\*

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## Abstract

Electron microscopic observations revealed that the serum ferric chloride mixture prepared in the range of pH 5.4 and 8.2 is of colloidal solution whose particles are composed of electron-dense iron colloid nuclei surrounded by protein cortex formed by the absorption around the iron colloid particles. The elevation of pH over 9.0 or the lowering below pH 3 in media results in the loss of the protein cortex enhancing the growth of each molecule to longer ones and to coagulate with each other. Ferritrat proved to have almost the same cortex surrounding the iron colloid as that of serum ferric chloride mixture, but the colloid particles were larger than those of the latter. Both Ferrobalt and the gelatin ferric chloride mixture are of colloid solution but the colloid particles are heterogenous both in size and shape and have no cortex part. Glufericon will be true solution of organic iron complex.

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**PATHOPHYSIOLOGICAL STUDIES ON FERRIC IRON  
PART 3. AN ELECTRONMICROSCOPIC STUDIES ON  
SERUM IRON COLLOID AND SOME  
IRON PREPARATES**

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In the former reports the author described about some properties of the iron serum mixture, S.I.C., which is prepared by adding ferric chloride or ferric ammonium solution to serum in a range of pH 5.4 to 8.2. From the observations on paperelectrochromatography iron was supposed to be combined with the protein belonging to albumin and globulin fractions, but the absorption spectrum obtained by this solution suggested that the iron may exist in a form of colloidal state in the presence of protein as protecting colloid. Therefore, the author attempted to solve these problems by observing the solutions under electron microscope. In the present paper the colloid character of this iron and its variation in size are described in relation to the electrophoretic patterns presented in the former reports. As the comparison some iron preparations on sale for anemic patients were also observed.

**MATERIALS AND METHODS**

As iron preparations the following six solutions were used.

*Sample 1.* Serum ferric iron mixture of a low concentration in iron; 20 cc of horse or rabbit serum was added with 10 mg Fe as ferric chloride and the pH of the solution was adjusted to pH 8.2 using a 3%  $\text{Na}_2\text{CO}_3$  solution. In this sample iron proved to move with protein by electrophoresis.

*Sample 2.* Serum ferric iron mixture of a high concentration in iron; 20 cc of horse or rabbit serum was added with 40 mg of iron in ferric chloride. The pH of media was the same as sample 1. On electropaper-chromatography iron proved to be mainly remaining at the starting line separating from protein.

*Sample 3.* Serum ferric iron mixture of a low concentration in iron; prepared by the same method as sample 1, but the terminal pH was adjusted at pH 9.0 or 9.5.

*Sample 4.* Serum ferric iron mixture of a low concentration in iron;

prepared by the same method as sample 1, but the pH was lowered to 3.0 temporarily by adding 1*n* HCl solution and then the pH is raised to 8.2.

*Sample 5.* "Ferritrat" of Nordmark in Germany diluted with distilled water so as the iron content is 0.5mg/cc.

*Sample 6.* "Ferrobalt" of Eizai, Osaka, (dextrin iron) diluted with distilled water so as the iron content is 0.5 mg/cc.

*Sample 7.* "Glufericon"; Shionogi, Osaka, diluted, 0.5 mg per cc in iron contents.

*Sample 8.* Gelatin iron ferric chloride mixture, donated by Dainihon Seiyaku, Osaka, iron contents, 0.5mg/cc.

Each of these samples was sprayed by using a splayer for paper chromatography on Formvar monomolecular membrane covering the mesh, No. 150, 1/8 inch in diameter, dried in decicator for two days, and photographed by the electronmicroscope, Hidachi HU-10 Type, with or without chromium shadowing. In a part of sample 1, after drying the preparation was exposed to 0.05% trypsin solution for one hour at room temperature, dried again and photographed without shadowing.

#### OBSERVATIONS AND RESULTS

As is demonstrated in Figs. 1a<sub>1</sub>, 1a<sub>2</sub> and Figs. 1b<sub>1</sub>, 1b<sub>2</sub>, iron colloid particles of almost the same in size, which are linked together presenting a picture like a bead-necklace, is detected under the electronmicroscope. Each particle is 300 to 400 Å in diameter having the electron-dense nucleus surrounded by transparent cortex. The electron-dense nuclear part seems to be composed of smaller particles about 30 Å in size which can be recognized clearly in some particles.

Treatment with trypsin resulted in the growth of each particle to larger ones by the coagulation of each particle forming a gross electron dense mass (Figs. 1c<sub>1</sub>, 1c<sub>2</sub>, 1c<sub>3</sub>).

In the samples 2, 3, and 4 a specific picture of each particle as in Figs. 1a, b growing larger ones could be seen and yet they had a tendency to form a rigid coagulated mass like coral and in these samples the colloid particles were irregular both in size and shape, representing a marked heterogenous picture (Figs. 2a, 2b, 3a, 3b, and 4).

Ferritrat showed a very similar picture to those of sample 1, presenting a fairly even grained picture, but the size of each colloid particle was larger in size than those found in sample 1 (Fig. 5a, b), 500 Å to 570 Å.

Ferobalt gave a rather heterogenous picture, a marked variety in colloid particles in size, 240 to 1200 Å (Fig. 6a, b).

Glufericon gave an amorphous or homogenous or plain crystals lack-

ing the characteristics of colloid solutions (Fig. 7a, b and c).

Gelatin-ferric chloride mixture gave a colloidal structure but the colloid particles were extraordinarily irregular both in shape and size (Fig. 8a, b,).

#### COMMENTS

From the results described above it has been clearly demonstrated that the serum ferric chloride mixture prepared in the range of pH 5.4 to 8.2 is of colloid solution and the colloid particles are almost constant in size and shape and composed of electron-dense nuclear part and the cortex part about 50 Å in width. These particles have a negative electric charge as they move with serum protein electrophoretically. Both the extreme lowering of pH of the media and the elevation of pH above 9.0 results in the loss of the cortex membrane with the growth of each particles to larger ones or the formation of gross masses coagulating with each other, and they lose their electric charge as revealed by paperelectrochromatography. From these observations it is reasonable to suppose that the cortex part of each colloid particle will be of protein adsorbed around the colloid particle of iron, by which the stability of the colloid system, electrophoretic movement and others can be understood. The loss of the electric charge of particle both in a high or low pH of media will be correlated with the loss of the protein membrane. It is well known that the protein molecule changes its charge by the changed pH of the media. This will be responsible for the membrane formation surrounding the iron particles in the limited range of pH and the loosening from them in the media of low or high in pH.

Treatment with pepsin, by which the protein cortex of the particles can be lost, gave the same effect as that in the elevating or lowering of pH in media exceeding the mentioned range.

Among the iron samples on sale for anemic patients Ferritrat only proved to have the cortex, but in this case the samples contained no protein, then this cortex must have been formed with some protecting colloid. The cortex was rather large in width comparable to that serum iron chloride mixture. Both of Ferrobalt and "gelatin ferric chloride mixture were proved to be of colloidal solutions but in these preparations the colloid particles are extremely heterogenous in size and shape and had no cortex corresponding to those of Ferritrat or serum ferric iron colloid. Glufericon proved to be a true solution of an organic iron complex. The biological activities of these three different types of compounds, those having cortex, those having no cortex and true solutions of iron complex are expected to be at a great variance and must be of great interest.

### SUMMARY

Electron microscopic observations revealed that the serum ferric chloride mixture prepared in the range of pH 5.4 and 8.2 is of colloidal solution whose particles are composed of electron-dense iron colloid nuclei surrounded by protein cortex formed by the absorption around the iron colloid particles. The elevation of pH over 9.0 or the lowering below pH 3 in media results in the loss of the protein cortex enhancing the growth of each molecule to longer ones and to coagulate with each other.

Ferritrat proved to have almost the same cortex surrounding the iron colloid as that of serum ferric chloride mixture, but the colloid particles were larger than those of the latter.

Both Ferrobalt and the gelatin ferric chloride mixture are of colloid solution but the colloid particles are heterogenous both in size and shape and have no cortex part. Glufericon will be true solution of organic iron complex.

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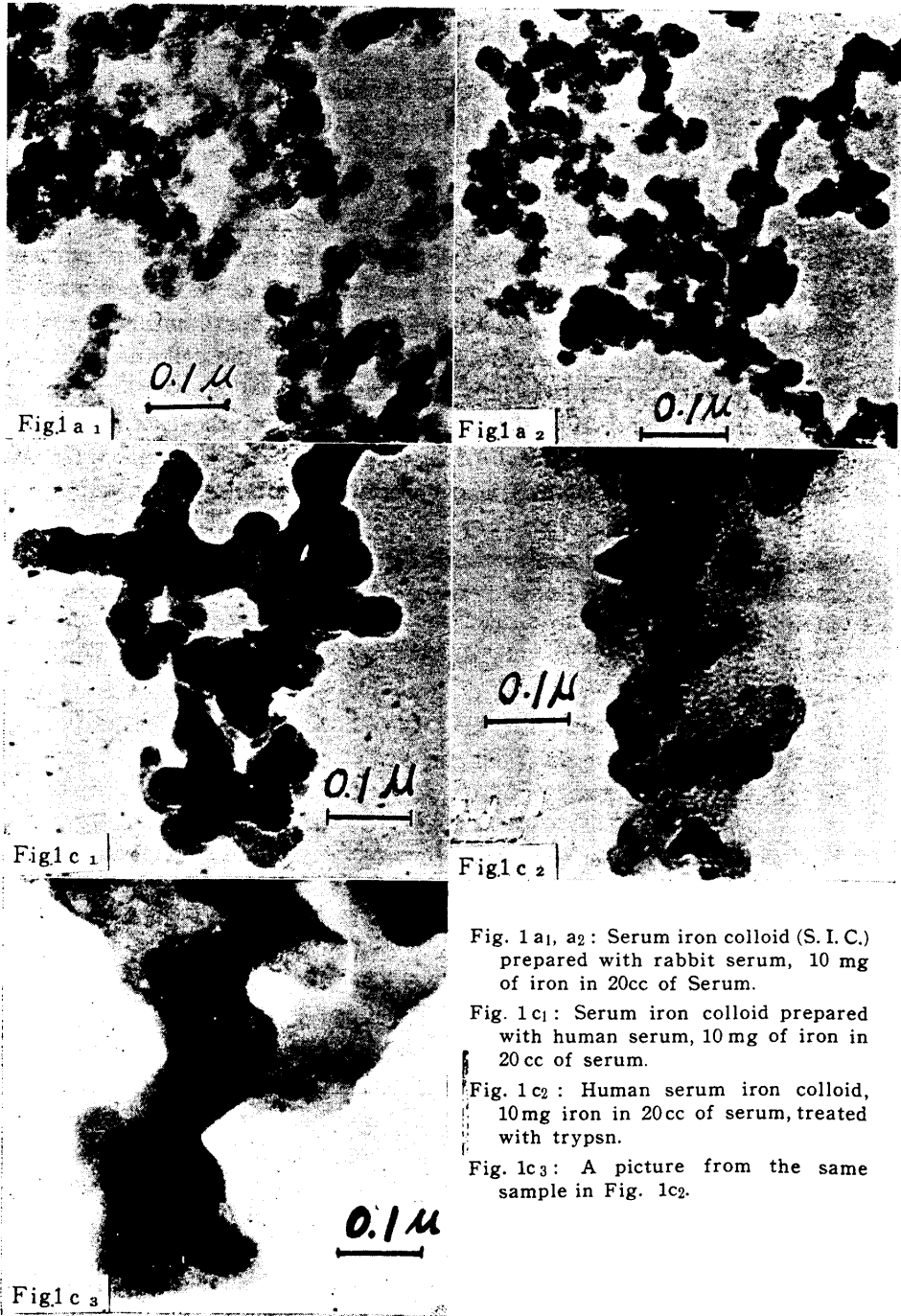


Fig. 1 a<sub>1</sub>, a<sub>2</sub> : Serum iron colloid (S. I. C.) prepared with rabbit serum, 10 mg of iron in 20cc of Serum.

Fig. 1 c<sub>1</sub> : Serum iron colloid prepared with human serum, 10 mg of iron in 20 cc of serum.

Fig. 1 c<sub>2</sub> : Human serum iron colloid, 10mg iron in 20cc of serum, treated with trypsn.

Fig. 1 c<sub>3</sub> : A picture from the same sample in Fig. 1c<sub>2</sub>.

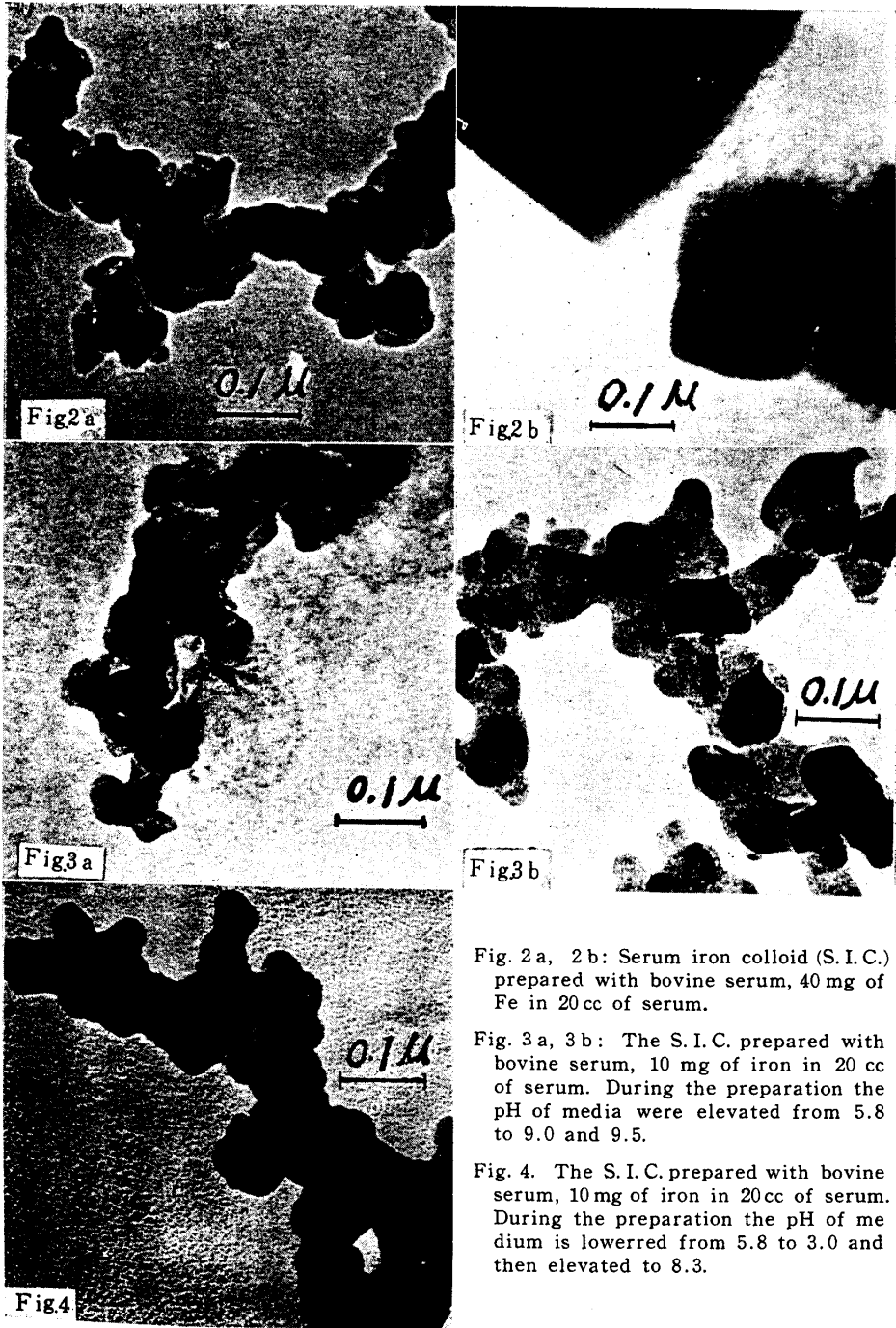
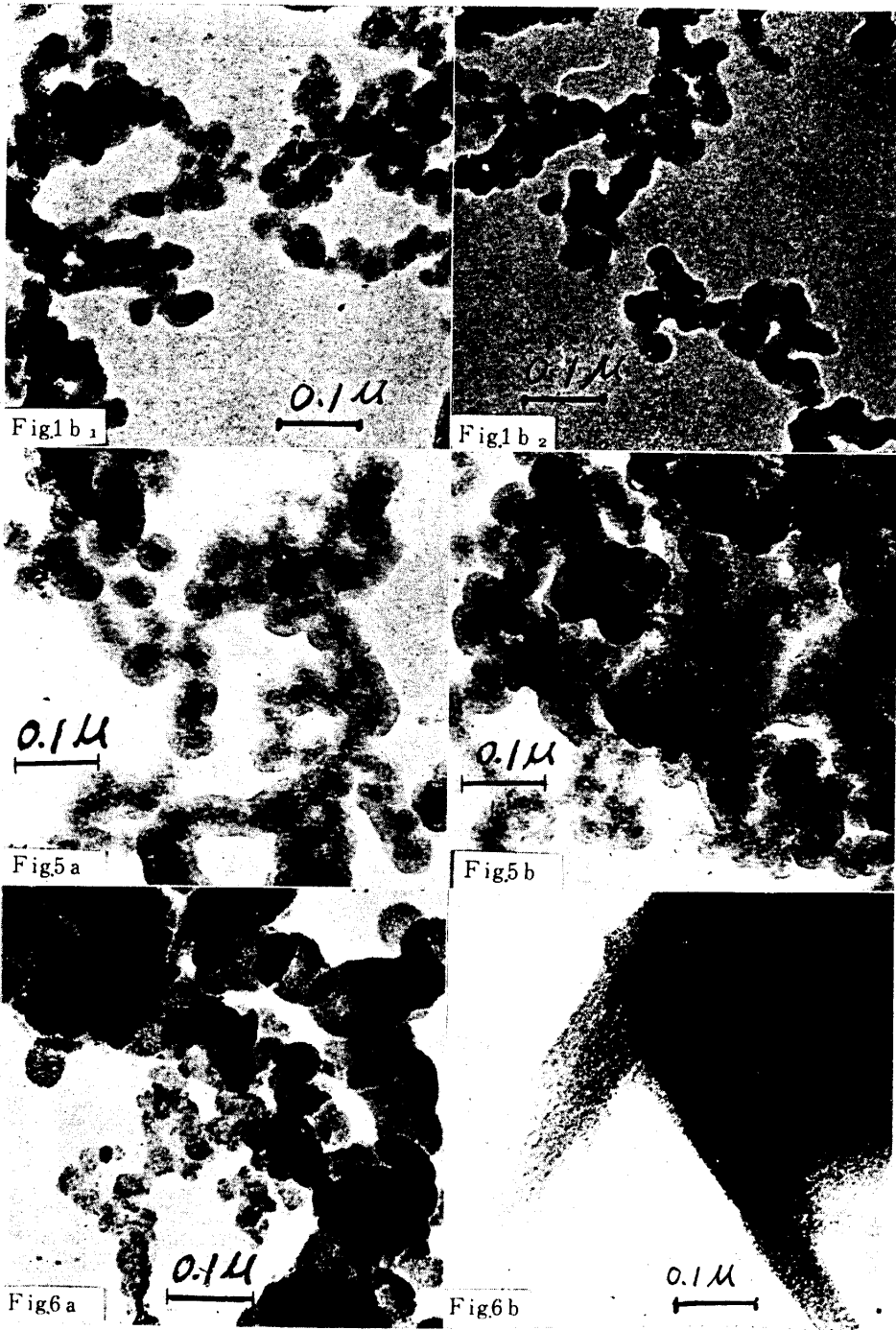


Fig. 2 a, 2 b: Serum iron colloid (S. I. C.) prepared with bovine serum, 40 mg of Fe in 20 cc of serum.

Fig. 3 a, 3 b: The S. I. C. prepared with bovine serum, 10 mg of iron in 20 cc of serum. During the preparation the pH of media were elevated from 5.8 to 9.0 and 9.5.

Fig. 4. The S. I. C. prepared with bovine serum, 10 mg of iron in 20 cc of serum. During the preparation the pH of medium is lowered from 5.8 to 3.0 and then elevated to 8.3.





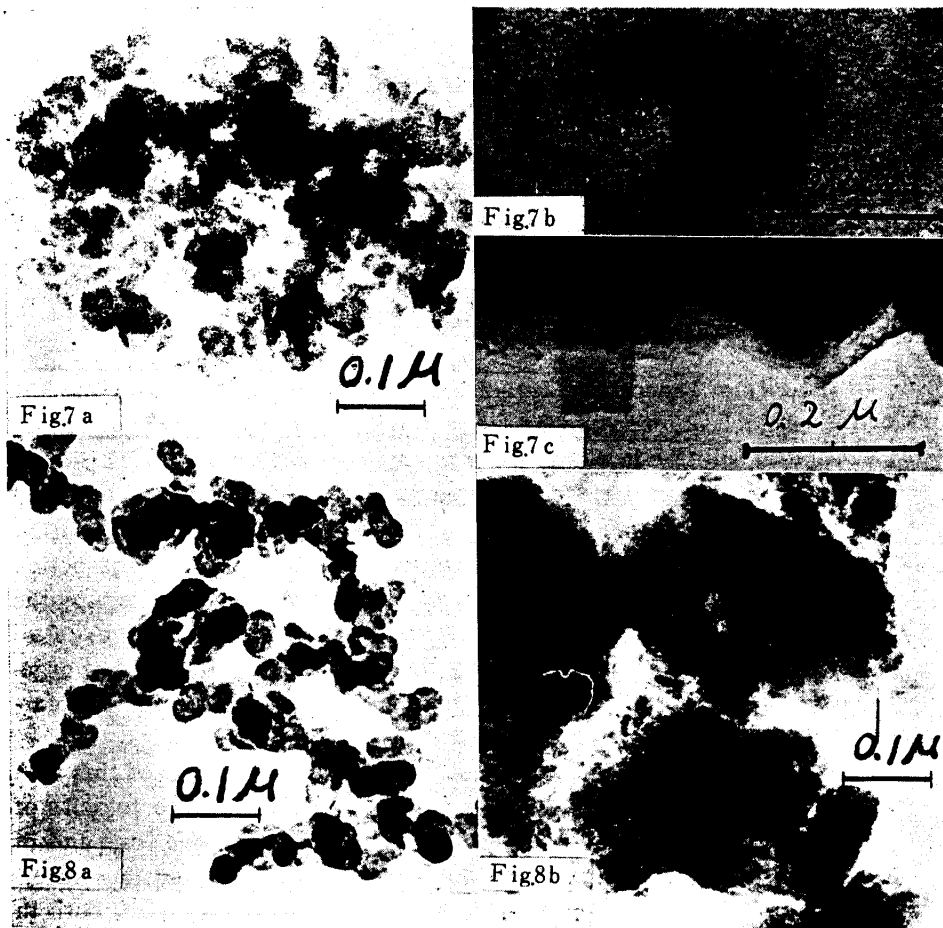


Fig. 1 b<sub>1</sub>, b<sub>2</sub>: Serum iron colloid prepared with horse serum, 10 mg iron in 20 cc of serum.

Fig. 5 a, b: Ferritrat of Nordmark, Germany.

Fig. 6 a, b: Ferobalt of Eizai, Osaka, (dextrin iron).

Fig. 7 a, b, c: Glufericon: Shionogi, Osaka.

Fig. 8 a, b: Gelatin iron ferric chloride mixtue donated by Dainihon Seiyaku, Osaka.