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Pathophysiological studies on ferric iron. Part

4. Bio-logical observation of serum iron colloid*

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Abstract

The iron introduced into vein in the form of the serum iron colloid is rapidly incorporated into ferritin and hemoglobin in a markedly high level with the increase in their amounts, without showing any ill effects. Experiments also show that there is another course of iron metabolism for the incorporation into ferritin and hemoglobin than the physiologic course by the aid of metal combining protein. This is true, however, only in the case in which the normal function of RES is retained. The incorporation of iron into ferritin and hemoglobin is accelerated in anemic animals and delayed in those having RES whose function is disturbed. From these results the author would suggest that the anemic patients may be given a quantity of iron in the form of serum iron colloid directly into vein without causing any side effects.

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PATHOPHYSIOLOGICAL STUDIES ON FERRIC IRON
PART 4. BIOLOGICAL OBSERVATION OF
SERUM IRON COLLOID*

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In the former reports the author described that the serum ferric chloride mixture, S.I.C., prepared by the method already mentioned is nothing but a colloidal solution in which the iron particles are covered with protein shell. As suggested in the former papers there is a possibility that such an iron compound as this serum iron colloid is produced in organism by the oral administration of iron in a large quantity, where the destruction of the mucous membrane occurs and iron can invade into organism by the physicochemical diffusion rather than the physiologic absorption. And yet the iron in such a compound may be used as the iron source of organism, because the oral administration of a quantity of iron shows a marked therapeutic effect upon anemic patients.

From this view point the author studied biological effects of this iron compound by introducing it directly into vein. At first the toxicity of this solution was tested by injecting a large quantity at one time and in the second experiment the iron metabolism was traced at various intervals after the injection of this colloid solution labeled with Fe^{59} both in blood and in several organs, especially in reference to hemoglobin and ferritin formation.

MATERIALS AND METHODS

As the test for toxicity two male dogs of about 9 kg of body weight were used. After the starvation for 12 hours they were anesthetized by the subcutaneous injection of urethan and morphine hydrochloride, 1 g and 10 mg per kg of body weight respectively. One dog was injected with 120 cc of serum iron colloid containing 100 mg iron into femoral vein with the homologous serum. To the other one an equal quantity of the

* The outline of this report was addressed at the Japanese Pathological Society in 1958.

homologous serum was injected. Throughout the period of experiment respiration and blood pressure both of femoral artery and portal vein were observed by kymograph using the Yamasaki's tambour¹ for two hours from the beginning of the injection.

For the observation of ferritin and hemoglobin syntheses seven male rabbits of about 2 kg body weight were used. They were all given of 20 mg iron for one animal intravenously in the form of serum iron colloid excepting one case in which only 2.5 mg of iron was injected with or without pretreatments.

Before the injection five rabbits were depleted of their blood from the ear vein, 30 cc per day, 90 cc in 3 days. Three of them were injected with 20 mg of iron in the form of serum iron colloid two to three days after the blood depletion. The two anemic rabbits were blocked of their reticulo-endothelial system (R. E. S.), one by injecting 100 cc of soot suspension intravenously, 50 cc per day for two days, 25 cc each, twice a day with the interval of three hours and other one by injecting 40 cc of gold sol intravenously, donated by Dainihon Pharmaceutical Company, 20 cc each, twice at 3 hours' interval. The soot or gold sol injection was given in the day after the last depletion, and on the following day the serum iron colloid was injected intravenously.

The serum iron colloid for rabbits was prepared by mixing rabbit's serum and ferric chloride by the method described in the first report in the range of pH 5.4 to 8.2. But in this instance a small amount of Fe^{59} was introduced as ferric chloride, i. e. at first FeCl_3 was added for the purpose of saturating the β_1 -metal combining protein of SCHADE² and LAURELL³, and then $\text{Fe}^{59}\text{Cl}_3$ was added, 80 μc , and finally FeCl_3 solution was added so as to make the total iron content to adjust to 0.5 mg per cc of serum.

Fe^{59} was of Oakridge National Laboratory $\text{Fe}^{59}\text{Cl}_3$, acidified with hydrochloric acid, normality 0.44, Fe 0.438 mg per cc, Fe^{59} $1.35 \pm 10\%$ mC per cc, Fe^{55} 0.0043 μc per cc, Co^{60} 3.2×10^{-6} μc per cc.

The soot suspension was prepared by rubbing gently the old Japan soot-block of superior quality on the stone plate with physiological saline solution. The thick solution obtained was centrifuged at 3000 rpm. for 30 minutes and the supernatant was used for injection.

During the experiment Fe^{59} level in serum and in red cells have been observed quantitatively at certain intervals. After the animals were killed at varied periods by the blood depletion from cardiac arteries and jugular veins, the distribution of Fe^{59} in every organ, tissues, and excreta was observed. The two control animals given 20 mg and 2.5 mg of iron were sacrificed 24 hours after the injection. In these animals the ferritin contents

of the liver and spleen were estimated. Determination of ferritin were carried out by the paperelectrochromatographic methods according to KEIDERING and WÖHLER⁴. For the paperelectrochromatography two different apparatuses as described in the second report were used. The papers used and the method were the same as those described in the first report, with exception of period of extension. On these papers autoradiography was carried out using Fuji Film for X-ray.

For the determination of Fe^{59} level contained in ferritin of the liver and spleen, and in hemoglobin in red cells, and in serum, scintillation counter of Kobe Kogyo Comp., scintillation probe, Model PS-1, scintillation mount Model E. A -13 and 1000 scaler Model, S. A, -1000A were used. The radioactivity of each organ, one gram in weight, or of blood samples were estimated at the distance of 11 cm for 5 minutes.

For the observation of the radioactivity of blood 2 cc of blood was taken from the ear vein and added a tracer of potassium oxalate and ammonium oxalate mixed powder. This blood sample was transferred into the tube for Sahli's hemoglobinometer till the level 100, centrifuged for 40 minutes at 3,000 rpm. The radioactivity of the serum was estimated on the supernatant. The sedimented blood corpuscles were washed with physiologic saline solution four times till the radioactivity of the washing solution disappeared completely. After counting the radioactivity of sedimented red cells, these cells were hemolized by adding 3 volumes of distilled water and exposed to supersonic wave by using the apparatus of Simazu Co. S-200, and then the radioactivity of heme and non-heme fractions obtained by the method of ZONDEK⁵ were estimated.

EXPERIMENTAL RESULTS

A) *Test for toxicity* : In the dogs given the intravenous injection of serum iron colloid in a large quantity at one time, 10 mg iron per kg body weight, the blood pressure in femoral artery showed a slight elevation momentarily after the injection and recovered to the normal level within 15 minutes. The portal pressure also elevated slightly, by 15 mm H_2O , all through the period of injections, but a few minutes after the termination of injection it turned back to the initial level. The respiration curve showed no abnormality. Further observations for two hours revealed no abnormalities both in the blood pressure and respiration (Fig. 1). An entirely similar result has been obtained on the control animal injected with the same volume of homologous serum as that of serum iron colloid. These results clearly show that the intravenous introduction of

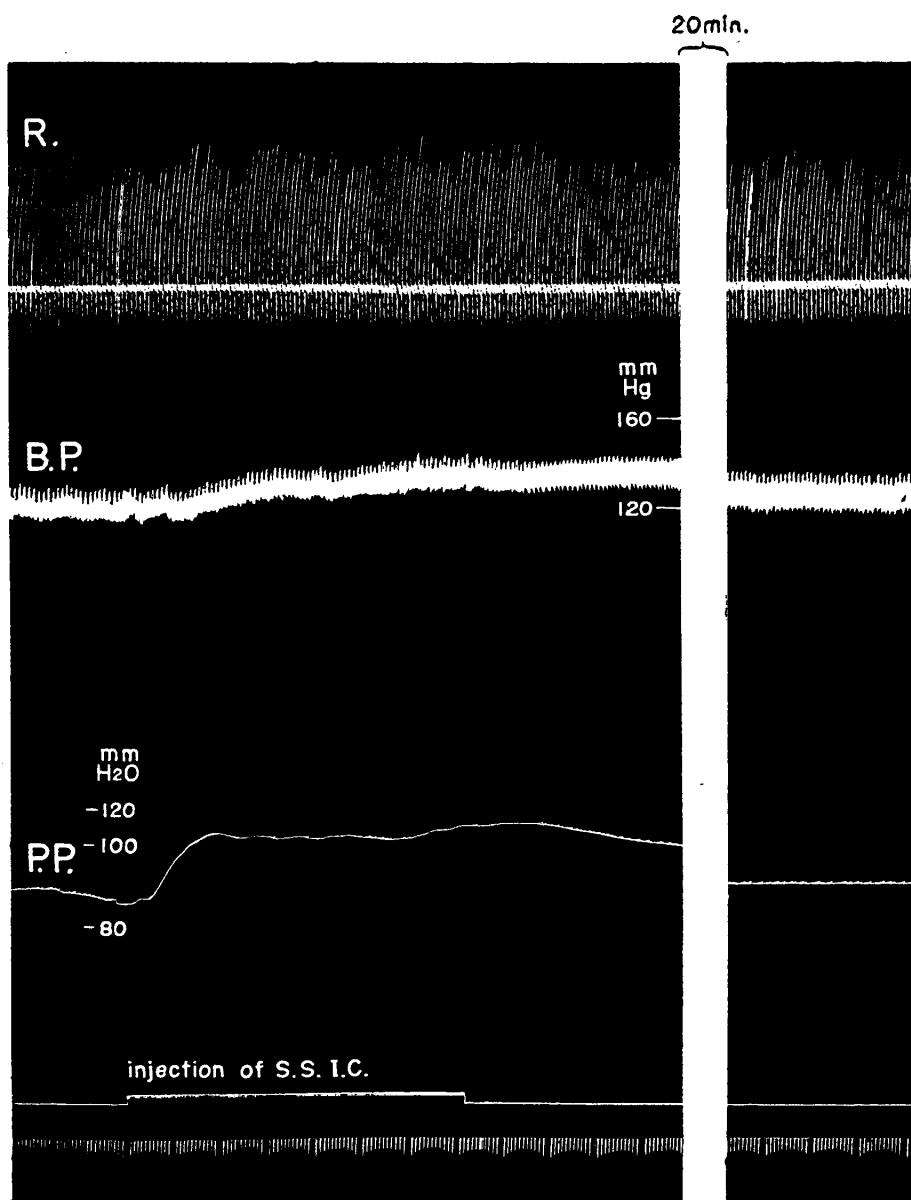


Fig. 1. Portal (P.P.) and arterial (A.P.) pressures and respiration (R) curves after the injection of the serum iron colloid (S.I.C.), 10 mg iron per kg of body weight, into femoral vein. An observation on a dog anesthetized by morphine urethane.

serum iron colloid causes no ill effects even in a large quantity.

B) Fe^{59} in serum after the injection of labeled serum iron colloid :
Hourly observations on the iron contents in serum detectable by its radio-

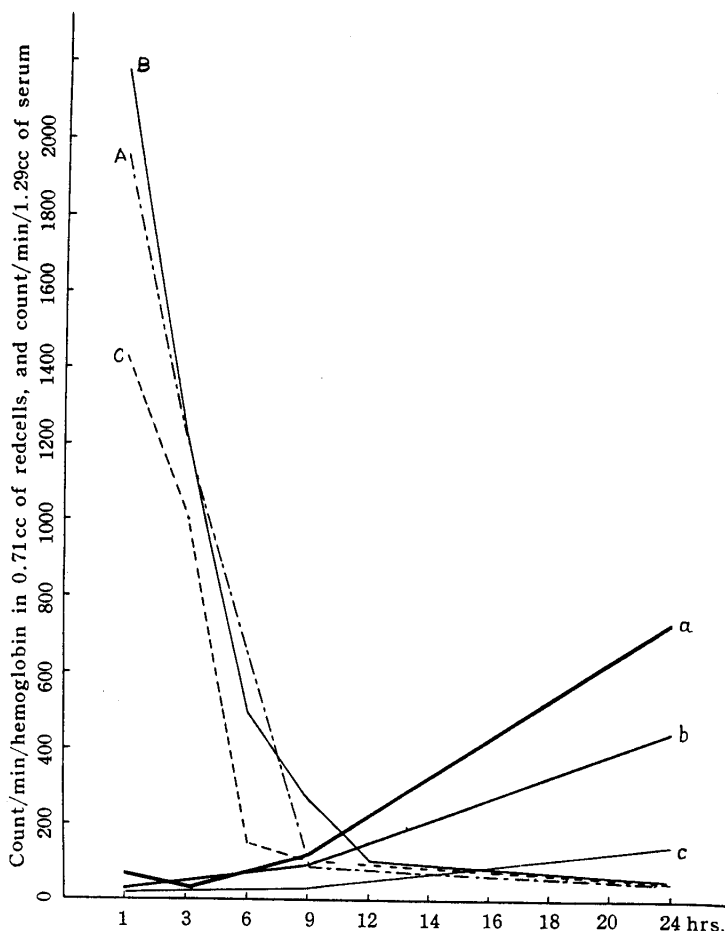


Fig. 2 The falling of the count in the serum and the rising incorporation of the Fe^{59} into hemoglobin after the intravenous injection of 20 mg iron containing $80 \mu c$ Fe^{59} , in the form of serum iron colloid (S. I. C.) into rabbit

A-a : given pretreatment of blood depletion from ear lobe

B-b : given pretreatment of depletion and gold sol block

C-c : given pretreatment of depletion and soot block

Curves a, b and c are obtained by connecting each value of $Hb(t)$, the count in Hemoglobin in a constant volume of the red cells.

$$Hb(t) = \text{estimated count} \times F(t) \times 35.5/Hb(t)$$

where $F(t)$: Ratio of the counts in heme iron to total (heme + non heme) iron

$Ht(t)$: Hematocrit at given time

35.5 : Mean value of the hematocrit of the rabbit before treatment

activity proved that the extremely elevated iron level in serum seen immediately after the injection decreased rapidly in the course of time, showing a marked decrease within 6 to 9 hours, reaching almost the initial level (Fig. 2).

C) *The incorporation of Fe^{59} in hemoglobin after the intravenous injection of 20 mg iron, containing $80 \mu c$ Fe^{59} , in the form of S. I. C. :* In the experiments of anemic animals the radioactivity of Hb increased gradually until 9 hours after the injection and showed a rapid increase during the subsequent 15 hours, reaching a plateau 3 to 4 days later (Figs. 2 and 3). The blocking of R.E.S. in anemic rabbits showed no effects on the decreasing rate of iron in serum, but a marked delay in the incorporation of iron into hemoglobin especially in the case whose R. E. S. being blocked with soot suspension (Figs. 2 and 3). In all cases the incorporation of iron into hemoglobin actually stopped after 3 or 4 days reaching a plateau where there was hardly any change during 9 to 13 days after the injection. The test on incorporation rate of labeled iron into the hemoglobin by the autoradiography combined with paperelectrochromatography proved that incorporation occurs as early as 3 hours after the injection (Fig. 4).

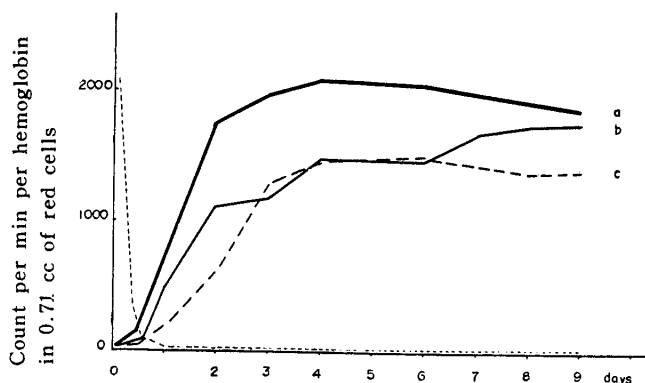


Fig. 3. The falling of the count in the serum (dotted line) and the rising in incorporation of Fe^{59} into hemoglobin after the intravenous injection of 20 mg iron, containing $80 \mu c$ Fe^{59} , in the form of serum iron colloid (S.I.C.) into a rabbit

a : given pretreatment of blood depletion from ear lobe

b : given pretreatment of depletion and gold sol block

c : given pretreatment of depletion and soot block

Curves a, b and c were drawn by the method described in Fig. 2.

D) *Serum iron after the injection of S. I. C. :* Observation on serum by paperelectrochromatography extended 60 minutes after the injection proved that the injected iron could be detected mainly in the area of

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Hb.

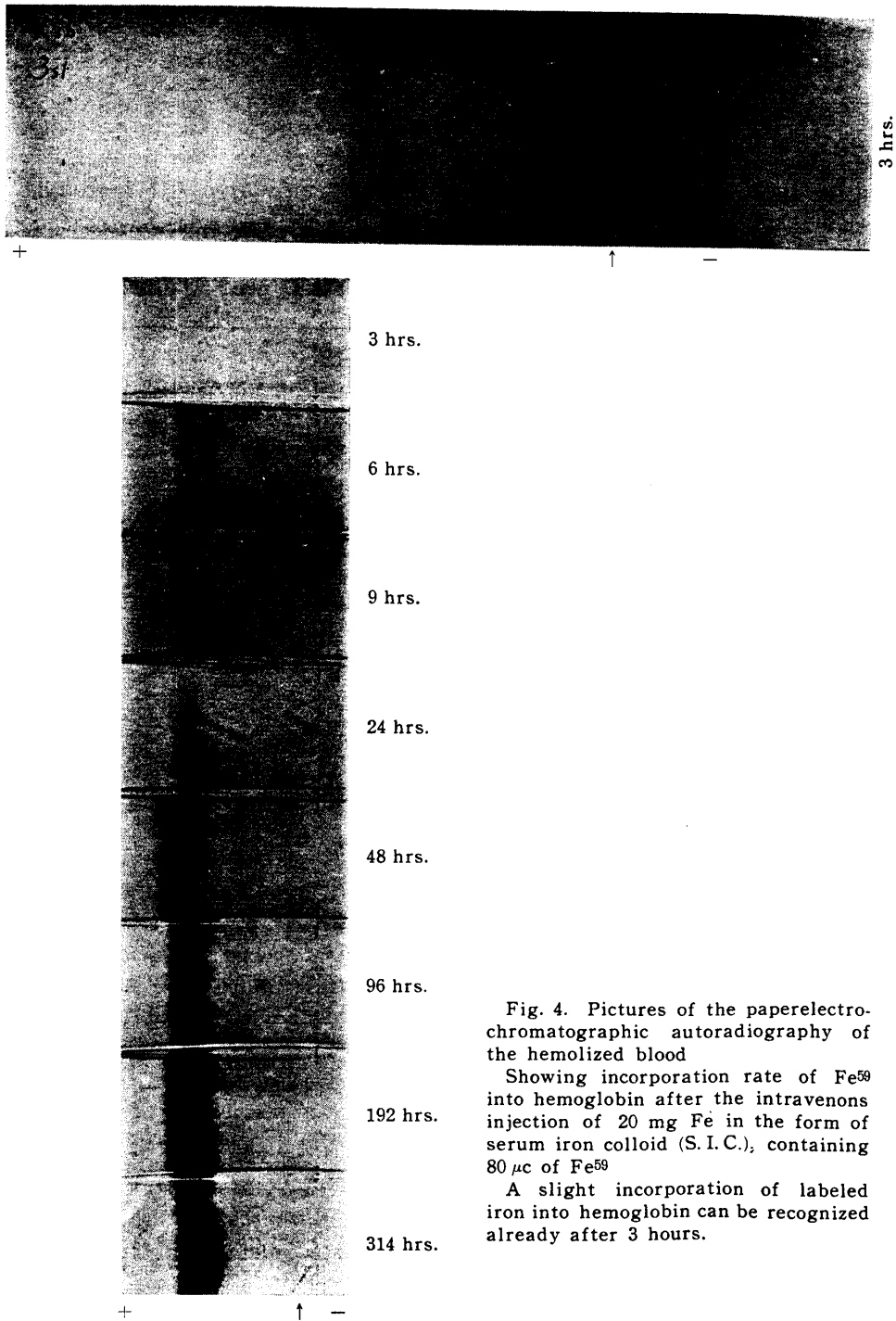


Fig. 4. Pictures of the paperelectrochromatographic autoradiography of the hemolized blood

Showing incorporation rate of Fe^{59} into hemoglobin after the intravenous injection of 20 mg Fe in the form of serum iron colloid (S.I.C.), containing $80 \mu\text{c}$ of Fe^{59}

A slight incorporation of labeled iron into hemoglobin can be recognized already after 3 hours.

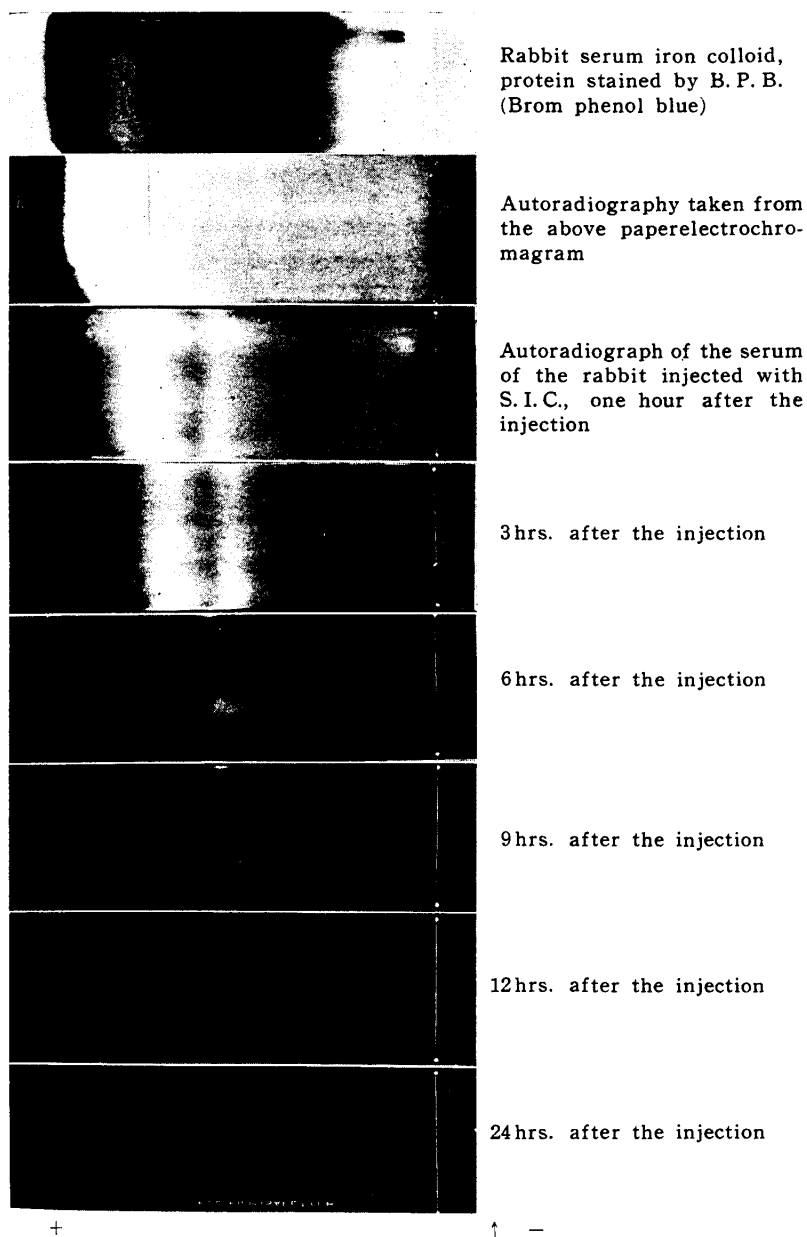


Fig. 5. The hourly change in the autoradiographic pattern of the serum after the intravenous injection of 20 mg iron in the form of serum iron colloid containing $80 \mu\text{c Fe}^{59}$. Pictures taken from the paperelectrochromatograms. The pictures of paperelectrochromatogram of S.I.C. and its autoradiograph are shown in the upper two bands.

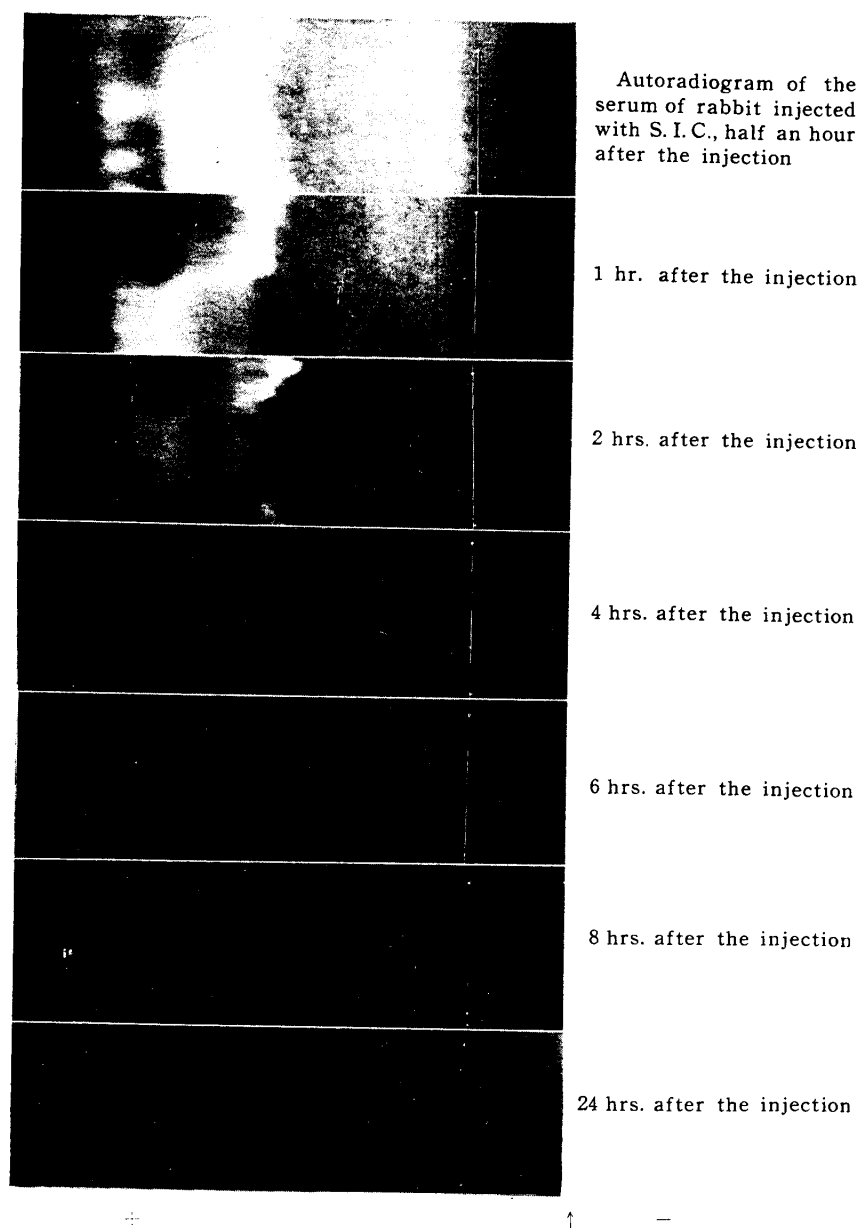


Fig. 6. The hourly changes in the autoradiographic pattern of the serum after the intravenous injection of 2.5 mg iron in the form of serum iron colloid (S. I. C.) containing $80 \mu\text{c Fe}^{59}$. Pictures taken from the paperelectrochromatograms.

fractions of albumin, α -globulin and β -globulin showing almost the same type as that of serum iron colloid. But in the course of time the iron decreased in its density in the area of albumin and α -globulin fractions, and after 6 hours iron was recognized only in β -globulin fraction disappearing in all other areas (Figs. 5, 6). This was especially marked in the case to which only a small quantity of the highly labeled serum iron colloid was given (Fig. 6).

E) *Distribution of Fe^{59} in various organs* : The radioactivity of Fe^{59} in various organs has been observed 24 hours after the injection of the labeled serum iron colloid, $80 \mu c Fe^{59}$ in 20 mg Fe, into a normal rabbit. As

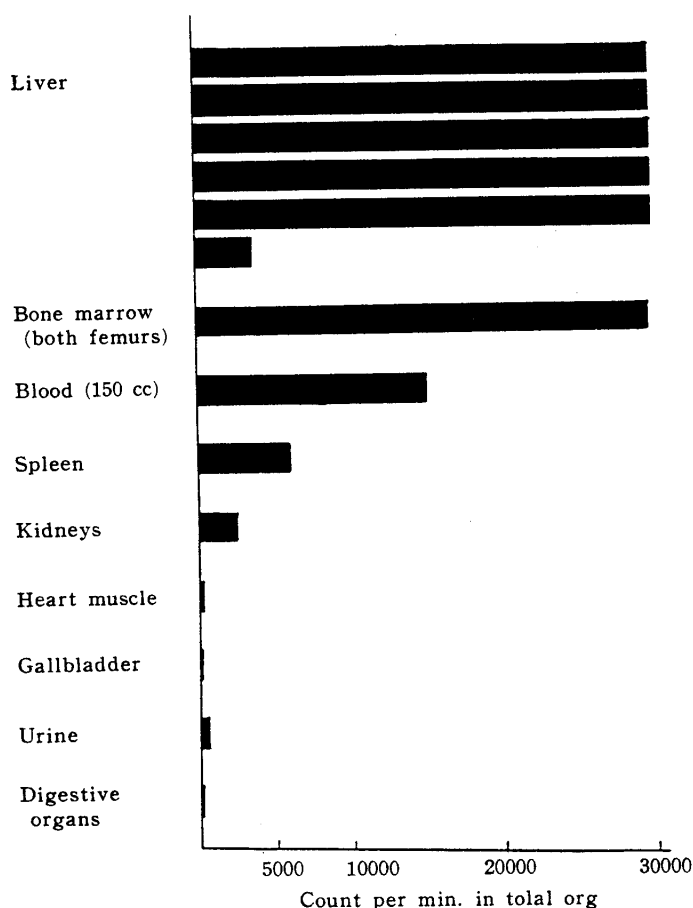


Fig. 7. The distribution of the labeled iron in the organs after the intravenous injection of 20 mg iron in the form of serum iron colloid containing $80 \mu c Fe^{59}$

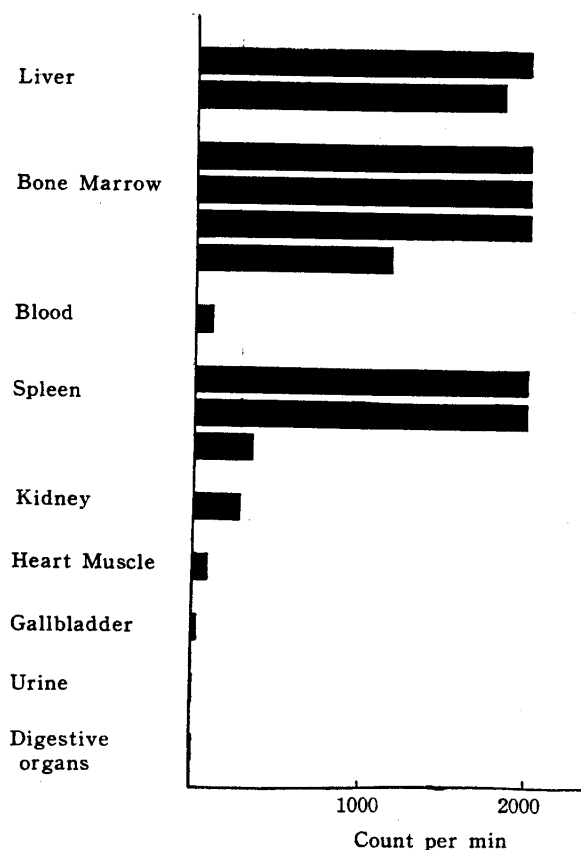


Fig. 8. The distribution of the labeled iron per gram in the organ in wet after the intravenous injection of 20 mg iron containing $80 \mu\text{c Fe}^{59}$ in the form of serum iron colloid

indicated in Figs. 7, and 8, the radioactivity was detected only in those in organs rich in RES, e. g. bone marrow, spleen and liver. Most of the labeled iron was detected in the liver, a moderate amount in the bone marrow and spleen and a small amount in the kidneys (Fig. 7), though in the contents of Fe^{59} per gram of each organ in wet the highest level was found in bone marrow, the moderate in spleen and liver, and the minimal in kidney (Fig. 8).

F) *Ferritin in the spleen and liver* : The amount of the labeled iron in the ferritin of liver and spleen after the intravenous injection of 2.5 mg or 20 mg iron in the form of serum iron colloid, containing $80 \mu\text{c Fe}^{59}$, is shown in Fig. 9. As a whole organ the incorporation of Fe^{59} into the fer-

Metabolism of Iron Injected into Vein

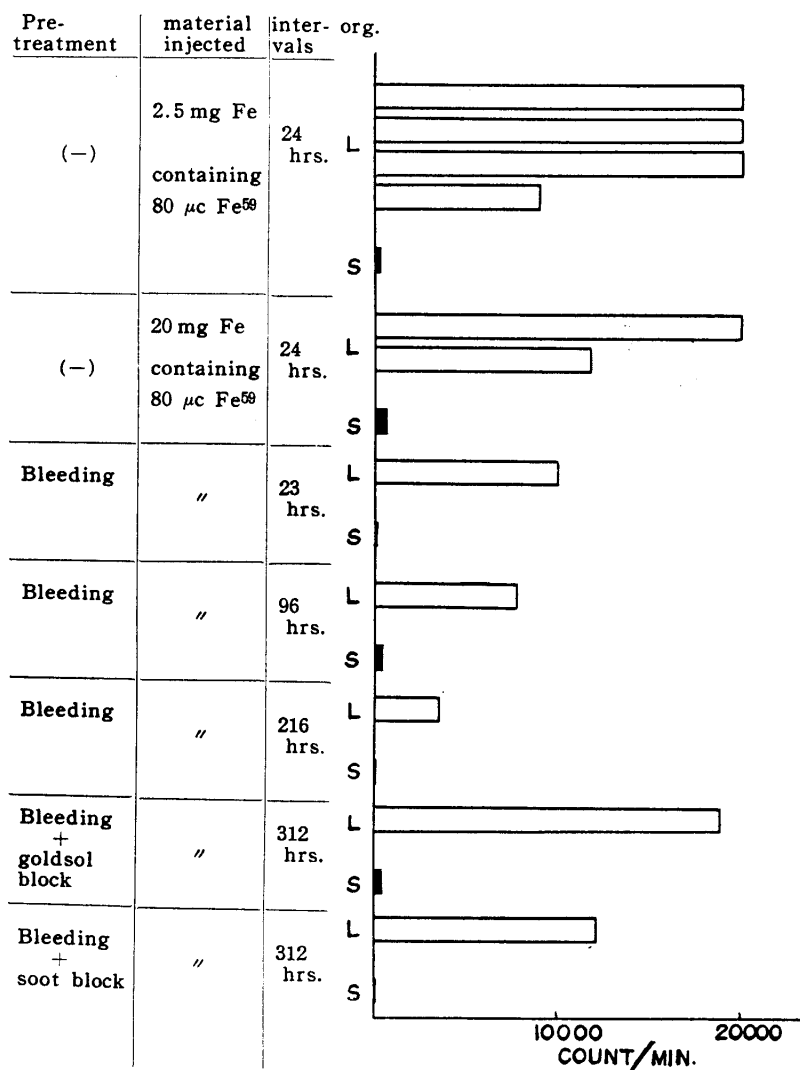


Fig. 9. The amount of the labeled iron in the ferritin of liver and spleen after the intravenous injection of 2.5 mg or 20 mg iron in the form of serum iron colloid containing 80 μ C Fe⁵⁹.

White bands L : liver, Black bands S : spleen,

ritin was found to be very low in level, especially marked in the case injected 80 μ C Fe⁵⁹ in 2.5 mg of iron. Bleeding resulted in the diminution of the Fe⁵⁹-incorporation into ferritin. Daily observation on the blood depleted animals revealed the decrease of Fe⁵⁹ in ferritin in the liver with

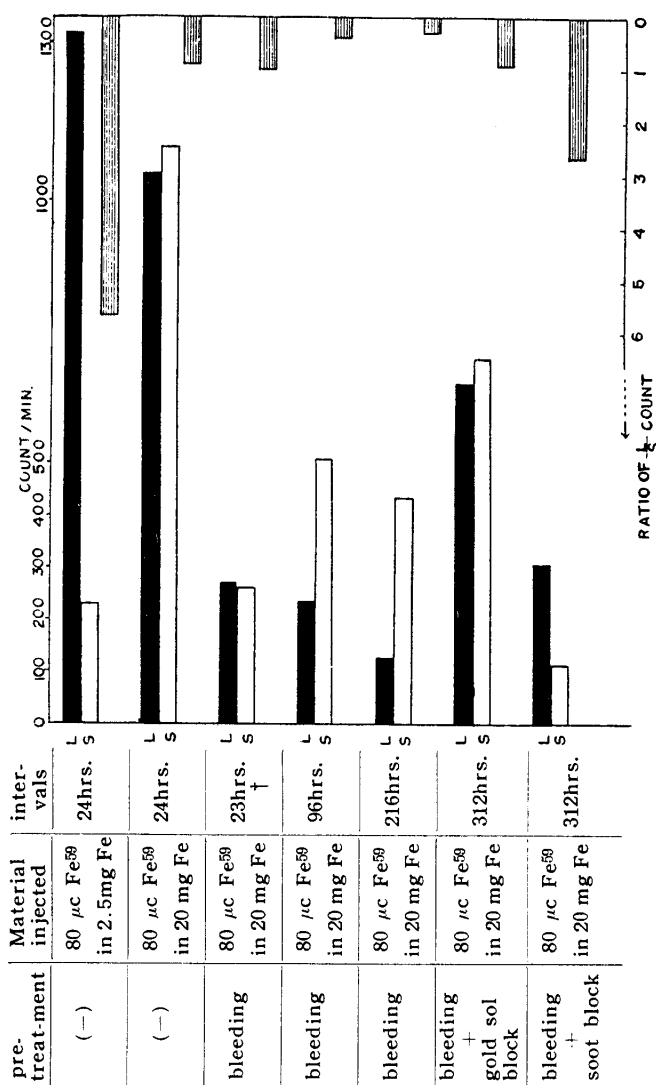


Fig. 10. The amount of the labeled iron in the ferritin of liver and spleen per gram in wet after the intravenous injection of 20mg or 2.5 mg iron in the form of serum iron colloid containing 80 µc Fe⁵⁹. Black bands, L : liver, white bands S : spleen, Hatched bands : the ratios, L/S.

the increased radioactivity in hemoglobin. Blocking of RES resulted in the delay in the diminution of the activity of ferritin in the liver (Fig. 9). As indicated in the figures when a small dose of iron, 2.5 m is given, a large quantity of labeled iron was detected in ferritin of the liver per gram and a small amount in that from the spleen. The injection of serum iron colloid in a quantity, 20 mg iron, resulted in the formation of labeled ferritin in an almost equal level both in the liver and spleen

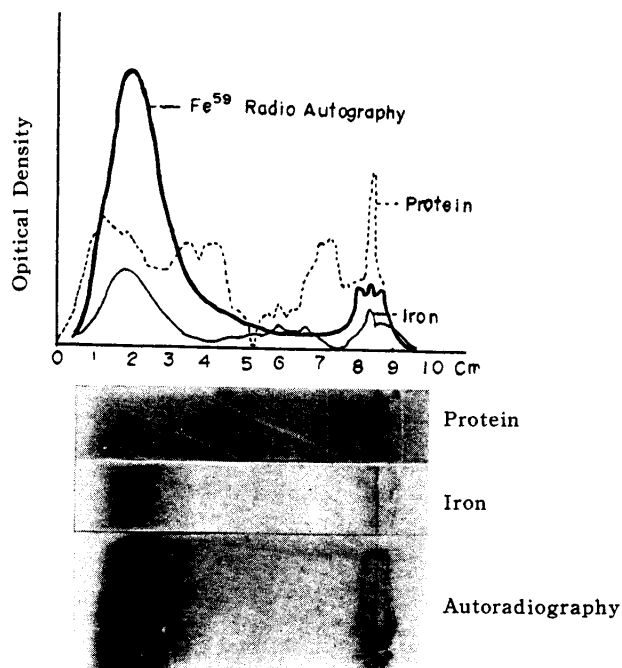


Fig. 11. Paper electrophoretograms for protein and iron, its radio-autogram and their optical densities observed in the ferritin from the rabbit liver 24 hours after the intravenous injection of 2.5 mg iron in the form of serum iron colloid containing $80 \mu\text{c Fe}^{59}$

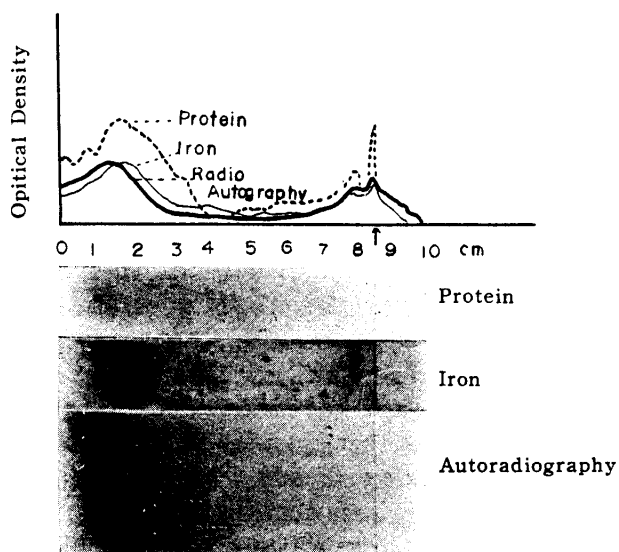


Fig. 12. The results obtained by the same method as in Fig. 11 on the ferritin of the rabbit spleen after the intravenous injection of 2.5 mg iron in the form of serum iron colloid containing $80 \mu\text{c Fe}^{59}$

per gram. In a case of the anemic rabbit died 23 hours after the injection showed only a minimal incorporation of Fe^{59} in the ferritin fraction both in liver and spleen per gram. The observations 4 and 9 days after the injection of serum iron colloid, $80\mu\text{c}$ in 20 mg of iron into the blood depleted animals showed a low level in ferritin in the liver per gram, but fairly a high level in the spleen per gram. Observations on the animals, whose RES. are blocked, 13 days after the injection of serum iron colloid, $80\mu\text{c}$, 20 mg of iron in each, proved a fairly high incorporation in the case of gold-sol block and relatively a low level in the soot-suspension block (Fig. 10).

Figs. 11 and 12. show the relation between the protein, total iron and the radioactive iron in the ferritin extracted from liver and the spleen respectively after intravenous injection of a small dose of iron, 2.5 mg. In these figures it is already seen most of the newly introduced iron is incorporated mainly into the ferritin of liver (Fig. 11) but only a small amount of iron in that of spleen, when a small amount of iron is introduced, though the incorporation increases in the ferritin of spleen when a large dose of iron is introduced at one time.

COMMENTS

Through the experiment in dogs the serum iron colloid has been proved to give no ill effect when it is introduced into vein even in a large quantity at one time; 10 mg per kg of body weight, KOBAYASHI⁹, one of my collaborators, has also proved this fact through the experiments on rats and rabbits. These facts show that this iron colloid could be utilized clinically for the patients with iron deficiency without causing any unpleasant side-effects differing from those iron compounds like ascorbic acid iron compound (*Heilmeyer* 1936), gluferricon and others.^{6,7,8}

Subsequent experiments have revealed that this iron colloid can be used very quickly for the hemoglobin and ferritin synthesis after the intravenous injection. The iron introduced into vein, which acts as to elevate the serum iron level enormously, decreases very rapidly in serum practically reaching the normal level after about 9 hours with a marked increase in the incorporation into ferritin and hemoglobin after 24 hours. The iron incorporation into hemoglobin occurs very slowly at first, though a slight incorporation occurs already 3 hours after the injection, and becomes marked especially from about 9 hours after the injection till 3rd to 4th experimental days, after which the hemoglobin level is retained at a certain level till 9th to 13th experimental days.

As the radioactivity of ferritin is minimized in liver and spleen 4 to 5 days after the injection of serum iron colloid, the turn-over of the iron should be very rapid and actually all of the iron introduced is incorporated into hemoglobin after 3 days in anemic animals.

The iron found in serum 30 minutes after the injection is of those detectable mainly in the site of albumin, α - and β -globulin fractions on paperelectrochromatograph. But MARUMO, a collaborator, succeeded in revealing that the iron is combined actually with serum albumin by the test with paperelectrochromatography on the protein separated from this iron protein fraction. In the course of time after the injection the serum iron level decreased quickly and after 6 hours the iron is found only at the site of β -metal combining protein fraction, and disappeared 24 hours after the injection. On anemic rabbits the decreasing rate of the serum iron level was almost the same as that in the normal animals.

Observations on the iron in tissues revealed that the iron is incorporated in a quantity into ferritin fraction of the liver and spleen after 24 hours with the marked increase in the amount of ferritin, especially in the liver. The test on the excretes showed that the iron is not lost by feces excretion. The electronmicroscopic observation by KOBAYASHI⁹ on the same material, taken at 24 hours after the injection, proved that the iron is incorporated into the mitochondria of the RES. cells of the liver increasing their electron density markedly. But in other animals treated similarly and killed 3 hours after the injection KOBAYASHI found iron in the RES cells of the liver in the form of colloid as indicated in my third report. With these observations, the fact that the iron incorporation into hemoglobin actually occurs at certain hours after the injection, and the increase of ferritin in organs is recognized in the stadium of the accelerated hemoglobin synthesis and that it decreases at the lowered hemoglobin synthesis, seems to show that iron is incorporated into hemoglobin not directly but through ferritin. In anemic animals the iron incorporation into hemoglobin is accelerated compared to that in the normal, with the lowered level of ferritin in the organs.

In the animals whose RES is blocked, the iron incorporation into hemoglobin is markedly delayed with a delay in the formation of ferritin. But the iron incorporation into hemoglobin reached to the level of the normal animals with the prolongation of 1 to 2 days, showing the recovery of the function of RES cells within these periods. Therefore, in this instance the disturbance in the function of RES by the injection of soot or gold sol seems to occur in the function of the ferritin formation but not in their phagocytic ability to iron colloid, because the decreasing

rate of the iron in serum after the injection, which signifies the phagocytosis by RES cells as described above, is almost the same as that in the control animals and yet it has been revealed histochemically that RES cells having soot can also actively phagocyte iron colloid.

In one case which died 23 hours after the injection of iron colloid the ferritin was found to be in a low level and the hemoglobin formation delayed, though the decreasing rate of iron level in serum was almost the same with those of other surviving animals. The cause of the death in this case will be of severe diarrhea but not of iron injection itself. Therefore, the delay in the iron incorporation into hemoglobin and the low level of ferritin will be due to the disturbance in the function of the RES in ferritin formation but not in the phagocytic ability. From these observations it is clear that the iron introduced into vein in the form of the serum iron colloid can be utilized directly and rapidly for the ferritin and hemoglobin syntheses so long as the function of the RES is in the normal state. And it is evidently clear that the iron in such a compound can enter into the physiologic metabolic cycle without passing the way of metal combining protein, when it is introduced into vein. This shows also a possibility that iron can be given to the patients with iron deficiency in a large quantity and most effectively with such a compound without any unpleasant side effects, though this point needs a definitive clarification by further clinical observations.

SUMMARY

The iron introduced into vein in the form of the serum iron colloid is rapidly incorporated into ferritin and hemoglobin in a markedly high level with the increase in their amounts, without showing any ill effects. Experiments also show that there is another course of iron metabolism for the incorporation into ferritin and hemoglobin than the physiologic course by the aid of metal combining protein. This is true, however, only in the case in which the normal function of RES is retained. The incorporation of iron into ferritin and hemoglobin is accelerated in anemic animals and delayed in those having RES whose function is disturbed.

From these results the author would suggest that the anemic patients may be given a quantity of iron in the form of serum iron colloid directly into vein without causing any side effects.

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