

## Transformation rate between ferritin and hemosiderin assayed by serum ferritin kinetics in patients with normal iron stores and iron overload

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### ABSTRACT

Ferritin iron, hemosiderin iron, total iron stores and transformation rate were determined by serum ferritin kinetics. The transformation rate between ferritin and hemosiderin is motivated by the potential difference between them. The transformer determines transformation rate according to the potential difference in iron mobilization and deposition. The correlations between transformation rate and iron stores were studied in 11 patients with chronic hepatitis C (CHC), 1 patient with treated iron deficiency anemia (TIDA), 9 patients with hereditary hemochromatosis (HH) and 4 patients with transfusion-dependent anemia (TD). The power regression curve of approximation showed an inverse correlation between transformation rate and ferritin iron, hemosiderin iron in part and total iron stores in HH. Such an inverse correlation between transformation rate and iron stores implies that the larger the amount of iron stores, the smaller the transformation of iron stores. On the other hand, a minimal inverse correlation between transformation rate and ferritin iron and no correlation between transformation rate and hemosiderin iron or total iron stores in CHC indicate the derangement of storage iron metabolism in the cells with CHC. Radio-iron fixation on the iron storing tissue in iron overload was larger than that in normal subjects by ferrokinetics. This is consistent with the inverse correlation between transformation rate and total iron stores in HH. The characteristics of iron turnover between ferritin and hemosiderin were disclosed from the correlation between transformation rate and ferritin iron, hemosiderin iron or total iron stores.

**Key Words:** transformation among iron stores, ferritin and hemosiderin, serum ferritin kinetics, hereditary hemochromatosis, chronic hepatitis C

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### INTRODUCTION

Sixty years have passed after the pioneering biochemical investigation on ferritin and hemosiderin iron by Shoden *et al.*<sup>1)</sup> in 1953. Since the introduction of radioimmunoassay method by Addison *et al.* in 1973,<sup>2)</sup> the studies on ferritin have progressed extensively,<sup>3-8)</sup> and a remarkable advance in the field of molecular biology was achieved disclosing the mechanism of iron metabolism; ferritin gene expression,<sup>9, 10)</sup> iron regulatory protein (IRP)-iron responsive element (IRE),<sup>11, 12)</sup> hepcidin-ferroportin axis<sup>13, 14)</sup> and others.<sup>15, 16)</sup> However, the studies on hemosiderin iron did not progress noticeably. To break through the limitation of biochemical methods for investigating ferritin and hemosiderin iron metabolism in humans, we adopted a biophysical method; serum

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ferritin kinetics, in which serum ferritin is used as an indicator of tissue ferritin iron level.<sup>17, 18)</sup>

Serum ferritin kinetics enabled us to disclose the dynamic behaviors of ferritin iron and hemosiderin iron<sup>17, 18)</sup> and as follows.

We confirmed iron pathways, from ferritin to hemosiderin in iron deposition and hemosiderin to ferritin in iron mobilization, those proposed by Shoden *et al.*<sup>1)</sup> However, the other iron pathways they proposed such as the direct iron deposition from intracellular iron to hemosiderin or the direct iron mobilization from hemosiderin to intracellular iron bypassing ferritin synthesis were considered unlikely.

We determined ferritin iron and hemosiderin iron at once from the serum ferritin decrease and increase curve and disclosed the decreasing and increasing phases of ferritin iron and hemosiderin iron<sup>18)</sup> in iron removal and in iron addition using patients with normal level of iron stores and iron overload. We confirmed that the amount of ferritin iron was slightly larger than hemosiderin iron when total iron stores were within normal level.<sup>17)</sup> However when ferritin iron was saturated in the range above around 5 g of total iron stores, ferritin iron was transformed into hemosiderin iron progressively in iron addition.<sup>1, 17, 19, 20)</sup> The response of ferritin iron to the decrease of iron density was instant, and the decrement of ferritin iron was recovered by ferritin synthesis using iron removed from hemosiderin in iron mobilization.<sup>17)</sup> We clarified that iron traced the same pathway to the opposite direction in iron mobilization and deposition.<sup>17)</sup> The above-described behaviors of ferritin iron and hemosiderin iron are characteristic of iron homeostasis showing the tendency for restoring a previous state of iron balance.<sup>19, 21)</sup>

In this article, we attempted to unveil the characteristics of iron metabolism in the transformation between ferritin and hemosiderin using serum ferritin kinetics.

### *Classification of iron status*

At first, we clarify our terminology for the classification of iron status as shown in Table 1.

**Table 1** Classifications of iron status according to storage iron levels

The term “Normal iron stores” (abbreviated to Normal iron in the table) means the level of iron stores between iron deficiency and iron overload regardless of disease and iron distribution in the body.

The border between the state of iron decrease within normal iron stores and iron deficiency is clear cut. However, that of normal iron increase and iron overload is not so clear; a transitional zone between normal iron increase and iron overload was assigned.

Iron deficiency ±symptom		<decrease	Normal iron	increase<	Iron overload ±symptom
Iron stores	g	<0.1		2.5~5.0<	
Serum ferritin	ng/ml	<12		250~500<	
TIBC*	μg/dl	>360		200>	

\*TIBC; total iron-binding capacity

### *Definition of the term “transformation rate”*

The term “transformation rate” is the abbreviation of the rate of transformation from ferritin into hemosiderin or that from hemosiderin into ferritin.

“Transformation rate” is defined as a rate of a transformed (hemosiderinized) ferritin iron value to a tissue ferritin iron value increased by iron addition or as a rate of a transformed (ferritinized; synthesized by removing iron from hemosiderin) ferritin iron value to a tissue ferritin value decreased by iron removal.

## MATERIALS

### *Patients*

Iron overload; 9 patients with hereditary hemochromatosis (HH); #1, 2, 4, 5,<sup>5)</sup> #6, 7,<sup>6)</sup> #3, 8, 9,<sup>7)</sup> and 4 patients with transfusion-dependent anemia (TD); #10 with myelodysplastic syndrome (MDS) and myelofibrosis (MF), #11 with aplastic anemia (AA), #12 with MF and #13 with AA.

Normal iron stores: 11 patients (#14–24) with chronic hepatitis C (CHC) in a steady state without anemia, and one patient (#25) treated for iron deficiency anemia (TIDA) whose hemoglobin was normalized after intravenous iron infusion therapy.

The patients used consist of the same as those in the previous study<sup>17, 18)</sup> and 3 more patients were added; one for HH (#6<sup>7)</sup>, TD (#13) and CHC (#8) each. The latter 2 patients added were cited from our record at The Nagoya University Hospital.

Patients with iron loss except for TIDA or with an uncertain transfusion record were excluded from this study.

The Ethics Committee of The Nagoya University School of Medicine permitted the use of patients following the study protocol.

### *Serum ferritin assay kit*

Products of Fujirebio Incorporated (Tokyo, Japan) and Denka Seiken (Tokyo, Japan).

### *Iron chelating agent*

Deferasirox (Exjade/ICL670), product of Novartis Pharma (Basel, Switzerland).

Computer: Windows 7 installed personal computer, Product of NEC (Tokyo, Japan).

## METHODS

### *Determination of total iron stores*

Iron stores in HH and CHC were determined from the total amount of blood removed by phlebotomy to the level of iron deficiency (serum ferritin <12 ng/ml). Patients were recommended to take a diet low in iron to minimize the effect of dietary iron absorption. In TD, iron stores were calculated from the iron content in the units of red cell transfusion. All the transfusional iron was thought to be stored in TD in a steady state.

The amount of iron in the removed blood was determined using iron/hemoglobin ratio and blood volume. In TIDA, injected iron other than the iron utilized for the normalization of hemoglobin was thought to be stored.

### *Iron removal*

Iron was removed by phlebotomy at a constant pace for HH and CHC. Around 10 mg/kg/day of deferasirox was administered orally for TD.

### *Detection of blood loss*

Intestinal blood loss was detected by macroscopic findings and fecal immunochemical occult blood tests.

### *Serum ferritin assay*

It was performed by an enzyme-immunoassay using chemical luminescence at Nagoya University Hospital and by one using latex agglutination at the National Hospital Organization,

Nagoya Medical Center. For each different assay system, an inter-assay system correction was performed using the same standard ferritin, except for the cases with HH.

Serum ferritin may render a value higher than the actual tissue ferritin iron level in patients with various inflammations, malignancies and hyperferritinemia cataract syndromes. Therefore, we excluded such suspected overestimation cases by clinical symptoms and examinations; such as c-reactive protein, transaminase and others. Despite such disadvantages, serum ferritin has been evaluated as the best index of tissue ferritin iron level.<sup>2-6, 17, 18)</sup>

#### *Initial and final serum ferritin*

The term “Initial serum ferritin” means the amount of pre-existing tissue ferritin iron before iron removal. It is obtained by extrapolating the serum ferritin decrease curve to zero time.<sup>17)</sup> The term “Final serum ferritin” means the amount of tissue ferritin iron after the last blood transfusion neglecting the unknown amount of pre-existing iron stores before starting blood transfusion.

#### *Selection of best-fit serum ferritin increase or decrease curve*

Serum ferritin was assayed at a constant interval. For the assay data, serum ferritin decrease or increase curves were produced using computer simulation with spreadsheet program by slightly changing the serum ferritin decrements or increments and proportionality constants, i. e. transformation rates. Among those curves, the one best fit to the actually assayed data was adopted.<sup>17)</sup>

#### *Determination of ferritin iron and hemosiderin iron*

The amount of ferritin iron and hemosiderin iron were determined from serum ferritin decrease or increase curve<sup>17)</sup> as described in the legend of Figure 1.

#### *Determination of transformation rate between ferritin and hemosiderin:*

It was determined from the best-fit curve of the decreasing or increasing assay-dots of serum ferritin as a ratio of transformed value to the iron removed or added value.

“Transformed value” means the amount of ferritin transformed into hemosiderin in iron addition or the amount of hemosiderin transformed into ferritin in iron removal.

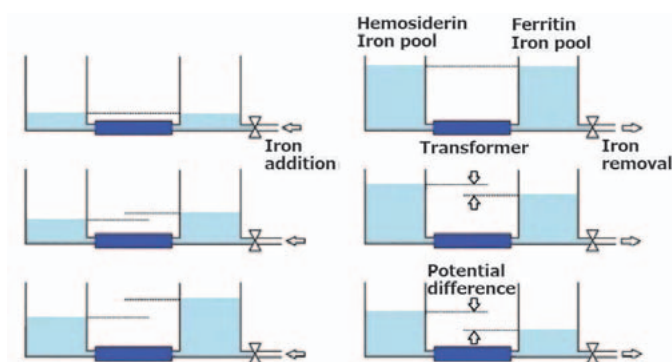
The difference between the initial serum ferritin in iron removal or final serum ferritin in iron addition and the serum ferritin assayed after a certain period of time was accounted for the potential for transformation between ferritin and hemosiderin.

#### *Distinguishing “correlation” from “no correlation”*

We set a borderline of reliable correlation at the R-squared value of  $-0.30$  for power regression curves of approximation.

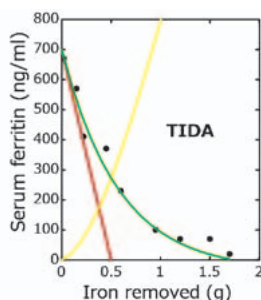
## RESULTS

A model of transformation between ferritin and hemosiderin in iron addition and removal is illustrated in Fig. 1.



**Fig. 1** A model for explaining the fundamental mechanism of transformation between ferritin and hemosiderin is introduced. The left side half shows the 3 stages of iron stores increasing in iron addition, and the right side half shows the 3 stages of iron stores decreasing in iron removal. The top stage of left and right side figures shows the equilibrium of potential between ferritin iron pool and hemosiderin iron pool. Ferritin iron is transformed into hemosiderin iron in iron addition or hemosiderin iron is transformed into ferritin iron in iron removal to maintain the equilibrium between ferritin iron and hemosiderin iron. When ferritin iron is condensed to its saturation level by iron addition, overbalanced ferritin iron is transformed into hemosiderin iron. When ferritin iron is decreased by iron removal, hemosiderin iron is transformed into ferritin iron by mobilizing iron from hemosiderin to compensate its deficit.<sup>17, 18)</sup> The potential difference between ferritin iron pool and hemosiderin iron pool is changed in the course of iron addition or iron removal. A difference in potential level between ferritin iron pool and hemosiderin iron pool motivates the transformation of iron stores. The boundary for transformation is named “Transformer”. The potential difference-dependent transformer determines transformation rate.

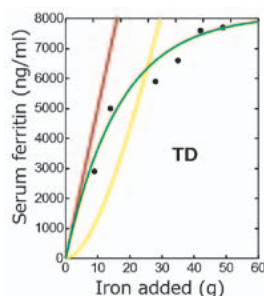
The serum ferritin decrease curve used for determining the amount of ferritin iron, hemosiderin iron and recovered ferritin iron in patient #25 with treated iron deficiency anemia (TIDA) is illustrated in Fig. 2.



**Fig. 2** Serum ferritin decrease curve in patient with treated iron deficiency anemia (TIDA), whose hemoglobin level was normalized after the intravenous iron administration. The rest of administered iron after the use for hemoglobin recovery, 1.7 gram of iron was stored temporarily. The patient fell into iron deficiency anemia again by persistent intestinal blood loss. The best fit green curve was selected by computer simulation to the series of decreasing serum ferritin assay-dots.

Decreasing concave green curve indicates the sum of decreasing component and increasing (recovering) component of serum ferritin. Red straight line indicates the curve of a net serum ferritin decreasing without increasing component. Yellow increasing curve indicates the cumulative serum ferritin recovered (increased) in the course of iron loss by bleeding. The intersection of the red straight line and the horizontal axis at 0.5 g indicates the sum of tissue ferritin iron removed, excluding the tissue ferritin iron increased. The end point of decreasing concave green curve on the horizontal axis at 1.7 g indicates the amount of total iron stores removed. The amount of hemosiderin iron removed was obtained by subtracting the sum of tissue ferritin iron removed from total iron stores removed ( $1.7 - 0.5 = 1.2$  g).<sup>9)</sup>

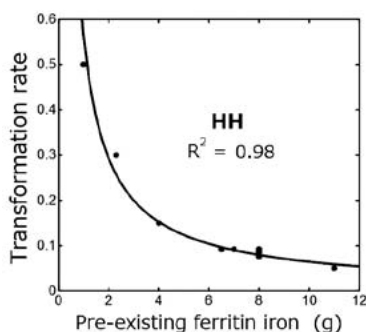
The serum ferritin increase curve used for determining the amount of ferritin iron, hemosiderin iron and transformed ferritin iron in patient #13 with transfusion-dependent anemia (TD) is illustrated in Fig. 3.



**Fig. 3** Serum ferritin increase curve following the repetitive constant blood transfusion in patient #13 with transfusion-dependent anemia (TD) with pre-transfusional iron stores, which were supposed to be the normal level from 81 ng/ml initial serum ferritin.

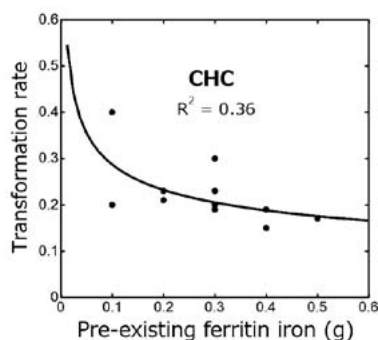
The increasing green curve was selected from serum ferritin assay-dots as mentioned in the Legend of Fig. 2. Increasing green convex curve indicates the sum of increasing component and decreasing (transforming) component of serum ferritin. The red straight line indicates the curve of a net serum ferritin increasing without decreasing component. At an amount of iron stores shown on the horizontal scale, a serum ferritin value on yellow curve (cumulative transformed ferritin iron = cumulative hemosiderin iron increased) is obtained by subtracting a value on green curve (sum of increasing and decreasing ferritin iron) vertically from a value on red line indicating cumulative net ferritin iron. The cumulative amount of hemosiderin iron transformed was obtained by subtracting the remaining tissue ferritin iron from the sum of ferritin iron synthesized by iron addition.

The power regression curve demonstrating an inverse correlation between transformation rate and ferritin iron in HH is shown in Fig. 4.



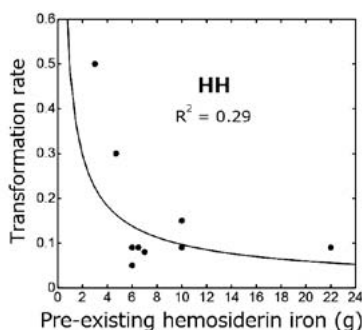
**Fig. 4** A well followed power regression curve shows a close inverse correlation between transformation rates and pre-existing ferritin iron in 9 patients with hereditary hemochromatosis (HH).<sup>5-7)</sup> The transformation rate is proportional to the  $-0.94$ th power of ferritin iron

A minimal inverse correlation between transformation rate and ferritin iron in CHC is shown in Fig. 5.



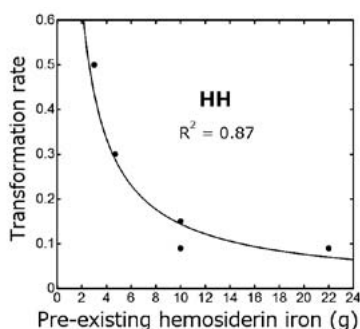
**Fig. 5** The power regression curve shows a minimal inverse correlation between transformation rates and pre-existing ferritin iron in 11 patients with chronic hepatitis C (CHC). The transformation rate is proportional to the  $-0.31$ th power of ferritin iron.

No correlation between transformation rate and hemosiderin iron was observed in 9 HH cases as shown in Fig. 6a. However, in the 2 HH cases (#8 and 9), transformation rates (0.30 and 0.50)



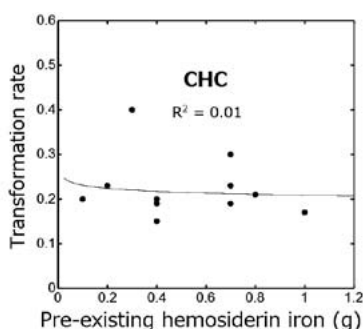
**Fig. 6a** No correlation is shown between turnover rates and pre-existing hemosiderin iron in 7 cases with hereditary hemochromatosis (HH)<sup>(5-7)</sup> with high grade iron overload. However, 2 HH cases (# 8 and 9) with low grade iron overload showed high transformation rates; 0.3 and 0.5 each as shown in Table 2.

were high and total iron stores (7 and 4 g) were low (Table 2). The power regression curve of approximation between transformation rate and hemosiderin iron demonstrated a close inverse correlation from a group of 5 HH cases as shown in Fig. 6b. Other 4 HH cases were excluded, because they had an unusually high ferritin per hemosiderin iron ratio in iron overload<sup>1, 17, 19, 20</sup> as explained later in the Discussion on the transformation rate.



**Fig. 6b** The power regression curve from 5 cases (#1, 2, 5, 8 and 9) with hereditary hemochromatosis (HH) shows a close inverse correlation between transformation rates and pre-existing hemosiderin iron similar to that of ferritin iron in Fig. 3. The transformation rate is proportional to the  $-0.92$ th power of hemosiderin iron.

No correlation between transformation rate and hemosiderin iron was observed in CHC as shown in Fig. 7.



**Fig. 7** No correlation is shown between transformation rates and pre-existing hemosiderin iron in 11 patients with chronic hepatitis C (CHC).

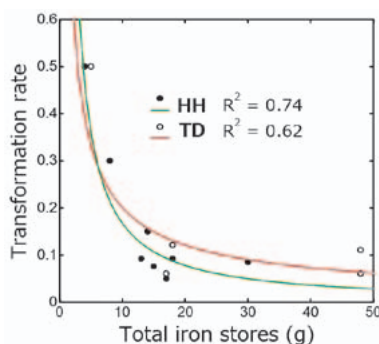
No correlation between transformation rate and total iron stores was observed in CHC (the figure similar to Fig. 7 is not displayed).

Transformation rate was high (0.3 and 0.5) in the 2 HH cases with pre-existing total iron stores below 7 g, but it was lower than 0.1 in 7 HH cases with pre-existing total iron stores more than 10 g.

Transformation rate was above 0.15 up to 0.50 in CHC and TIDA as shown in Table 2.



Two curves of HH and TD showing an inverse correlation between transformation rate and total iron stores are illustrated in Fig. 8.



**Fig. 8** Two power regression curves resembling one another with an inverse correlation between transformation rates and pre-existing total iron stores in hereditary hemochromatosis (HH),<sup>5-7)</sup> and between transformation rate and final total iron stores in transfusion-dependent anemia (TD) are shown. The transformation rate for HH is proportional to the  $-1.1$ th power of pre-existing total iron stores, and that for TD is proportional to the  $-0.72$ th power of final total iron stores.

The amount of ferritin iron was larger than that of hemosiderin iron in 3 HH cases (#3, 4 and 6) or equal in 1 case #7 among 9 HH cases, but only 1 CHC case (#22) among 11 as shown in Table 2.

Transformation rate, ferritin iron, hemosiderin iron, total iron stores and pre-existing serum ferritin in HH and CHC or final serum ferritin determined in TD and TIDA are summarized in Table 2.

**Table 2** (1) Hereditary hemochromatosis (HH)

Patient No.	Sex	Serum ferritin ng/ml	Transformation rate	Ferritin iron g		Hemosiderin iron g	Total iron stores g
1.	m	10000	0.09	8	<	22	30
2.	m	6000	0.09	8	<	10	18
3.	m	1600	0.05	11	>	6	17
4.	m	5000	0.08	8	>	7	15
5.	m	4000	0.15	4	<	10	14
6.	m	3000	0.09	7	>	6	13
7.	m	4000	0.09	6.5	=	6.5	13
8.	m	2200	0.30	2.3	<	4.7	7
9.	m	1800	0.50	1	<	3	4
Average 1800~10000			0.16	6.2	<	8.5	14.6

**Table 2** (2) Transfusion-dependent anemia (TD)

Final iron stores will be affected by an unknown amount of pre-existing iron stores in the case with low final iron stores in TD, although the amount of pre-existing iron stores seems to be within the normal level before falling into anemia.

Patient No.	Sex	Serum ferritin ng/ml	Transformation rate	Ferritin iron g		Hemosiderin iron g	Total iron stores g
10.	f	8000	0.11	12	<	36	48
10.	f	<i>8000</i>	<i>0.06</i>	<i>16</i>	<	<i>32</i>	<i>48</i>
11.	m	1800	0.50	4	>	1	5
12.	m	5800	0.06	4	<	13	17
13.	f	<i>7700</i>	<i>0.12</i>	<i>6</i>	<	<i>12</i>	<i>18</i>

The data determined from serum ferritin increase curve in TD (#10 and 13) are shown in italics.

**Table 2** (3) Chronic hepatitis C (CHC)

Patient No.	Sex	Serum ferritin ng/ml	Transformation rate	Ferritin iron g		Hemosiderin iron g	Total iron stores g
14.	m	500	0.17	0.5	<	1.0	1.5
15.	m	152	0.19	0.4	<	0.7	1.1
16.	m	225	0.21	0.2	<	0.8	1.0
17.	m	200	0.30	0.3	<	0.7	1.0
18.	m	200	0.23	0.2	<	0.7	0.9
19.	m	145	0.15	0.4	=	0.4	0.8
20.	m	216	0.20	0.3	<	0.4	0.7
21.	m	128	0.19	0.3	<	0.4	0.7
22.	m	132	0.23	0.3	>	0.2	0.5
23.	m	81	0.40	0.1	<	0.3	0.4
24.	m	28	0.20	0.15	=	0.15	0.3
Average	28~500		0.23	0.3	<	0.5	0.8

**Table 2** (4) Treated iron deficiency anemia (TIDA)

Patient No.	Sex	Serum ferritin ng/ml	Transformation rate	Ferritin iron g		Hemosiderin iron g	Total iron stores g
25.	f	700	0.16	0.5	<	1.2	1.7

## DISCUSSION

### *Transformation rate*

Transformation between ferritin and hemosiderin is motivated by the potential difference between them. The potential difference between ferritin iron pool and hemosiderin iron pool is variable in the course of iron addition or iron removal. The boundary for the transformation between ferritin and hemosiderin is named “Transformer” (Fig. 1). The potential difference-dependent transformer determines the transformation rate.

Storage iron turnover rate<sup>19, 22)</sup> estimated from plasma iron turnover rate<sup>22-27)</sup> has a unit mg/day.

On the other hand, transformation rate has no such unit by the cancellation of serum ferritin unit ng/ml between numerator and denominator.

Ferrokinetics is performed while not changing body iron level under the dynamic equilibrium of body iron. On the other hand, the transformation rate is determined by changing iron density gradient in iron addition or iron removal by serum ferritin kinetics.

Transformation rate will stay unchanged, as far as iron is added or removed in constant pace as regular blood transfusion or phlebotomy.

The power regression curve of inverse correlation between transformation rate and total iron stores in HH was similar to that in TD (Fig. 8), where pre-existing iron was stored not artificially in HH, but final iron was stored artificially in TD. The above-described finding implies that transformation rate is not set congenitally, but it is decided according to the increasing or decreasing curve form of serum ferritin, which depends on the ratio between ferritin iron and hemosiderin iron.

The ferritin per hemosiderin iron ratio changes according to the change of total iron stores; in the normal storage iron range, the ratio of ferritin iron is larger than that of hemosiderin iron, but the ratio is reversed in iron overload range, because the increase of ferritin iron is nearly saturated, but the hemosiderin iron increases linearly by the transformation of ferritin iron into hemosiderin iron in the progress of iron addition in the iron overload range.<sup>1, 17, 19, 20</sup> The ratio was also different in disease states but changes in iron stores do not seem to have affected the transformation rate, as the inverse correlation between the transformation rate and the iron stores in HH could not be extended to that of CHC.

#### *Transformation rate and ferritin iron*

The power regression curves indicate a close inverse correlation between transformation rate and ferritin iron in HH (Fig. 4). The above-described finding implies that the smaller the amount of ferritin, the larger the transformation rate of ferritin or vice versa. In other words, the increase of ferritin strengthens the resistance to ferritin synthesis.

Transformation of ferritin into hemosiderin increases in response to the condensation of ferritin in loading iron in general. However, the transformation started before the condensation of ferritin iron in CHC.

A minimal inverse correlation between transformation rate and ferritin iron in CHC (Fig. 5) suggests the difficulty in storing iron in the form of ferritin in the cells with CHC.

#### *Transformation rate and hemosiderin iron*

The data-points of HH cases shown in Fig. 6a can be divided into two groups; one having high transformation rate and small iron stores (#8 and 9), and the other 7 cases having low transformation rate and large iron stores (#1, 2, 4 and 5). No correlation was observed between transformation rate and hemosiderin iron in 7 HH cases with large iron stores. The low transformation rate in the 7 HH cases with high hemosiderin iron suggests that their hemosiderin iron were expelled from the realm of iron turnover. However, the 2 HH cases (#8 and 9) with high transformation rate and low grade iron overload indicated an inverse correlation between transformation rate and hemosiderin iron. The borderline of iron stores between the two groups may correspond to the alternation level of principal storage iron; ferritin or hemosiderin.<sup>17, 18)</sup>

The power regression curve of approximation from 5 HH cases in Fig. 6b was similar to the curve in Fig. 4, indicating the similarity of iron turnover between hemosiderin and ferritin.

No correlation between transformation rate and hemosiderin iron in CHC with relatively increased hemosiderin iron suggests the derangement of iron turnover in CHC (Fig. 7).

Generally, the amount of hemosiderin iron is smaller than that of ferritin iron in the range

of normal iron stores.<sup>1, 17)</sup> However, the ratio of hemosiderin iron to ferritin iron was high in CHC, even having total iron stores within normal range.

The relative increase of hemosiderin iron and decrease of ferritin iron in CHC will be preferable for protecting inflammatory cells from iron toxicity.<sup>28)</sup>

Colloidal iron injected intravenously for the treatment of iron deficiency anemia is immediately cleared by the phagocytes resulting in the high hemosiderin iron to ferritin iron ratio probably due to the limitation of iron storing capacity in the suddenly iron overloaded phagocytes.

#### *Transformation rate and total iron stores*

Although the temporary radio-iron uptake and release in the liver and spleen was demonstrated by the animal experiment, such a movement of storage iron was unable to be demonstrated by body surface monitoring over the liver and spleen due to high background radioactivity within 2 days after intravenous radio-iron injection.<sup>24-27)</sup>

Intravenously injected radio-iron entering the iron storing tissue refluxes into plasma leaving a small amount of radio-iron fixed in the iron storing tissue, and the red cell radio-iron utilization (RCU) reaches above 80% by the additional fixation of refluxed radio-iron to red cell mass in normal subjects.<sup>19, 22-27)</sup> Thus, RCU exceeds the 2/3 ratio of red cell iron to total body iron. On the other hand, radio-iron entering the iron storing tissue refluxes little into plasma leaving a large amount of radio-iron fixed in the iron storing tissue, and RCU falls below 70% in iron overload.<sup>19, 22-27)</sup>

Above-described findings are consistent with the inverse correlation between transformation rate and total iron stores.

The transformation rate is not determined congenitally, but it is inversely correlated to the amount of total iron stores in HH and TD (Fig. 8), but not in CHC. No correlation between transformation rate and total iron stores in CHC suggests the derangement of iron metabolism in the cells with inflammation.

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Conflict of Interest declaration: none.

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